

**VA NEBRASKA-WESTERN IOWA HEALTH CARE
SYSTEM**

Omaha, NE (636)

Animal Research Facility (ARF)

STANDARD OPERATING PROCEDURES

A MANUAL

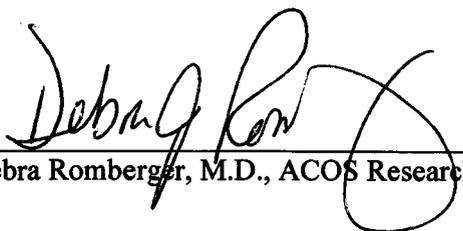
for

Principal Investigators

IACUC Members

& Staff

Revised January 2014


Debra Romberger, M.D., ACOS Research

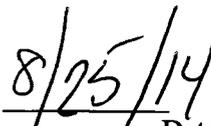

Date

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Table from Section Y of ACORP Main Body (Ver. 4)

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

Item	SOP		Approval Date
	Title	ID	
C.2.c	<i>Each Experimental Procedure</i>		
M.1	<i>Housing Type</i>		
M.2	<i>Enrichment</i>		
U.4.a	<i>Disposition of dead animals</i>		
U.4.b	<i>Contacting PI/Staff</i>		
V	<i>Special Procedures/Husbandry</i>		

Omaha VA Animal Facility SOPs

<u>Page</u>	<u>Title</u>	<u>ID</u>	<u>Approval Date</u>
26	Standard Housing	XXII, A	2014
27	Housing singly	XXII, C	2014
26	Enrichment	XXII, A, 4	2014
18	Euthanasia, CO ₂	XIII, A, 3	2014
18	Euthanasia, Exsanguination	XIII, A, 4	2014
8	Disposal of Animals found dead in cage	IX, 4	2014
38	IVIS SOP	Appendix E	2014
40	ABSL2 SOP	Appendix F	2014

POLICIES AND MONITORING ANIMAL RESEARCH FACILITY

Animals housed in the Omaha VA Animal Research Facility (ARF), which are ARF approved, shall be monitored and under surveillance as detailed below. The Omaha VA Research Facility is in compliance with standards defined in the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Academy Press 2011.

I. GENERAL PRINCIPLES OF ANIMAL CARE:

Each investigator utilizing the animal care facility should be familiar with the principles governing the humane use and care of laboratory animals. It is important that investigators and research technicians be familiar with policies and procedures in this document. Any variances or changes required for any experimental design must be justified in a protocol or amendment and be approved by the Institutional Animal Care and Use Committee (IACUC) prior to initiating the experiment.

Animals are to be housed only in areas designated as animal rooms. As recommended in the aforementioned references, each species will be housed in separate rooms. Animals may be moved to designated laboratory areas for experimental manipulations for periods not to exceed 12 hours. If housing is required for longer than the 12-hour period, a letter of justification will be written to the IACUC for approval with a copy in the ARF files. Animals may not be returned to the animal room from the laboratory area without prior approval. Experimental manipulation should be performed in the surgery prep (R-124), surgery rooms (R120/R119) whenever possible or animal room without disturbance to the other animals with approval by ARF.

To prevent spread or carrying of viruses, all research employees using or entering the animal facilities should not have rodent species as pets in the home, laboratory, office or ARF. Under no circumstances, may animals be removed from the ARF to become pets.

Monitoring of cages/pens/rooms will be done by the ARF supervisor.

All persons entering the ARF animal room(s) will be required to wear a lab coat, surgery smock or scrubs as a covering of street clothing [when handling animals, cages and accessories within the ARF.] This covering is to be worn only when working in the ARF and exchanged for a different lab coat when working in the laboratory.

II. PROCUREMENT AND RECEIPT OF ANIMALS:

Animal procurement must be from authorized vendors only. All rodents must be ordered only from vendors with barrier produced, health monitored breeding colonies. Each purchase order should state that the animals have not been exposed to, nor recovered from, the specific infectious agents and parasites for that species. Purchase orders requesting animals from vendors who do not meet with the above standards will not be processed until justification is made by the investigator and approval has been granted. Questions about vendors should be directed to the Supervisor of the ARF (Ext. 3272).

A "Request for Housing" form will also be completed and approved by the ARF supervisor each time animals are ordered. All orders for animals to be housed in the VA Animal Facility must be approved and processed by the supervisor of the ARF or designee.

A. Purchase of Rodents from Non-commercial Sources

Rats and mice can only be purchased from non-commercial sources based only upon scientific merit. Price will never be justification for purchasing from non-commercial sources.

Rodents purchased from non-commercial sources will have the following stipulations:

1. One week quarantine before any use. Animals may be utilized after the first week, but appropriate measures must be taken to prevent exposure of other populations of the same species.
2. Sentinels will be provided immediately at PI expense.
3. Serology will be done on sentinels 3 weeks
4. Animals will be released from quarantine when negative serological results are available. Positive

- animals will be euthanized as soon as possible.
5. When possible the animals should be placed in laminar flow hoods and/or microisolator cage tops should be used for both rats as well as mice.
 6. Serological Tests to be ordered listed below plus additional if needed.
 - a. Rats:
 - i. RCV/SDAV
 - ii. NS1 (Generic Parvovirus)
 - iii. RPV
 - iv. RMV
 - v. KRV
 - vi. H-1
 - vii. RTV
 - viii. Sendai
 - ix. PV
 - x. Mycoplasma pulmonis
 - xi. REO3
 - xii. LCMV
 - b. Mice:
 - i. MHV
 - ii. MVM (MMV)
 - iii. NS1 (Generic Parvovirus)
 - iv. MPV (MPV 1-5)
 - v. MNV
 - vi. TMEV
 - vii. EDIM
 - viii. Sendai
 - ix. Mycoplasma pulmonis

All investigators with animals housed within the ARF must have an approved protocol by the VAMC IACUC. Space availability is assessed at the time of protocol review. Room assignment will be the responsibility of the ARF Supervisor or designee. Final space assignment will be determined after funding acceptance of protocol for funding.

Animal(s) sent in error for any reason or any animal not used in the completion of a study will be turned over to the ARF for distribution. Investigators will be informed of extra animals and distributed on a first come basis. These animals will be assigned to a protocol and deducted from the total animal use for that protocol. Those investigators forfeiting any animals will be given credit on the original protocol. A "Transfer of Animals" form will be completed at the time of transfer.

Any tumor cell lines or tissue cultures brought into the research facility for implantation into an animal species must have verified assurance that the cells are not infected with viruses. Vendors and investigators should have all specimens checked at vendor's expense.

B. Procedure for Live Animal Shipping

When the need arises to ship live animals to another institution or commercial lab for disease testing many factors are involved. The PI, Veterinarian, ARF Supervisor and the receiving institution all need to be involved in the decision. Consideration of weather enroute as animals are vulnerable when left on tarmacs and shipping docks, arrival site availability, number and condition of animals to be shipped, costs, reason for shipment, and methods of transportation are some of the factors to be considered prior to the decision of shipping. All requests for animal shipping are discussed on a case--by-- case basis.

Below are steps to transport animal by air, the ARF Supervisor will arrange shipping details.

1. The VA live animal shipping vendor will be contacted to arrange shipping. They ship on

commercial airlines and ground transportation. If the primary shipping company is not available alternative transportation options will be discussed on a case--by-- case basis.

2. The receiving institution is advised of the projected arrival time and company involved to insure animal safety and that correct procedures are in place.
3. Steps 1 and 2 usually take a minimum of 2 days to finalize all details and receive the purchase order number so the process should be started as early as possible.
4. Animals are packaged by ARF personnel (in coordination with study personnel) and the shipping vendor picks up at ARF Facility.
5. Receiving institution or shipping vendor advises shipping institution when animals are safely delivered.

III. ANIMAL DISPOSAL:

All small animal carcasses shall be placed in small plastic bags (provided by the ARF) then placed in the freezer located in the ARF. If animal carcasses are collected before disposal - combined weight should not exceed 20 pounds per container. Larger carcasses should be bagged separately - not to exceed maximum of 60 pounds. When contents exceed this limit, it is the responsibility of the Principal Investigator to make arrangements for disposal.

IV. ANIMAL CHARGES:

Expenses for animal procurement are the total responsibility of the investigator. Expenses for animal maintenance will be managed by the Omaha VAMC Research Office. With the assistance of the veterinarian, as well as the Administrative Office, the IACUC is notified of per diem charges and forwards these charges to the R&D Committee. These charges will be used to determine the specific costs to be reimbursed by investigators to the Research Service. This per diem will reflect the actual costs of animal maintenance as well as established rates in the local area. These rates are used by the ACOS to compile the quarterly charges billed to the investigators. The final charges will reflect per diem charges, source of project funding (VA versus non-VA) and total VA support of the ARF.

Notification will be a written request, subject: "Request for Animal Housing" submitted prior to the animals being ordered or introduced into ARF. Please address the length of stay expected with the title and VA I.D. number of the protocol under which the animal subjects will be utilized. The "Request for Animal Housing" form may be obtained from the ARF Supervisor or by using online IACUC forms. Space accommodations will be reviewed at the time of the request. Animals delivered without prior approval will be refused entry into the ARF.

All investigators with animals housed within the ARF must have an approved protocol by the VAMC IACUC.

Animals housed within the VA ARF will be subject to per diem charges. Charges will be billed on a quarterly basis. Expenses for all special feed, caging, specialty items and testing will be borne by the investigator.

PER DIEM CHARGES ARE AS FOLLOWS (FY 2014)

mouse (single)	@ .65 ea	rat (single)	@ .75 ea
mouse (group)	@ .50 ea	rat (group)	@ .60 ea
rabbit	@ 3.00 ea		
swine	@ 10.00 ea		

Consideration to rate reduction will be given in individual cases.

V. ARF PERSONNEL:

Ellis G. Jensen, DVM, and Sarah Mongan, D.V.M., (consultant veterinarians): 24 years affiliation - routine biweekly and emergency visits (24-hr call) to the Animal Care Facility.

Robert L. Wiegert, B.S., LATG, Supervisor of Animal Care Facility with 21 years experience – certified through the National AALAS as a Laboratory Animal Technologist.

T. Norman Thomas, Animal Caretaker – 15 years experience with laboratory animals, certified through the National AALAS as an Assistant Laboratory Animal Technician.

Ryle Oliver – Part time 2 years' experience

VI. ARF SECURITY SYSTEM:

A security alarm system is in force. Anyone entering the ARF area after hours/weekends/holidays must adhere to the guidelines as follows: All persons must sign-in upon entry and record the time of entry. Make sure the alarm is in off mode while you are in the facility. If for any reason you must exit the ARF, check the sign-in list for others within the area at that time. If you are the last to exit, reset the alarm, record the time and exit. If you are not the last to exit, sign-out and record time leaving (DO NOT RESET ALARM). Do not leave alarm in off position when no one is present.

Proximity card readers are installed on ARF doors. All persons are required to carry the proximity card issued to them at all times. The ARF is secured 24 hours year around. Monitoring of this system is collected by computer. Lost cards should be reported immediately. If any card is used by an unauthorized person, the registered name will remain responsible. In case of an electronic emergency – an emergency release button has been installed at the main entrance by the elevator lobby.

Security cameras (continuous 24 hours surveillance) are located on each floor to monitor all corridors, entrance into Building 15 and the lobby area on first floor. Records are kept via computer located in police control center.

VII. VETERINARY CARE:

A. The veterinarian or his/her designee shall:

1. Observe animals daily for health and welfare.
2. Prevent, control, diagnose and treat diseases or injury.
3. Provide guidance to users (investigators) regarding handling, immobilization, anesthesia and euthanasia.
4. Monitor surgery programs and post surgical care.

B. There will be direct and regular contact between animal care facility personnel and veterinarians. Any unusual or extraordinary situations are to be communicated immediately to the ARF supervisor and the attending veterinarian. The ARF supervisor may be contacted at 402-995-3272 or 402-213-7733 and the veterinarian may be contacted at 402-333-3847 during normal working hours and 402-697-1776 after normal working hours, weekends and holidays.

VIII. MEDICAL SURVEILLANCE, Investigator Animal Care/Responsibility:

A. All investigators responsible for animals will provide the animal care facility personnel with his/her telephone number and that of his/her designee's name and telephone to facilitate answering questions about animal care. In cases of animal health problems, the investigator or designee are required to be available to respond to the animal care facility personnel and veterinarian concerning specific problems regarding his/her animals.

1. In cases of post-procedural care and post-operative care of animals, instructions will be given to animal caretakers regarding special attention to the animals.
2. Animal caretakers will report **immediately** any abnormalities of activity, behavior or illness to the ARF supervisor who will report the findings to the veterinarian and investigator or designee for expeditious attention. In the absence of the supervisor, the Animal Care Technician will contact the principal investigator or designee. If the principal investigator/designee cannot be contacted, the caretaker will call the veterinarian.
3. The investigator, in consultation with the veterinarian, will resolve any acute or long term care problems.
4. In the event that the investigator or his designee is not available, the veterinarian will initiate treatment or action based solely on his/her judgment.
5. Husbandry Logs: Logs will be kept in animal rooms which include temperature/humidity records, record of cage and water bottle changes, as well as general animal condition and specifics regarding any animal that might require attention from the veterinarian, the PI or the technician. The logs for the limited access rooms will be kept on the outside of the room.

IX. MEDICAL SURVEILLANCE, ARF animal monitoring:

A. The care of animals may be short, intermediate and long term. Short-term care is defined as use of animals within a 45 day period. Intermediate term does not exceed a 90 day period. Long-term care will include all experimental animals used for a period greater than 90 days as designated by the project protocol.

1. Animal care will include daily observation for welfare, illness, injury, behavior and mortality.
2. Reporting to the veterinarian of abnormalities as in Section A, 1-4 above.
3. All unusual situations, repetitive problems or improprieties will be submitted by the veterinarian or ARF supervisor to the IACUC for review with appropriate action(s) initiated and communicated to the investigator.
4. When animals are found dead in cages they will be placed in freezer. The Investigator and ARF Supervisor will be notified. Investigators that want other arrangements must indicate on each individual ACORP.

X. INFECTIOUS DISEASES:

- A. Identification of disease will warrant immediate attention, including isolation, notification of both investigator and veterinarian and treatment of the infectious agent.
- B. Isolation procedures will be initiated to reduce spread of any infectious disease to other animal colonies in the facility.
- C. Investigator will have the responsibility to eliminate the source(s) of infection, bear the costs of identification and, in cooperation with the veterinarian, eliminate the problem.

XI. COMMON CLINICAL SIGNS INDICATING PAIN, DISTRESS OR DISCOMFORT IN EXPERIMENTAL ANIMALS:

- A. **CARDIOVASCULAR** – Heart rate altered; pulse strength affected; peripheral circulation decreased, blue and cold extremities (ears, paws).
- B. **RESPIRATORY** – Abnormal breathing pattern, rate and depth altered, labored, panting; nasal discharge.
- C. **DIGESTIVE** – Body weight loss or poor growth; feces altered in volume, color or consistency (e.g., black with blood, pale, lack of bile pigments, undigested food; diarrhea/constipation); jaundice, salivation, vomiting (except in rats)

- D. **NERVOUS and MUSCULOSKELETAL** (locomotor) – Twitching, fitting, tremors, convulsions, paralysis, pupils dilated, shivering hyperaesthesia, reflexes sluggish or absent; unsteady gait, lameness, muscle flaccidity, rigidity or weakness, protecting affected area such as “boarding” abdomen or reluctant to move a limb (e.g. arthritis).
- E. **MISCELLANEOUS** – Any abnormal swelling, protrusion (hernia, rupture) or abnormal discharges from natural orifices; raised body temperature. Dehydration, sunken eyes, skin tents, urine specific gravity, increase/ decrease in volume and porphyrin staining.

XII. GUIDELINES FOR ANESTHESIA AND ANALGESIA IN LABORATORY ANIMALS

This section contains information on the administration and dosage of common analgesic, tranquilizing and anesthetic agents for laboratory animals. It is strongly recommended that the advice of veterinary staff or other experts be sought when such drugs are to be administered to uncommon species or when agents not listed in the tables will be used.

Unnecessary exposure of personnel to gasses from volatile anesthetics should be avoided. Expired gases should be vented to the exterior or absorbed in activated charcoal.

A. ANESTHESIA: PRINCIPLES OF MANAGEMENT OF ANESTHESIA:

1. GENERAL:

Sedatives, analgesics, and general anesthetic agents must be utilized for the control of pain and distress unless contrary to the achievement of the objectives of the study in which case strong justification must exist.

Anesthetic agents frequently affect the cardiovascular, respiratory and thermo-regulatory mechanisms, in addition to the central nervous system (CNS). Every effort should therefore be made to maintain the circulation, respiratory blood gases and the body temperature of the anesthetized subject within normal physiological limits. The use of endotracheal intubation ensures that the airway remains patent and free from obstruction.

Hypothermia may occur as a result of anesthesia in small animals. This may result in death or a greatly prolonged recovery from the anesthetic. Hypothermia may be counteracted to some extent by placing the animal on a warm water circulation unit or other devices that assist in conserving body heat.

2. HANDLING THE ANIMAL:

The animal should always be handled gently and calmly. Struggling and excessive fright should be kept to a minimum. Prolonged excitation will disturb the circulatory and metabolic state of the animal and induce a degree of shock. Furthermore, attempts to anesthetize struggling animal present physical problems in addition to enhancing the likelihood of an abnormal response.

3. DISSOCIATE ANESTHETICS:

Dissociate anesthetics produce a state of chemical restraint and anesthesia characterized by a form of muscle rigidity and an apparent dissociation of the mind from the external environment. The eyes remain open; various reflexes, including the blinking reflex remain intact. Adequate respiration is normally maintained, an increase in heart rate and blood pressure frequently occurs.

Ketamine is the most commonly used member of this group. Depth of anesthesia is dose related. It produces activation of the limbic system and depression of the thalamo-neocortical system rather than dose-related general CNS depression. It is rapidly metabolized to norketamine in the liver via the hepatic P450 microsomal system. The metabolites are mainly conjugated and excreted in hepatic or renal function. Side effects of ketamine hydrochloride include excessive salivation which may be controlled with atropine; a tendency toward

convulsions and a recovery characterized by excitement, disorientation, and hallucinations, which may be controlled by tranquilizers and barbiturates. In all cases, a smooth recovery will be facilitated if the animal is left undisturbed in a quiet relatively darkened environment.

Ketamine can be used alone or in combination with the non-narcotic, sedative-analgesic-muscle relaxant xylazine to produce anesthesia with a wide margin of safety in many species. Ketamine does not provide analgesia. It is best to limit the use of Ketamine or the Ketamine xylazine combination for chemical restraint and for relatively non-invasive procedures of short duration.

4. BARBITURATES:

Barbiturates differ from tranquilizers and narcotics in that increasing the dose progressively increases the depth of depression until a state of general anesthesia is reached. Their primary use is in the induction and/or maintenance of general anesthesia. Barbiturates are potent respiratory depressants and their effects on the cardiovascular system are variable. At intermediate dosages, excitement is sometimes induced.

The barbiturates are grouped according to duration of action into long acting (e.g., phenobarbital); short or intermediate acting (e.g., pentobarbital) and ultra short acting (e.g.; thiopental, thiamylal, methohexital). Only the short and ultra short acting drugs are commonly used for anesthesia. Anesthetic duration with short/intermediate barbiturates is approximately 2-4 hours. The effects of ultra short barbiturates range from 15 to 30 minutes.

Variation in dose response and duration of effect of barbiturates is extreme within and between species.

Whenever possible, barbiturates should be administered intravenously, slowly to effect. Administration by other routes, except IP in rodents, is far less predictable. Subcutaneous injection is contraindicated.

5. INHALANT ANESTHETICS:

Inhalant anesthetics give a relatively rapid onset and recovery. The high degree of controllability over anesthetic depth and the relatively constant response of a very wide variety of species are advantages of these agents. However, specialized equipment and constant monitoring of the animal are required with use of inhalant anesthetics.

The depth and duration of effect in inhalation anesthesia can be controlled by the anesthesiologist through the manipulation of drug concentration and pulmonary ventilation. Differences in anesthetic solubility determine the speed with which gas concentration builds up in the arterial blood. As highly soluble gases require more time to build up a significant concentration in the blood, they result in a more prolonged induction and recovery. The reverse is true of the highly insoluble gases which are therefore more controllable as their blood concentration can be rapidly changed; however, for this reason they are more hazardous.

Inhalant anesthetics can be administered by means of a simple nose cone for the performance of short procedures. However, for more extended surgery and in most larger species the following special equipment should be available: a source of carrier gas oxygen; a vaporizer for the volatile anesthetics; a breathing system from which the anesthetic mixture is inhaled and a mask or endotracheal tube for connecting the breathing system to the animal.

Inhalant anesthetics commonly used in experimental animal surgery include:

6. NITROUS OXIDE:

Potent analgesic; a weak general anesthetic; highly insoluble in lipids. Induction of a state of general anesthesia with this gas alone is difficult, but it is particularly useful in conjunction with other injectable and inhalant agents. Respiration and circulation are minimally affected. The gas is non-irritating and non-explosive and has a sweet odor. It is usually used in mixture with oxygen of 1:1 to 4:1 to supplement other anesthetics such as methoxyflurane or halothane.

7. ISOFLURANE:

This inhalant agent released for clinical use in 1981 which has properties approaching those of an ideal anesthetic. Non-flammable (in anesthetic concentrations), non-irritating, non-toxic, and relatively insoluble in blood. This low solubility results in rapid induction and recovery and permits the level of anesthesia to be altered quickly and precisely requires use of a precision vaporizer.

8. LOCAL ANESTHETICS

Local anesthetics such as bupivacaine hydrochloride (Marcaine) and lidocaine hydrochloride (Xylocaine) may be used by themselves to block the nerve supply to a limited area for the performance of minor or rapid procedures. Local anesthesia is also frequently used as an adjunct to various sedative and hypnotic agents in somewhat more prolonged and invasive procedures, as in a caesarian section. Local anesthetic agents may be used for the regional infiltration of a surgical site, field blocking, nerve blocks, and for epidural and spinal anesthesia. Expert (veterinary) assistance should be sought in the initial use of the last three procedures.

B. ANALGESICS AND GUIDELINES FOR POST-OPERATIVE ANALGESIA

1. THE INVASIVENESS OF THE PROCEDURE:

- a. Are body cavities invaded?
- b. Are sensitive tissues involved (bones or teeth)?
- c. Is significant tissue destruction or inflammation produced?

2. THE DEGREE OF SEVERITY OF PAIN EXPECTED:

- a. Comparison to similar procedures in people; would a reasonably stoic person tolerate the postoperative period without analgesics?
- b. Behavior of the animal during postoperative period; e.g., level of activity, appetite, roughness of hair coat, etc.

3. GENERAL CONSIDERATIONS:

Procedures expected to cause more than slight or momentary pain (e.g., pain in excess of a needle prick or injection) require the appropriate use of pain-relieving measures unless scientifically justified in an approved animal care and use protocol.

Management of pain in animals requires that pain either be anticipated and prevented (pre-emptive), or recognized and alleviated (post-inductive).

Procedures that have historically produced a high pain score (based upon literature reviews or pilot study results) require a prospective plan for post-operative analgesic treatment of all study animals that will undergo this manipulation.

4. Categories of Pain:

Minimal to Mild Pain	Mild to Moderate Pain	Moderate to Severe Pain
Catheter implantation	Minor laparotomy incisions	Major laparotomy/organ incision
Tail clipping	Thyroidectomy	Thoracotomy
Ear notching	Orchidectomy	Heterotopic organ transplantation
Superficial tumor implantation	C-section	
	Embryo transfer	

Orbital sinus venotomy Superficial lymphadenectomy Ocular procedures Multiple ID antigen injections Intracerebral electrode implantation Vasectomy Vascular access port implantation	Hypophysectomy Thymectomy	Vertebral procedures Burn procedures Trauma models Orthopedic procedures
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5. Identification of Pain:

a. Mice:

- reduced grooming
- reduced level of spontaneous activity
- piloerection
- hunched posture
- squint-eyes
- pale eyes (if albino)
- increased aggressiveness when handled
- distance themselves from cage mates
- reduced food/water intake

b. Rats:

- reduced level of spontaneous activity
- increased back arching, horizontal stretching, abdominal writhing, falling/staggering, poor gait and twitching
- decreased grooming
- porphyrin secretions (ocular/nares)
- squint-eyed
- pale eyes (if albino)
- piloerection
- reduced food and water intake
- increased aggressiveness when handled

c. Rabbits:

- reduced activity
- failure to groom
- reduced food and/or water intake
- squint-eyed
- pale eyes (if albino)
- changed posture, tucking of abdomen, tensing of muscles
- guarding, attempt to hide, or aggressiveness
- grinding of teeth

d. Swine

- . abnormal gait
- . reduced food/water intake
- . hunched back
- . tucked up abdomen
- . failure to move with fluid motion

6. Non Pharmacological Considerations:

Management of pain in animals can be enhanced by providing appropriate housing, handling, and restraint and by utilizing appropriate experimental techniques. This is especially true when surgery is part of the protocol. A skilled surgeon who utilizes proper surgical techniques can minimize complications of surgery and tissue trauma, which contribute to postoperative comfort. Surgical complications such as infection, seromas, hemorrhage and inflammation induce painful and stressful sensations. Selection of appropriate suture materials and utilization of proper instrumentation both can help to alleviate postoperative trauma as well as perioperative care, which emphasizes maintenance of homeostasis. For instance many animals have inflammatory reactions to surgical gut and silk sutures that can be avoided by use of newer synthetic suture materials, which are less likely to produce inflammatory responses (Flecknell, 1966; Swindle et. al., 2002; Thurmon et. al., 1996).

Housing appropriate for the species may reduce post-procedural discomfort. Animals housed in a stressful situation can be more vulnerable to pain. For example, animals generally require an increased environmental temperature to recover quickly from anesthesia. Wet bedding materials may also contribute to hypothermia and increase the chance of infection. Animals, which have been habituated to handling and husbandry routines, may experience less distress. Husbandry may have to be modified to provide animals with easier access to food and water if defects such as spinal cord trauma have been induced. Use of nesting materials, soft food, bandaging and other types of nursing care, such as expressing the bladder in animals with spinal cord dysfunction, may also be indicated as adjuncts to analgesics (Flecknell, 1996; Swindle et. Al., 2002).

Diet may contribute to post-procedural recovery. For example, soy-containing diets have been demonstrated to help alleviate pain in rats with chronic sensory disorders. Consuming a soy-containing diet prevents development of tactile and heat allodynia, but not mechanical hyperalgesia in rats with partial sciatic nerve ligation (Shir et. al., 2001). Softened food or foods with high caloric content may be helpful in assisting animals with oral lesions or debilitating procedures.

Essential to any program of post-procedural care is training to make investigators aware of species-specific requirements and appropriate experimental techniques that may reduce the discomfort level of the animals.

7. The duration of the postoperative pain or discomfort expected

a. Postoperative analgesia is desirable for most surgical procedures involving penetration deeper than the skin and subcutaneous tissues. It is recognized, however, that the unique properties of some anesthetics may meet the analgesic requirement for some procedures. For procedures involving invasion of bones, joints, teeth or significant destruction or inflammation in other tissues or body cavities opened, it is the responsibility of the investigator to make sufficient justification in his or her animal use protocol if postoperative analgesics cannot be used.

The following categorical examples may be useful to investigators in determining the necessity for supplementary postoperative analgesia in procedures involving experimental or instructional use of animals:

b. No postoperative analgesia required:

Procedures likely to cause mild or no postoperative pain or discomfort, e.g.:

- * injections of substances of low irritation potential
- * relatively non-invasive catheter or electrode placement
- * skin incisions, suture or wound clip placement

- * subq pump implantation

c. Short-term postoperative analgesia desired:

Procedures likely to cause mild to moderate pain or discomfort of short duration, e.g.

- * castrations, including ovariectomies
- * invasive electrode or catheter placement
- * extraocular surgery

The postoperative analgesia associated with methoxyflurane anesthesia may be adequate for many of these procedures.

d. Prolonged postoperative analgesia required:

Procedures likely to result in severe or prolonged pain or discomfort, e.g.:

- * extensive dissection of soft tissues
- * major entry into the pleural or peritoneal cavity
- * intraocular surgery
- * adrenalectomy and hypophysectomy in rodents
- * orthopedic or dental surgery
- * Thoracotomy, hepatectomy

8. ANALGESIC AGENTS

a. Procedure for storage, use and inspection of controlled substances

- Investigator brings prescription for controlled substance to R104. Robert Wiegert or Fred Hamel takes prescription to VA pharmacy to obtain substance and place in R104.
- Substance is labeled, logged in, paperwork setup and stored until needed.
- When needed an investigator may 1) take bottle and paperwork for short period if doing large numbers of animals or over weekends; 2) take syringe (s) only as needed.
- Hospital will inventory only in R104 when conducting narcotic inspection. All controlled substances in Research will be kept in R104.
- Controlled substances will be storage in a double locked cabinet. The cabinet has a stainless steel sheet in place of glass on outside doors.

b. Narcotics: Narcotics produce potent hypnotic and analgesic effects including a significant depression of the cardiovascular and respiratory systems and an alteration in the thermoregulatory mechanism. The euphoria and addiction associated with narcotics in humans is not a problem in animals when the drugs are used properly

i. Buprenex – buprenorphine is indicated for the relief of moderate to severe pain post-operative in dogs and rodents. Pharmacological effects occur as soon as 15 minutes after intramuscular injection and persist for 6 hours or longer. Peak effects usually are observed at 1 hour. Dosage: Canine – 0.01 – 0.05mg/kg SubQ or IM injection. Rats .02-.05 mg/kg, SC q 6-12h or IM injection, Mice 0.05-.1 mg/kg, SC q 8-12 h. In rodents the Buprenex can also be added to a jello preparation for postoperative pain and be taken orally. It is preferred to inject once and then follow up with the oral jello preparation.

ii. Morphine is most frequently used clinically for the control of post-operative pain its use is complicated by undesirable gastrointestinal effects. As a premedicant, its stimulatory effect on the vagus nerve may induce an abnormal slowing of the heart beat (bradycardia)

unless atropine is given in advance.

iii. **Meperidine (Demerol; Pethidine)** has effects similar to morphine as very little gastrointestinal stimulation is induced. This drug has also proven useful as a post-operative sedative.

iv. **Fentanyl** is a very potent short-acting narcotic. Patch delivery system is appropriate analgesia. Applied to clipped/clean skin area. Demerol 20R/Buprenex is given preceding patch placement. Side effects are dry mouth, dilated pupils and detoxifying.

v. **Oxymorphone hydrochloride (Numorphan)** – A semi-synthetic narcotic analgesic with a potency approximately 10 times that of morphine. There are limited reports of its use as a pre-anesthetic and post analgesic.

vi. **Butorphanol tartrate (Stadol)** – A synthetic narcotic agonist-antagonist which is 3-5 times more potent than morphine and causes about the same degree of respiratory depression as morphine. Provides greater sedation than nalbuphine. Sedative effect last longer than analgesic effect. Analgesia duration = 3-5 h

vii. **Acetylsalicylic acid (aspirin):** The salicylate analgesics relieve only mild to moderate pain. They do not relieve deep visceral pain or sharp intense pain.

c. **NARCOTIC ANTAGONISTS:** Effective “antagonists” such as nalorphine hydrochloride and naloxone hydrochloride are available to reverse the effects of narcotics. These agents do not reverse the sedative or depressant effects of other drugs.

9. Suggested Analgesic Formulary

a. MICE:

Minimal to Mild	Mild to Moderate	Moderate to Severe
Local anesthesia	Local anesthesia	Local anesthesia
Butorphanol 1-5 mg/kg, SC q4h	Buprenorphine .05-0.1 mg/kg, SC q8-12h	Buprenorphine *1 .05-0.1 mg/kg, SC q8-12h
Carprofen 2.5 – 5.0 mg/kg, SC	Carprofen 2.5 – 5.0 mg/kg, SC q24h	Carprofen *1 2.5 – 5.0 mg/kg, SC q24h
		Morphine 2 – 5 mg/kg, SC q 2-4 h

*1 Severe pain may be better addressed by the addition of a NSAID to an opioid.

b. RATS:

Minimal to Mild	Mild to Moderate	Moderate to Severe

Local anesthesia	Local anesthesia (adjunct to systemic analgesics)	Local anesthesia (adjunct to systemic analgesics)
Butorphanol 2 mg/kg, SC once	Buprenorphine 0.02 - .05 mg/kg SC q6-12h	Buprenorphine *1 .05 mg/kg, SC q6-8h
Carprofen 2.5 – 5.0 mg/kg, SC once	Carprofen 2.5 – 5.0 mg/kg, SC q24h	Carprofen *1 2.5 – 5.0 mg/kg, SC q24h
Meloxicam 1 mg/kg, SC once	Meloxicam 1-2 mg/kg, SC q24h	Meloxicam 1-2 mg/kg, SC q24h
		Morphine 2.5 – 10 mg/kg, SC q 2-4 h Severe Pain

c. RABBITS:

Minimal to Mild	Mild to Moderate	Moderate to Severe
Local anesthesia	Local anesthesia (adjunct to systemic analgesics)	Local anesthesia (adjunct to systemic analgesics)
Ketoprofen 3 mg/kg, SC once	Buprenorphine 0.01-0.05 mg/kg SC, IM, IV q6-12h	Buprenorphine .05 mg/kg SC, IM, IV q6-12h
Butorphanol 0.1 – 0.5 mg/kg, IM, IV q4h	Butorphanol 0.1 – 0.5 mg/kg, IM, IV q4h	Morphine 2-5 mg/kg, SC q2-4h
Carprofen 4.0 mg/kg, SC 1.5 mg/kg, PO once	Carprofen 4.0 mg/kg, SC, q24h 1.5 mg/kg, PO, q12h	Fentanyl Patch 25 ug/h transdermal, q72h
Meloxicam 0.2 – 0.3 mg/kg, SC, PO once	Meloxicam 0.3 – 1.5 mg/kg, PO q24h	

d. SWINE:

Minimal to Mild	Mild to Moderate	Moderate to Severe
Local anesthesia	Local anesthesia (adjunct to systemic	Local anesthesia (adjunct to systemic analgesics)
	to systemic	

	analgesics)	
	Carprofen 2mg/kg SC q24	Fentayl/Transdermic Patch 15-25kg - 50-100 ug/hr
	Buprenorphine 0.005-0.02 mg/kg SC,IM,IV q6-12hr	Fentayl 2-6 mg/kg/hr CRI
		Morphine 0.2mg/kg IM 20 mg max dose

XIII. EUTHANASIA:

For the welfare of our animals, the Research Service, Omaha VA Medical Center, has reviewed and adopted guidelines concerning euthanasia as described in the AVMA Guidelines on Euthanasia.

Euthanasia is the act of inducing painless death. Criteria to be considered for a painless death are: rapidly occurring unconsciousness and unconsciousness followed by cardiac or respiratory arrest. Several criteria were used in evaluating methods of euthanasia: 1) ability to produce death without causing pain, distress, anxiety or apprehension; 2) time required to induce unconsciousness; 3) reliability; 4) safety of personnel; 5) irreversibility; 6) compatibility with requirement and purpose; 7) emotional effect on observers or operators; 8) compatibility with subsequent evaluation, examination, or use of tissue; 9) drug availability and human abuse potential; 10) age and species limitations; and 11) ability to maintain equipment in proper working order.

The facial expressions and body postures indicate various emotional states of animals. The need to minimize animal distress, including fear, anxiety, and apprehension, must be considered in determining the method of euthanasia. Distress vocalization, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Whenever possible, other animals should not be present when euthanasia is performed, especially euthanasia of the same species. Gentle restraint, preferably in a familiar environment, careful handling, and talking during euthanasia often has a calming effect on companion animals. Some of these methods may not be effective with wild animals or animals that are injured or diseased. When restraint may cause pain, injury or anxiety to the animal or danger to the operator, the use of tranquilizers, analgesics, and/or immobilizing drugs should be considered.

A. METHODS APPROVED BY VAMC IACUC:

1. Fatal Plus: Intravenous administration is preferred means because the effect is most rapid and reliable. Intrapulmonic (lungs) injection should be avoided. Skill is required for intra cardiac injection. Intra cardiac injection may be used on depressed, anesthetized, or comatose animals. If the animal to be euthanatized is excitable or vicious, use of analgesics, tranquilizers, Ketamine, xylazine or other tranquilizers/sedative is recommended before administration of euthanatizing agent.

Dosage: 1mg/10 pounds any species

2. Sodium Pentobarbital. Accurate records must be kept in logbook in the ARF supervisor's office and ordered and dispensed under the DEA number of the ACOS/Research. Monitoring or verification of death is lack of heartbeat, lack of respiration, lack of eye response, pale bluish gums and tongue with onset of rigor mortis. Animal should be rechecked for the above signs after 15 minutes before disposal (Open chest to ensure death).

Dosage: 1) Rodents – via IP or cardiac – 50-60 mg/kg. 2) Rabbits – 40mg/kg IP to

induce anesthesia followed by intra cardiac puncture of 1cc per 2.2kg. (Open chest to ensure death)

3. Carbon Dioxide: Rodents will be euthanized in their cage in room R118.

The room is equipped with a CO₂ Tank, Regulator and Flow meter to insure delivery of correct volume of CO₂ according to AVMA current standards. A plastic cover is placed over the rodent cage; CO₂ is introduced at 20 to 30% cage volume/minute for five minutes. The animals are removed, chest opened to insure death and animals are placed in freezer located in room. ARF staff will periodically empty the freezer for hospital to incinerate as medical waste. Directions are on plastic cover used for euthanasia and technicians are trained by ARF Supervisor before use.

The correct setting to introduce CO₂ is on the plastic cover and all personnel involved in euthanasia are trained.

4. Exsanguination (anesthetized animals only): Rabbits and other laboratory animals may be exsanguinated to obtain hyper immune antisera, but because of the anxiety associated with extreme hypovolemia, exsanguination should be done only in sedated or anesthetized animals. Monitoring or verification of death is lack of heartbeat, lack of respiration, lack of eye response, pale bluish gums and tongue with onset of rigor mortis. Animal should be rechecked for the above signs 15 minutes –before disposal. Open chest to insure death.

5. Decapitation: Decapitation is most often used to euthanize rodents and small rabbits. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides neurobiologist brain tissue for study. In the latter case, the head is immediately placed in liquid nitrogen to halt metabolic processes. Although it has been demonstrated that electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate that this activity does not infer the ability to perceive pain, and in fact conclude that loss of consciousness develops rapidly.

This technique is conditionally acceptable if performed correctly, and should be used in research settings when its use is required by the experimental design and approved by the IACUC. All equipment should be kept in good working condition and serviced on a regular basis to ensure sharpness of blades.

The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. Those responsible for the use of this technique must ensure that personnel who perform decapitation have been properly trained to do so. Decapitation will be done with anesthesia unless prior approval by the IACUC.

6. Cervical dislocation: Cervical dislocation is used to euthanize mice, and immature rats and rabbits. For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the bases of the tail or hindlimbs are quickly pulled, causing separation of the cervical vertebrae from the skull. For immature rabbits, the head is held in one hand and the hindlimbs in the other. The animal is stretched and the neck is hyper extended and dorsally twisted to separate the cervical vertebrae from the skull. Monitoring or verification of death is lack of heartbeat, lack of respiration, lack of eye response, pale bluish gums and tongue with onset of rigor mortis. Animal should be rechecked for the above signs after 15 minutes – before disposal. Chest opened to ensure death. Cervical dislocation will be done with anesthesia unless prior approval by the IACUC.

XIV. LONG-TERM CARE MONITORING:

A. The animal care facility will have available the following laboratory services for the assessment of their animals.

1. Microbiology.
2. Hematology.
3. Chemistry.
4. Gross and histopathology.

B. Monitoring of cages/pens/rooms will be conducted by the ARF personnel on a daily basis. Record of monitoring will be kept in the animal rooms or for limited access room on the outside of the room via clip boards.

Monitoring of the water temperature of the cage washer/bottle washer is recorded every month. Temp-plates for registering 180°F are placed within a wash and removed at completion. Records of monitoring are kept within the ARF office. A steam booster has been added to the bottle washer to maintain 180°F for the final rinse. The cage washer is equipped with an "absolute 180°F" temperature before rinse cycle will activate.

Appropriate repair will be initiated if the indicator strips do not show proper sterilization temperature and all will be properly documented in the washer log.

C. In accordance with AAALAC/NIH guidelines for "preventive medicine," the following are recommended as minimum surveillance testing procedures for animals in the facility.

1. Mice:

MVH, Sendai and other necessary screening for infectious disease at specified intervals (i.e., six months) to be performed on a representative portion of mice entering the Animal Research Facility and on mice being housed in the Animal Research Facility.

2. Rats: Appropriate viral screening at 6 month intervals or as indicated by population surveillance.

3. Rabbits: Long term, H/H test at intervals when other procedures are performed.

4. Swine: Currently from specific disease free vendor. They are usually only in ARF for short periods. Long term surveillance as needed.

D. Other testing will be performed as necessary based on clinical judgment of investigator and/or veterinarian

XV. MEDICAL RECORDS:

"Medical Records" of animals (except rodents) will be kept in the office of the ARF and will include statement of animal condition, alteration of conditions, surgical procedures, purpose of animal use and any other procedure to which the animal is exposed. Each entry in all medical records should be initialed or signed by individual making the entry. Clinical laboratory records will be kept with the chart in the animal facility for review and entry of observation by animal caretakers, technicians, investigators and veterinarian, and be subject for review by the IACUC on request. Rodent surveillance records are filed together and kept in the ARF office

XVI .SURGERY ROOM/PROCEDURES:

A. Three areas have been set up for surgery. Large animal (swine etc.) will be performed in R-119. Small animal surgery such as rats, rabbits and etc. will be performed in R-120. Surgery area (R122 / X-ray) will be used only as requested with justification. Two different species will not be surgically manipulated at the same time in the same room. "Survival" surgeries will be done aseptically and only one at any time.

B. A formal schedule will be maintained by the ARF supervisor. Scheduling will be done as needed by the technicians. The ARF supervisor will maintain a schedule 4-8 weeks in advance where needed. This schedule will determine a fair and equal utilization of this VA Research Common Core Facility. Schedule sheet is to prioritize the preliminary schedule. The log record provided will formalize the surgery suite usage. Anyone who uses the Surgery Suite must record time in and out. This is kept to document the actual usage of the O.R. This facilitates justification of new equipment/space. This record will also facilitate responsibilities of general cleaning/maintenance. Each Investigator is responsible for leaving the area clean and readied for the following surgery.

C, In general, the surgical suite can only be reserved by one investigative group limited to a half day block on a given date. If no other group requires the use of the surgical suite on a given day, one investigative group can use it the entire day.

D. Should there be overlap for need to utilize the surgical suite, the senior investigator from each group is directed to work out any scheduling conflicts in an appropriate and gracious manner. All scheduling problems that cannot be settled by the senior investigator in consultation with the ARF supervisor will be resolved by the Associate Chief of Staff for Research and Development.

E. Non-Survival Surgery

1. Clean is the key word

- a. Work space
 - b. Instruments
 - c. Wear exam gloves
 - d. Wear clean lab coat or similar
 - e. Shave hair
2. Surgical plane of anesthesia acquired before surgery begins.
 3. Euthanized by exsanguination or additional "overdose" anesthetic, chest opened to ensure death.
 4. Designated Lab area or surgery room.

F. Survival Minor Surgery-Any Species

1. Does not expose a body cavity and causes little or no physical impairment.
2. Technique includes (but, not limited to):
 - a. Sterile instruments
 - b. Sterile gloves
 - c. Surgical skin prep (shave hair and scrub)
 - e. Surgical plane of anesthesia
 - f. Clean lab coat/smock- surgery smock not worn outside of surgery room or covered with lab coat if you have to leave surgery room. (Example: do not prep the animal in the same smock that you are going to do surgery in.)
3. All skin sutures, staples, and clips removed at the proper time.
4. Additional sterile technique possibly needed depending upon situation.
5. Designated surgery room, unless prior approval by IACUC.

- G. Survival Major Surgery – Rodents – Penetrates and exposes a body cavity or produces substantial impairment of physical or physiological function. (e.g. laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation).**
1. Aseptic technique includes but is not limited to:
 - a. Sterile instruments
 - b. Sterile gloves
 - c. Surgery cap and mask
 - d. Clean smock or lab coat – not worn or covered when not in surgery room
 - e. Surgical prep of animal with hair clipped and skin scrubbed
 - f. Appropriate cutaneous suture, staples, and clip removal.
 2. Surgical plane of anesthesia with monitoring of depth.
 3. Appropriate post operative care including, but not limited to:
 - a. Observation of animal until awake and stable.
 - b. Analgesia
 4. Designated surgery rooms only, unless prior approval from IACUC for special needs.
- H. Survival Major Surgery – Animals more sentient than rodents. (Rabbits-Swine). Surgical procedures where body cavities are opened, orthopedic procedures performed, or where permanent physical or physiological effects are produced.**
1. Sterile Technique
 - a. Surgeon scrub-gown, glove, and mask
 - b. Sterile instruments
 - c. Animal surgical prep with skin clipped free of hair and scrubbed
 - d. Animal surgical area draped properly
 2. Surgical plane of anesthesia
 3. Animal intraoperative monitoring and records (dog and greater)
 4. Postoperative monitoring and records (dog and greater)
 5. Analgesia
 6. Appropriate skin suture, staple, and clip removal
 7. Only an IACUC approved surgeon
 8. Dedicated surgery room only.
- I. VA Technician Training for Survival and Non Survival Surgeries – The following are the major points that need to be covered for training of technicians on the surgical techniques necessary to fulfill their role for both survival and non-survival surgeries.**
1. Animal care and restraint of the species selected
 2. The appropriate use of drugs needed for the procedure
 - a. Pre-anesthetic drugs
 - b. Anesthetic administration and monitoring
 - c. Pain Control
 - d. Antibiotic Therapy
 - e. Record keeping
 3. Training for the surgical procedure by person approved by the PI and the IACUC.
 4. Demonstrate competency with the surgical procedure on a suitable number of cases, before doing them alone. The number of procedures done with observation by the trainer is determined on an individual basis depending on complexity of the procedure along with a positive outcome for the procedure.
 5. Procurement of the proper equipment needed to do the surgical procedure, as well as proper training in the use of that equipment.

XVII. BLOOD SAMPLE AMOUNTS

- A. Total circulating blood volume can be estimated as 5.5-7% of body mass – for example, for a 30 gr mouse is 1.65-2.10 ml, of which 1% (0.017-0.021 ml) can generally be removed safely every 24 hours, or 10% (0.17-0.21 ml) every 2-4 weeks.

XVIII. QUARANTINE PROCEDURES:

- A. All animals are to be quarantined for a period of two weeks immediately after receipt with the exception of rodents.
1. “Proven Source” rats will be placed in the animal housing room on arrival and health monitored for 3 to 4 days prior to entry into a study. Animals from a “New Source” (Vendor) will be placed in an isolation room for 10-14 days. Observation, monitoring and clinical testing will take place during this time. Introduction to the common rat room will take place only after this time period and with negative test results.
 2. When necessary, a room will be designated “Isolation Room” for animals that will be for long-term isolation (more than 4 weeks). The room is to be entered only by ARF personnel and designated technicians who are properly gowned, gloved and foot coverings.
 3. Each species will be housed in separate rooms.
 4. All species are to be closely watched and given food and water during the quarantine period.
 5. When infectious hazard is recognized, the animal (all species) involved will be isolated from all other animals by placing it in an isolation unit or a separate room and the veterinarian informed immediately, for instructions as to the care and/or clinical laboratory tests to be obtained. If separate isolation is not possible, the entire room is to be quarantined and the veterinarian notified.
 6. A full complement liquid diet may be administered during the quarantine period, 48 hours after arrival, if no complications with the animals are noted.

XIX. WEEKEND/HOLIDAY STANDARD OPERATING PROCEDURES:

- A. All rooms are checked for temperature, humidity, lights, etc. For individual species see below.

Rodents: All cages are check for animal health, adequate water, food and bedding. New pups are noted in breeding colonies.

Rabbits: Each water bottle will be replaced with a fresh bottle of water. Food dish cleaned and refilled with daily food ration. Unless otherwise instructed (by means of oral or written instruction or information written on cage cards).

Pigs: Water supply checked, cage cleaned, daily food provided.

- B. Lock all doors on the first floor and reset alarm system just before exiting.

XX. MONITORING THE CARE AND USE OF ANIMALS

A. Institutional Animal Care and Use Committee (s) (IACUC)

See VA Handbook 1200.7, IACUC Standard Operating Procedure (SOP) and local policy R & D – 002.

B. Physical Restraint

When restraint devices are deemed necessary for a study, the restraint device or appliance is carefully investigated and must be approved by the IACUC. If restraint is used, animals are monitored closely by the technicians and/or animal caretakers. If the animals are placed in the device for several days at a time, the veterinarian checks for developing lesions, ulcers, discomfort, illness and weight loss with necessary treatment provided.

C. Multiple Major Surgical Procedures

1. Institutional Policy

Under special circumstances, multiple major surgical procedures on single animal may be permitted when such a procedure is a necessary component of a research project. Permission for such procedures is by full IACUC discussion followed by a vote. The principal investigator may come before the committee to defend his proposal. Notification is completed in the same manner as regular protocols.

2. Approval Procedure

Such procedures must be approved by the veterinarian in charge and the IACUC. In all cases, approval will be based on the programs that employ adequate anesthesia and to alleviate post-surgical pain with assurance of adequate postoperative care. The rationale for approving multiple procedures is that the surgeries must be interrelated to the particular study that is being done. At no time is cost-saving ever allowed to be criteria for multiple surgeries. Monitoring of the multiple surgery animal is done via the principal investigator, research technicians, veterinarian, animal care personnel and when necessary the IACUC.

3. Food or Fluid Restriction

a. Experimental Situation

Overnight removal of food for surgery or testing purposes is allowed. Protocols with food/fluid restriction must have prior approval from the IACUC.

b. Justification/Extent/Monitoring

Food may be restricted for the purpose of prevention of aspiration during surgery procedures. This would include removal of food the evening before a scheduled surgery. Replacement of food would take place as soon as the animal recovers the anesthesia and is upright. Animals are monitored closely after all surgeries for complete recovery.

Water (fluids) is not restricted at any time without IACUC prior approval.

XXI. OCCUPATIONAL HEALTH AND SAFETY OF PERSONNEL

A. Hazard Identification and Risk Assessment

The VAMC employs a full-time Industrial Hygienist to assist with any questions or problems that may arise. This person is the OSHA contact for the VAMC.

The IACUC and the R&D must approve all experimentation involving hazardous agents. Other OVAMC committees may also have oversight and approval privileges as well. Projects are approved only after the SAS committee has been satisfied that adequate safeguards are in place or available. Policies for use of various hazardous agents in the animal facility are on file in the ACOS/Research office with copies for investigators use when pertinent to their protocol. Currently written policies for use of radioactive substances, radioactive spills and emergencies, use of chemical carcinogens and infectious agents are in each research lab.

Policy is to review the qualifications of the principal investigator at the time of protocol review, making sure he/she has full knowledge of the handling, use and precautions of the product used. The facilities to be used will also be inspected for assurance.

B. Personnel Training

1. Description of Special Qualifications and Training for Work With Hazardous Agents in Animals

Any personnel working with a specific hazardous material must present verification of qualification before procedures begin. It will be the responsibility of the P.I. to insure that the recommended practices are followed and the staff involved are

completely informed of the risks of this agent and appropriately trained in its use.

2. Description of Educational Programs

When certain hazardous agents are used in animals (e.g. pathogenic organisms, carcinogenic, radioactive materials, etc.), the specific principles must be followed to prevent infection for contamination of other animals within the facility and humans.

The principal investigator is responsible for any hazards created by research or teaching activities. The P.I. must anticipate possible problems where personnel, students or animals might be involved, practice responsible measures, and see that all personnel who might be affected are properly indoctrinated in advance.

The manuals for AALAS certification, all current Laboratory Animal Science publications, USDA Federal Guidelines, Guide for the Care and Use of Laboratory Animals, some small animal surgery and necropsy publications. The AALAS Learning Library and AALAS publication are available and used for training.

Special hands on training will be conducted by Robert Wiegert, LATG, (ARF Supervisor) on an "as needed" basis.

C. Personal Hygiene and Protection

1. Personal Protective Equipment/Work Clothing Provided

Protective equipment/clothing are provided and worn (when work assignment requires) includes, but is not limited to, medical center provided shirts, trousers, steel-toe safety shoes, steel-toe rubber boots, aprons, disposable gloves, goggles, masks, ear protection, head covers, shoe covers and back support equipment.

VAMC laundry. All laundry is picked up and washed separately from hospital laundry. Items are returned directly to Research for storage.

2. Provisions for Washing Hands, Changing Clothes, Wearing Work Clothes Outside Facility

All animal rooms, except the large animal rooms, are equipped with sink (foot peddle on/off controls), Bacti-Stat hand soap, paper towels and foot peddle control waste basket. A common sink in the hallway (R140) can be used with large animal rooms. Each floor in the Research building has rest rooms for male and female employees. The first floor (ARF area) restrooms are equipped with lockers and shower facilities. Employees of the ARF are to change into a uniform on arriving and change back into street clothes on leaving for the day. Lab coats are provided when a persons needs to exit the ARF area but remain in the VAMC complex. Lab coats worn by the research technicians will be kept separate from those worn in the animal facility.

3. Eating, Drinking, and Smoking Policies

Eating, drinking, personal items are restricted from all animal rooms. Lockers (men – R103/women – R110) are provided for personal items, refrigerator (R102), microwave (R102) are provided to keep or cook food items. Breaks and lunch can be eaten in R102/R104 (office areas). Smoking is prohibited within the complex of the VAMC system. Designated areas (outside) have been made for those who wish to smoke.

D. Medical Evaluation and Preventive Medicine for Personnel

1. Description of Program; Personnel Included

Personnel included in the Occupational Health Program (OHP) are those involved in the direct care of animals and their living quarters as well as those individuals who have direct contact with animals (live or dead), their viable tissues, body fluids, or waste. This includes all Animal Facility staff, some investigators and laboratory assistants, some personnel in Engineering Service, Security and Building Management Service. Occupational Health administers rabies vaccine as needed

He/She also administers care and tetanus, if needed, in case of injury.

2. Aspects Relating to Hazardous Agents

All animal room doors are to remain closed. At completion of activities the doors will be locked for security reasons. Any room containing hazard related material will be clearly marked. Each animal cage shall be properly identified as to the animals it contains, the biologic materials used and the date of exposure. All personnel entering the room must be properly attired as instructed by the P.I. These articles of clothing must be left in the room when exiting. All material from said room must be disposed of or sterilized in the proper manner.

Personnel potentially exposed to radioactive materials are required to wear disposable gloves and protective clothing. Personnel exposed to potentially infectious agents, Class I bio-hazard, are required to wear protective clothing. Personnel potentially exposed to chemical carcinogens are given explicit instructions in the policy set forth and listed as Omaha VAMC Policy and Guidelines for the Use of Chemical Carcinogens.

E. Animal Experimentation Involving Hazards

1. Description of Institutional Policies

Policies and procedures involving hazards are on file in the ARF office and in each laboratory within the Research Facility.

2 Description of Oversight Process and Husbandry Practices

The IACUC and the R&D must approve all experimentation involving hazardous agents. Other OVAMC committees may also have oversight and approval privileges as well. Projects are approved only after the IACUC committee has been satisfied the adequate safeguards are in place or available. Policies for use of hazardous agents in the facility are on file I the ARF office with copies for investigators use when pertinent to their protocol. Currently written policies for use of radioactive substances, radioactive spills and emergencies, use of chemical carcinogens and infectious agents are attached. VA policies which covers all aspects is required in each research laboratory.

3. Containment of Hazardous Agents

See Hazardous Agents Policy located in each lab.

a. Monitoring of Autoclave

The autoclave testing and documentation is done approximately 1-2 times per month with interior indicators of sterilization. As a further safety measure, time- temperature monitoring strips are required with each bio-hazards material run in the autoclave. Log books, strips and tape are kept. The log book will be reviewed and records kept in R107.

4. Scavenging of Anesthetic Gases

Safety procedures for using volatile anesthetics and provisions for waste anesthetic include the use of a gas scavenging device. Badges are used to ensure equipment and correct procedures limit exposure.

F. Facilities, Procedures, and Monitoring

1. Description of Requirements for Showers and Change Facilities

Showers, locker and change facilities are available for ARF staff on the first floor of the Research Building (R-103/men – R-110/women).Animal caretakers are to immediately change from street clothes to uniform wear upon arrival. Before leaving at the end of the day they are to shower, store soiled clothing in container supplied, apply street clothes and exit the facility.

2. Description of Procedures that Reduce Potential for Injury

Safety equipment is supplied to each animal caretaker and lab technician as required by each position. The animal caretakers are supplied with steel-toed safety shoe and boots, safety glasses, ear protection, face shields, back supports, respirators and the usual items such as gloves, masks and etc. Any specialty needs are supplied as needed.

Instructions for use of each item is included in the orientation and reiterated during the required safety classes taken each year. Close monitoring by the ARF supervisor making sure equipment is worn and worn appropriately.

XXII. ANIMAL ENVIRONMENT, HOUSING, AND MANAGEMENT

The Guide for the Care and Use of Laboratory Animals is used for references and considerations to determine adequate cage or pen size or housing densities

A. Physical Environment

1. Primary Enclosures

a. Mice are housed in group caging whenever possible. Population density is determined by the weight of each animal and size of cage available. At no time will the numbers exceed 12 per enclosure. Polypropylene cage is the choice material to insure visual contact. Cage size and population most used at OVAMC are: 11 $\frac{1}{2}$ " x 7 $\frac{1}{2}$ " x 5" = 5 or less count or 19" x 10 $\frac{1}{2}$ " x 5 $\frac{1}{8}$ " = 12 or less count.

b. Rats are housed in group housing. Population density is determined by the weight of each animal, ongoing procedure and size of cage available. At no time will the numbers exceed 12 per enclosure. Polypropylene and stainless steel hanging cages are the choice materials for housing and placed in a manner in which visual contact can be made. The polypropylene cage size most used is 19" x 10 $\frac{1}{2}$ " x 7". Stainless steel cages used for single housing measures 7 $\frac{3}{4}$ " x 8 $\frac{1}{4}$ " x 11" and group cages 17 $\frac{1}{8}$ " x 10 $\frac{5}{8}$ " x 7 $\frac{1}{8}$ ".

c. Rabbits are housed in single units with visual contact whenever possible. Stainless steel (dog cages) measuring 48 x 34 x 32 inches.

d. Swine are housed in pens. In R115 they are 35 sq.ft., Pens in R145 are 30 sq.ft .

2. Temperature and Humidity

Rooms are on one of two separate HVAC systems. Each system supplies 100% fresh air. Room temperature is controlled by thermostat within each animal room.

Rooms are positive or negative air pressure, up to 15 air changes per hour. Both systems have energy recovery capacity. The air handling equipment is checked three times per day by maintenance staff and monitored by an automatic control system.

Each individual animal room supplied with a Min/Max thermometer and is checked and recorded twice daily (once on weekends and holidays) by the animal care personnel.

3. Lighting

Lights are waterproof encased-suspended fluorescent fixtures with automatic waterproof timer controls. Lighting measurement is 100% candles. No outside lighting exists. An override switch is in place for light if entrance is required after timer has turned off. The override could be set for up to one hour before it resets to the off position. This is to prevent lights being left on after hour use.

4. Enrichment

1. Mice – PVC pipe (1.75 inch ID X 5 inch), Crink-I’Nest, and Kleenex Clear plastic square bottle with hole on top is also available

2. Rat – PVC Pipe (2.5 inch and 3 inch ID X 6 inch)

3. Rabbit – Resting Board, Bell (attached to ceiling), Roller Skate Wheel, and PVC pipe- all sizes Occasional diet treats – lettuce, carrot, apple, fresh hay, ----

5. Swine – Various rubber items (balls, large dog chew toys, etc.) Large heavy rubber floor mat for comfort and play. Occasional diet treats – apples, hat etc.

B. Visual Contact

1. Rodents are housed in group caging whenever possible. Single housing is clear so visual is possible. Stainless steel caging facing for visual contact.
2. Rabbit caging is arranged so animals have visual of each other at all times.
3. Swine are socially housed or in pens next to each other for visual and verbal contact.

C. Housing Social Animals Singly

The Guide recommends that social animals be housed in stable pairs or groups unless they must be housed alone for experimental reasons or because of social incompatibility (Guide, p. 51 and 64). Exemptions due to study restrictions must be justified by the PI and approved by the Institutional Animal Care and Use Committee.

The attending Veterinarian has the authority to restrict social housing for medical reasons. If this should be necessary, the case will be reviewed at least monthly and the requirement noted in the medical record, which will be made available to the IACUC for review during its semiannual review of facilities. Alternative enrichment will be provided whenever possible.

Every effort will be made to find compatible pairs for social animals. If compatibility of a particular animal(s) cannot be established despite comprehensive attempts, alternative enrichment will be provided.

1. Social animals may be housed singly when approved by the IACUC for experimental reasons with scientific justification.
2. Any social animal may be housed singly at veterinary discretion for medical or social incompatibility for as long as the condition continues. The IACUC will be notified of each housing change.
3. The ARF staff may house any animal singly for medical reasons or social incompatibility, but must immediately contact the VMO for permission to continue the separation. The IACUC will be notified of each housing change.
4. All rodent males that have been used for breeding should be housed separately for their own protection. Unless with females.
5. Housing guidelines –
 - a. PI/research staff members are responsible for ensuring pair/group housing.
 - b. Compatible rodents are paired or group-housed (2 or more rodents per cage) directly from vendor.
 - c. Animals that arrive individually remain single-housed until a suitable group or pair is established by researcher.
 - d. PI/researchers indicate number housed per cage and pairing/breeding preferences under special instructions on ACORP.

Our monthly Animal Facility Inspection Checklist for IACUC meetings will include a line item to keep IACUC aware of individually housed social animals.

D. Food

1. Type and Source
 - a. The following products purchased from Northwest Feed & Grain Co.
8625 Military Road, Omaha, NE

i. Rats/Mice: Rodent Laboratory Chow #5001, Mouse Diet 501 (Purina Mills, Inc.)

ii. Rabbits: Lab Rabbit Chow HF 5326 (Purina Mills, Inc.)

iii. Swine; Ground corn from Mead

2. Storage Facilities of Vendors

a. Northwest Feed & Grain: These buildings are constructed of steel siding and floors of concrete. The feed is stored on plastic pallets or shelves. Vermin control is baiting for rodents and spray for roaches and etc. The building is non-heated and non-air conditioned.

b. South Omaha Terminal Warehouse: This building is constructed of concrete block walls with wood floor. Feed is stored on plastic pallets. Vermin control is baiting for rodents and spray for roaches. The building is non-heated and non-air conditioned.

3. Storage in Animal Facilities

Shipments of feed and bedding are stored in room R-129. The room is monitored daily for temperature. All feed and bedding are placed on pallets. Feed is rotated so new feed is used last. The temperature and humidity are controlled the same as the remaining animal facility. Vermin control is by inspection. If a problem should occur, a work order is placed with the OVAMC EMS.

4. Storage in Animal Rooms

Once a bag of feed is opened, it is placed into a lidded, plastic-bag lined metal or plastic container.

5. Food Preparation Areas

A food preparation area is located in room R-132. Special diets are prepared and stored in this area. Two refrigerator/freezers are for this use only.

6. Quality Control Procedures

a. Rotating Stock: Feed stored not only by shipment date received but also according to milling date if it varies within a shipment. Any feed supplies are refused if milling date and usage time period overlap the recommended time. All feed supplies are placed in manner which milling dates are easily accessible for earliest date usage.

b. Monitoring Milling Dates: Feed supplies are ordered in a manner in which the on-hand supply is almost depleted. Feed is not accepted with a "short term" milling date. If a bag of feed remains with a "short term" date, an agreement has been made with the supplier that such feed will be picked up and replaced with a later milling date.

c. Chemical Contaminants, etc: Most feed supply companies now package the feed within bags that include triple lined or plastic liner with several layers of heavy paper for protection. Any bag opened or soiled in any way at time of arrival will be refused at that time.

E Bedding

1. Type, How Used, and How Selected

a. Corn cob (1/8") – This bedding is used for all rodents in need of contact bedding.

b. Dehydrated Alfalfa – This product is used for odor control when mixed with Bed-O-Cobs under wire bottom cages.

c. Crink-I'Nest/ Kleenex is also used with bedding.

2. Storage Facilities

a. Corn cob (1/8") are stored on pallets in room R-129. This area is kept vermin free by inspection. Corn cob (1/8") bags are opened and emptied into a container with tight fitting lid and placed in R129. Rodent cages are filled in this room and transported to animal rooms as needed.

b. The combination of Corn cob (1/8") and Dehydrated Alfalfa is mixed (1-3) and placed into a container in R129. Excreta pans are prepared in this room and transported to animal rooms as needed.

c. Crink-1 Nest is kept in R129

3. Quality Control Procedures

Corn cob (1/8"), Dehydrated Alfalfa and Crink-1 Nest are examined closely upon arrival for any stains appearing on outside of bags. If a fluid penetration is apparent or the bag is opened, refusal will take place immediately.

F. Sanitation

1. Cage Sanitation/Bedding Change

a. All cages/racks using contact and non-contact bedding are changed twice weekly for conventional cages or once a week for ventilated cages unless otherwise requested by the investigator and approved by VMO/IACUC.

b. Rabbit cages are cleaned and flushed daily. Animals are moved to another sanitized cage and cage sanitized every two weeks.

c. Swine pens are cleaned daily. Sanitized between occupants.

2. Location Where Soiled Bedding is Removed

Excreta pan and cages are transported to the cage wash area. Environmental Dump Station. Trash containers are double lined with plastic bags (1.5 ml). Pans and box cages are emptied, rinsed and placed on racks for the cage washer process. All bags are placed at the west dock for pickup that day by the Grounds Crew.

3. Cage Washing/Sanitizing Procedures

Mechanical (Steris) washer is used for small animal cages, racks and excreta trays – High pressure sprayer is used for large animal cages and also for room sanitization.

The cage and bottle washer will be monitored in an orderly fashion utilizing temperature strips and regular documentation in a log book. Appropriate repair will be initiated if the indicator strips do not show proper sterilization temperature and all will be properly documented in the washer log.

4. Cleaning/Sanitizing Agents

Labsan 100 – Alkaline Detergent

Labsan 230C – Acidic Detergent

Labsan 256Q – Quat Disinfectant

Labsan Glass Pro – alkaline glassware Detergent

Above agents purchased from Sanitation Strategies

POLICY APPENDIX A: MICE

Animal Husbandry and Care Practices

MICE

A. **Animal health monitoring:**

1. **Means by which health status of animals is evaluated at time of arrival:** Inspect shipment, and determine that species shows no obvious signs of illness.(e.g. clear eyes, nose clean, coat in good condition, normal appearance of feces).
2. **Means of animal identification:** Identification card is placed on each cage for every animal. (Identification card with investigator name, vendor, date received, protocol number, species, sex, strain, weight, USDA/VA number. The date of death is recorded at the time of disposal for each animal.) All cards are returned to the ARF office at time of completion.
3. **Program of quarantine, stabilization and/or conditioning prior to placement in study:** Animals are quarantined for a period of two weeks after receipt, in a room separate from those already in the facility, until the health of the newly received animals has been evaluated. See page 2, "Procedure for Purchase of Rodents from Non-commercial Sources"
4. **Diagnostic tests performed prior to placement in study:** Diagnostic tests are to be performed and recorded by the investigator. All results are to be accessible for review on demand.
5. **Routine immunization and treatment practices:** N/A

B. **Dietary factors:**

1. **Description of animal diet:** Rodent Laboratory Chow 5001, Mouse Diet 5015 used for breeders (Ralston Purina.)
2. **Frequency and method of feeding:** Gravity feed feeders monitored daily.
3. **Frequency and method of providing water:** Bottles will be replaced with fresh water on all cage changes.. Used bottles will not be refilled and replaced on cages because of possible cross contamination.

C. **Animal environment:**

1. **Description of primary enclosure:**
 - a. **Dimensions:** Polycarbonate cages – 5x11x6 ^{3/4}”; 6^{1/2}x14x12”; 7^{3/4}x17^{1/2}x9
Availability of housing – Polycarbonate suspension cage racks (6 units) 5 animals per cage, 210 animals per rack (unit). Number of animals per cage as required by the “Guide
 - b. The addition of ventilated racks as primary enclosures, with polycarbonate cages, maintains similar cage populations. They allow for changes in frequency of bedding changes. All ventilated racks are monitored for performance and maintained when required.
 - c. **Cage material:** Stainless steel cages, polycarbonate tubs.

- d. **Bedding type:** Bed-O-Cobs in plastic tubs.
- e. **Enrichment:** PVC pipe (1.75 inch ID X 5 inch), Crink-1'Nest, and Kleenex
Clear plastic square bottle with hole on top is also available

2. **Cage sanitation:**

- a. **Frequency of cleaning:** Cage changes 2 times per week with fresh bedding. Once per week in ventilated racks.
- b. **Method of cleaning:** Cages are mechanically washed and sanitized by Steris equipment. "Thermolabel" temperature-sensitive tape attached to cage/rack for periodic check of water temperature to assure sanitizing temperature of 180°F is reached.

Bottles will be replaced with each cleaning. All sipper devices are placed in a sodium hypochlorite solution, then a hot water rinse before placing into the bottle washer. Bottle are not to be removed, filled and replaced to insure cross contamination does not occur.

3. **Room sanitation:**

- a. Floors are flushed on cleaning days. Rooms sanitized on a monthly basis.

4. **Environmental control:**

- a. **Room temperature range:** Rooms are maintained between 68°-74°. HI/LO temperature gauge is placed in small animal rooms for monitoring area.
- b. **Heating and cooling:** Temperature and humidity thermostatically controlled. Design criteria for system:

<u>COOLING</u>	<u>HEATING</u>
75 FDB	75 FDB
50% RH	50% RH

- c. **Number of air changes per hour:** Air is to be 100% outside with 10-15 changes per hour. Exhaust is to the outside. HEPA pre and post filters are used in the supply and exhaust systems.

POLICY APPENDIX B: RATS

Animal Husbandry and Care Practices

Rats

A. Animal health monitoring:

1. **Means by which health status of animals is evaluated at time of arrival:** Inspect shipment, and determine that species shows no obvious signs of illness.(e.g. clear eyes, nose clean, coat in good condition, normal appearance of feces).
2. **Means of animal identification:** Identification card is placed on each cage for each animal. (Identification card with investigator name, vendor, date received, protocol number, species, sex, strain, weight, USDA/VA number. The date of death is recorded at the time of disposal for each animal. All cards are returned to the ARF office at the time of completion.)
3. **Program of quarantine,** stabilization and/or conditioning prior to placement in study: Animals are placed in the animal room for evaluation and monitoring 3-4 days before placement into study.
See page 2, "Procedure for Purchase of Rodents from Non-commerical Sources"
4. **Diagnostic tests performed prior to placement in study:** Diagnostic tests are to be performed and recorded by the investigator. All results are to be accessible for review on demand.
5. **Routine immunization and treatment practices:** N/A

B. Dietary factors.

1. **Description of animal diet:** Rodent Lab Chow 5001 (Ralston Purina).
2. **Frequency/Method of feeding:** Gravity Feed Feeders monitored daily.
3. **Frequency and method of providing water:** Water bottles are placed on individual cages. Monitored daily, replaced with fresh bottle as needed. Fresh bottles are placed on each cage at least twice weekly.

C. Animal environment.

1. **Description of primary enclosure (pen, cage):**
 - a. **Dimensions:** Group Caging 19x10x8 in. Single Animal Caging 7 x 9 $\frac{3}{4}$ x 7 3
Polycarbonate cages -19x10x8. The cage size and number of occupants depends on the size of animal(s) as recommended in the "Guide".
 - b. **Cage material:** Stainless steel cages, polycarbonate tubs.
 - c. **Bedding type:** Bed-O-Cobs direct and 1-3 Bed-O-Cobs/Alfa Cobs indirect.
 - d. **Enrichment:** PVC Pipe (2.5 inch and 3 inch ID X 6 inch)

2. **Cage sanitation:**

- a. **Frequency of cleaning:** Bedding and/or tray liners under cages changed twice per week. Cage/rack rotation as required or every two weeks.
- b. **Method of cleaning:** Mechanical rack/cage washing and sanitizing equipment. (Bazil) "Thermolabel" temperature-sensitive tape attached to cage/rack for periodic check of water temperature for sanitizing to assure temperature of 180°F reached.

Bottles will be replaced twice weekly. All sipper devices are placed in a sodium hypochlorite solution then a hot water rinse before placing into the bottle washer.

3. **Room sanitation:**

Floors are flushed and squeegeed daily. Room sanitized monthly.

4. **Environmental control.**

- a. **Room temperature range:** Room temperature is maintained between 68_o-74_o. HI/LO temperature gauge is placed in small animal rooms for monitoring the area. Temperature and humidity thermostatically controlled. Design criteria for system:

<u>COOLING</u>	<u>HEATING</u>
75 FDB	75 FDB
50% RH	50% RH

- b. **Number of air changes per hour:** Air is to be 100% outside with 10-15 changes per hour. Exhaust is to the outside. HEPA pre and post filters in the supply and exhaust systems.

POLICY APPENDIX C: RABBITS

Animal Husbandry and Care Practices

A. Animal health monitoring:

1. **Means by which health status of animals is evaluated at time of arrival:** Inspect shipment, and determine that species shows no obvious signs of illness.(e.g. teeth, clear eyes, nose clean, coat in good condition, normal appearance of feces).
2. **Means of animal identification:** Identification card placed on each cage for each animal. (Identification card with investigator name, vendor, date received, protocol number, species, sex, strain, weight, USDA/VA number. Any tattoo or special markings are recorded on the I.D. card. The date of death is recorded at the time of disposal for each animal. All cards are returned to the ARF office at the time of completion.) Ear tattoos are applied upon request by the P.I.
3. **Program of quarantine, stabilization and/or conditioning prior to placement in study:** All “Proven Source” rabbits are placed in the animal housing room upon arrival. Animals are monitored and evaluated for 10 – 14 days before introduction into study. Animals from other vendors will be placed in a separate room for observation and testing before introduction into regular animal room.
4. **Diagnostic tests performed prior to placement in study:** Animals to be entered into long term studies (4-6> weeks) – CBC on entry into facility.
5. **Routine immunization and treatment practices:** Freund’s Complete Adjuvant will be used for initial injections only. All booster injections are to be made with incomplete Freund’s. Injections are to be made 1-2 inches lateral to the dorsal midline. The number of injections sites are determined by total volume to be injected with no more than 0.1ml per injection site.

B. Dietary factors:

1. **Description of animal diet:** Lab Rabbit Chow (High Performance). Fed from rotated stock to insure fresh diet.
2. **Frequency and method of feeding:** Fresh pellets in 1 quart bowl daily.
3. **Frequency and method of providing water:** Water bottles are placed on individual cages. Fresh water bottles daily.

C. Animal environment:

1. **Description of primary enclosure (i.e., pen, cage, or room):**
 - a. **Cage dimensions:** Caging is Vollrath stainless Steel (48 x 34 x 32) dog cages with vinyl coated cast iron resting boards.
 - b. **Cage material:** Stainless Steel
 - c. **Bedding type:** N/A

d. **Enrichment:** Resting Board, Bell (attached to ceiling), Roller Skate Wheel, and PVC pipe- all sizes Occasional diet treats – lettuce, carrot, apple, fresh hay, ----

2. **Cage sanitation:**

a. **Frequency of cleaning:** Daily

b. **Method of cleaning:** Cages are cleaned and flushed daily. Animals a

c. **Room sanitation:** Floors are flushed and squeegeed daily. Rooms sanitized on a weekly basis.

D. **Environmental control:**

1. **Room temperature range:** Rooms are maintained between 68° -74° temperature. HI/LO temperature gauge is placed in small animal rooms for monitoring. Temperature and humidity thermostatically controlled.

COOLING
70 FDB
50% RH

HEATING
70 FDB
50% RH

2. **Number of air changes per hour:** Air is to be 100% outside with 10-15 changes per hour. Exhaust is to the outside. HEPA pre and post filters in the supply and exhaust system.

POLICY APPENDIX F: SWINE

Animal Husbandry and Care Practices

A. Animal health monitoring:

1. **Means by which health status of animal is evaluated at time of arrival:** Animal caretakers inspect shipment for any visual signs of illness and that all animals are in acceptable condition.
2. **Means of animal identification:** Identification card placed on each cage. Ear markings are applied upon delivery if requested by the investigator. (Identification card with investigator name, vendor, date received, protocol number, species, sex, strain, weight, USDA/VA number. The date of death is recorded at the time of disposal for each animal on the card. All cards are returned to the ARF office at the time of completion.)
3. **Program of quarantine, stabilization and/or conditioning prior to placement in study:** Animals received by a "Proven Source" will be placed into the animal housing room and observed and monitored for 7-10 days. Those animals purchased from unknown sources are quarantined for a period of two weeks immediately after receipt in a room separate from those already in the facility until the health of the newly received animals has been evaluated.
4. **Diagnostic tests performed prior to placement in study:** Animals to be entered into long term studies, (> 4-6 weeks) – CBC on entry into facility.
5. **Routine immunization and treatment practices:** As needed regarding shipping records/background.

B. Dietary factors:

1. **Description of animal diet:**
Ground corn/ protein
2. **Frequency and method of feeding:**
Feed will be placed in clean rubber pans once a day
3. **Frequency and method of providing water:**
Automatic waterers are checked daily. Water pans provided if needed

C. Animal environment:

1. **Description of primary enclosure (i.e., pen, cage, or room):**
 - a. Pens or rooms
 - b. **Bedding type used.** Rubber pads
2. **Cage sanitation:**
 - a. **Frequency of cleaning:** Pens are cleaned daily.
 - b. **Method of cleaning:** Sanitized between uses.
3. **Environmental control:**

- a. **Room temperature range:** Temperature and humidity thermostatically controlled
- | | |
|----------------|----------------|
| <u>COOLING</u> | <u>HEATING</u> |
| 75 FDB | 75 FDB |
| 50% RH | 50% RH |
- b. **Number of air changes per hour:** Air is to be 100% outside with 10-15 changes per hour. Exhaust is to the outside. HEPA pre and post filters in the supply and exhaust system.

POLICY APPENDIX E.

Procedures for the IVIS Lumina Series

Rodents that are used in the IVIS Lumina Series 3 must meet the following conditions. IVIS must also be cleaned as listed below.

Animal requirements-

Animals must be healthy when placed in IVIS. Live rat and mice used in the IVIS, must be housed in the Omaha VA Medical Center Animal Research Facility for a minimum of days 21 days after which sentinel blood serum will be tested for diseases listed below. Animals may be used when all results are negative.

Rat	Mouse
RCV/SDAV	MHV
NS1 (Generic Parvovirus)	MVM (MMV)
RPV	NS1 (Generic Parvovirus)
RMV	MPV (MPV 1-5)
KRV	MNV
H-1	TMEV
RTV	EDIM
Sendai	Sendai
PVM	Mycoplasma pulmonis
Mycoplasma pulmonis	
REO3	
LCMV	

Should any room/colony become positive during a routine serology tests they will not be allowed in the IVIS until room/colony tests negative. The frequency of serology testing may change if emerging issues dictate, at the investigators expense.

Tissue requirements –

Tissue from negative animals housed in the Omaha VA Medical Center Animal Research Facility may be used.

Tissue from facilities outside the Omaha VA Medical Center Animal Research Facility must provide the Animal Research Facility with a negative serology report, see list above, from the outside facility room animals were housed in. Tissue will be allowed in after ARF Supervisor and VMO review and approve report.

IVIS Lumina K Series 3 must be sanitized, see below, between each animal and tissue test to prevent the spread of disease. This is necessary to safeguard continued negative disease serology between rooms and institutions.

Optical Imaging with the IVIS Lumina K Series 3

The IVIS Lumina K Series 3 allows researchers to use real-time luminescence and fluorescence imaging to non-invasively monitor and record cellular and genetic activity within a living organism.

Animal preparation and scanning

Animal preparation will include the inhalation of isoflurane anesthesia along with oxygen through a nose cone, which will be used during imaging to immobilize the animal until the scan is complete. The administration of isoflurane should not exceed vaporizer settings of 2% for mice and 4% for rats. The carbon filters on the anesthesia scavenging system should be monitored after each use and replaced if there is an increase of 50 grams or more above the starting weight.

Living Image® Software from Xenogen will be used for image acquisition. Scan times vary between 0.5 second and 3 minutes.

Post-anesthesia recovery:

A heating pad may be used to maintain the animal models body temperature during post-anesthesia recovery.

Cleaning the IVIS Lumina K Series 3

The IVIS Lumina K Series 3 should be cleaned after each session of use

The approved cleaning solutions listed below will not damage the internal finish of the IVIS®

1. Cidexplus® Solution (3.4% Johnson & Johnson Medical glutaraldehyde)
2. 70% methyl alcohol/30% deionized water solution.
3. 70% ethyl alcohol/30% deionized water solution.
4. Sporicidin® Sterilizing Solution Sporicidin International (1.56% phenol)
- 5 Clidox-s® Disinfectant Pharmacal Research Laboratories, Inc.

Do not use any solution not included in this list. In particular, avoid strong bases, bleach, or acids that may potentially damage the unit and compromise its operation.

Do not spray cleaning solutions in the imaging chamber. Targets and others accessories should be taken out of the imaging chamber to be cleaned.

It is recommended that you use a lint-free wipe, such as Scott Pure® wipe or a Kaydry EX-L® wipe to minimize the presence of particulate matter in the imaging chamber. After saturating a lint-free wipe, clean the internal surfaces using a gentle circular motion. Do not pour or spray the solution directly onto internal surfaces. Rinse surfaces using a wipe saturated with sterile deionized water. Do not allow puddles of water to remain on the surfaces. To avoid any phosphorescence from the cleaner, be sure that the surfaces are dry before using the imaging chamber. Be careful not to smudge the camera lens and optical filters.

SOP for ABSL2

The following information is taken from-

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition

<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

"Animal Biosafety Level 2 (ABSL2)

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.

As every different type of agent has unique hazards and individual experiments involve any number of procedures close communication is needed between PI and ARF before and during procedures.

All ACORP's that involve the use of ABSL2 facilities must have the following information, most of which is already required at various places on the form.

1. Specific hazards to ARF personnel.
2. Special equipment and PPE required inside animal room.
3. Animal handling, husbandry, etc. that are unique to hazard.
4. List of special animal training required, all personnel to be trained, and who will train
5. Detailed procedures for decontamination of animal wastes, room and equipment.
6. Information for sign to be placed on animal room.
7. ABSL2 animals, if used in IVIS, must document IVIS SOP procedures.

ARF Procedure (SOP) - Subject to changes if necessary

1. Assign a room with appropriate equipment.
2. Restrict access to trained personnel.
3. Ensure PPE is appropriate.
4. Post signs when agent is present with information above. (Need to incorporate symbol)

5. When agent is present –

- A. Only authorized personnel (PI and trained technician's) allowed into room. ARF personnel do not enter room, clean cages left in hallway as needed.**
- B. All soiled bedding is removed from cages in room, bagged and autoclaved**
- C. All equipment (cages, water bottles, etc.,) is disinfected with a bleach solution by authorized personnel in animal room before taken to wash room to be cleaned and sanitized.**

6. Room is sanitized as needed.

Universal biohazard symbol



The actual symbol shall be no smaller than 10 cm by 10 cm and no larger than 40 cm by 40 cm. Unless otherwise specified, the width of the symbol should be approximately one quarter the width of the surface on which it appears. The symbol and its background must be in contrasting colors.

