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 Last Revised: January 31, 2014
 Approved by: Michael Fallon D.V.M. _____ (initials)

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STANDARD OPERATING PROCEDURE

Rodent Anesthesia, Analgesia and Post-operative Care

Written by: Sandra Meyer, RVT, LATG
 IACUC Approval: March 13, 2013
 Last Revised: January 27, 2011
 Approved by: Michael Fallon D.V.M. _____(initials)

1. Purpose: To document and describe the reason we treat pain in animals, the proper techniques and drug combinations used in rodents for anesthesia and analgesia, and the post-operative care of rodents.

Animal Component of Research Protocol (ACORP)

Pain meds (analgesics) should be administered as outlined in your ACORP (*new lab staff should read the ACORP before beginning any work on the ACORP*). The administration of analgesics, anesthetics and post-operative animal observations must be documented on the VMU standardized rodent post-op record. ***ALL post-op records must be maintained in a log book within your animal procedure lab for the IACUC to review during the semi-annual review of animal use protocols.*** If you do not have the form, see Sandy in room 4A108 for a copy of the form. Immediately following surgery you are required to place a "POST OP" card on the cage card holder for each cage containing post-op animals. You will then initial the card each day after checking on and observing the animal. If you need post op cards see Sandy in room 4A108.

WHY TREAT PAIN?

In addition to the obvious humanitarian and ethical aspects of treating pain and suffering, many other benefits are associated with good pain management. Untreated pain increases the risk for secondary complications. Pain leads to changes in the neuroendocrine system, including a surge in catecholamines, which increases the risks for cardiac arrhythmia, vasoconstriction, and hypertension. There is a decrease in gastrointestinal motility, which increases nausea and leads to inappetence. Pain is associated with a high circulating concentration of cortisol, which leads to immunosuppression and increases the risk for infection and sepsis. Insulin resistance and an increase in catabolic hormones lead to increased healing times, catabolism, and weight loss. Finally, if pain is not appropriately managed, physical changes occur in the spinal cord and brain that may lead to long-term pain syndromes, such as phantom limb pain and neuropathic pain (nerve pain).

Definitions

- **Anesthesia:** loss of sensation and consciousness.
- **Analgesia:** insensibility to pain without loss of consciousness.
- **Wind-up:** spinal cord nerve cells undergoing repeated stimulation and activation (noxious stimuli transmission during surgery).
- **Central sensitization:** state of hyperexcitability of dorsal horn neurons as a result of repeated afferent impulses.
- **Onset time:** the length of time it takes before a drug reaches systemic therapeutic levels.
- **Duration of action:** the length of time the drug remains active after reaching systemic therapeutic levels.
- **IP:** Intraperitoneal
- **SC:** Subcutaneous
- **IM:** Intramuscular

2. Anesthetic goal: to block the perception of pain and to immobilize the animal to permit manipulation.

3. Analgesic goal:

3.1 Pre-emptive analgesic (drug given before surgery) to prevent the initiation of "wind-up" (see above) so that "central sensitization" does not occur. The analgesic drug must be bound to the relevant receptors prior to the first incision and remain effective throughout the surgical procedure until the end of the wound closure. An important benefit is that anesthetic doses may be reduced when analgesics are used preemptively, which reduces cardiopulmonary side effects of the anesthesia. An example of a commonly used preemptive analgesic is Meloxicam (Non-Steroidal Anti-inflammatory Drug-NSAID) and/or buprenorphine (opioid). If preemptive analgesics are not administered- central sensitization may occur, making postoperative control of pain more difficult and requiring larger doses of postoperative drug (increasing the chance of drug side-effects). Meloxicam (see dose pg 4) is administered immediately following anesthetic induction (pre-emptive) --before making an incision, this will allow time for the drug

to become systemic while you are prepping the skin for the incision. Post-op use: if buprenorphine (see dose pg 4) was not administered pre-emptively it should be administered immediately following surgery (before the animal wakes up from anesthesia). Buprenorphine has a short duration of action (>8 hours in rodents) and must be administered a minimum of twice a day (AM/PM). Meloxicam is administered once a day in the AM.

Analgesics (Buprenorphine, Meloxicam or other) must be administered in all major survival surgeries (penetration of body cavity or bone) unless scientific justification for not administering analgesics has been provided to and approved by the IACUC.

3.2 Post-operative analgesic (drug given after surgery) to continue to block the pain receptors during the “inflammatory” phase of healing (most painful phase). Analgesics should be administered for a minimum of three days for rodents following a major survival surgery (i.e. anything more than a skin incision).

4. Considerations:

4.1 Animal- Variability(in anesthesia and analgesics) may be seen in the responses of some rodents of different stocks/strains/genotype.

4.2 Procedure- Length of the procedure and the level of pain or post-operative complications that might result.

4.3 Procedure location- Inhalant anesthetic administration requires an oxygen source, flow meter, and precision anesthetic vaporizer (anesthesia machine).

4.4 Post-operative Care- Choose an anesthetic that has a low potential for a long or difficult recovery from anesthesia such as isoflurane delivered through a precision anesthetic vaporizer (best and allows for a quick recovery). The body temperature of a rodent can drop very rapidly under anesthesia, it is important to support the anesthetized rodent with a heat source, preferably a water circulating pad, circulating air pads, etc. (circulating to prevent thermal burns). Continue to provide warmth to the animal during recovery from the anesthesia, a warm animal will recovery more rapidly than an animal with low body temperature. It may be necessary following surgical procedures to keep the rodents’ cage on a heating pad --**set on LOW**—overnight, however, you must notify veterinary staff (Sandy) if you plan to keep rodent cages on heating pads overnight. The cage should be placed so that only half of the cage is on the heating pad, this will provide a thermal gradient and allow the rodent to remove itself from the heat if it becomes too warm. After surgery the rodent should always be placed into a **NEW/CLEAN** cage to enable the monitoring of feces production following surgery (an indicator of how well the animal is eating and recovering). Immediately following surgery to encourage the animal to eat, you should soak food pellets in warm tap water for **20 minutes**, pour off the excess water and place the food on the cage floor (easy access for the rodent). ***Rodents housed under sterile conditions must have the food soaked by cutting open a purified Hydropac water pouch (at the top) then place “sterilized” food pellets into the pouch for 20 minutes and pour off the excess water before placing the food into the cage.*** The water soaked food inside the cage will encourage the animal to eat, an animal that eats well will heal faster than an animal that stops eating. All rodents that are ambulating and have recovered from the anesthesia must be placed back into the animal housing room the day of surgery. Rodents should not remain in your lab overnight and should always be returned to the animal facility housing room following any procedure. Heat sources may be used in the animal housing room if the animal requires heat overnight. Rodents recovering/waking up from major survival surgery or other procedures requiring 30 or more minutes of anesthesia should have their cage placed on a heating pad or inside an incubator until the animal is fully ambulating inside the cage. The anesthesia recovery/waking up process should be monitored closely until the animal is fully ambulating (standing on all four limbs and walking inside the cage). Never place an animal back into the animal room to recovery -without monitoring the animal until ambulating.

4.5 Inhalant anesthetics- Whenever inhalant anesthetics are used, appropriate measures must be taken to prevent exposure of human personnel to anesthetic vapors. This may be accomplished by performing the procedure in a properly ventilated fume hood or by using a variety of scavenging systems (e.g. vacuum waste gas to the outside of building, or f/air scavenging canister).

5. Multi-modal Pain Therapy: Even with the pre-emptive use of analgesics, control of severe pain (even with high doses of a single analgesic drug) may not be complete. This leads to the concept of “multimodal pain therapy”, in which **two or more types/classes of analgesics are used**. Because clinical pain arises from a combination of central and

peripheral hypersensitivity and involves a multiplicity of pathways, mechanisms and transmitter systems, the use of more than one type of analgesic drug is more likely to relieve pain than the use of a single drug. Using more than one class of an analgesic agent will act at different points on the pain pathway, an example of this would be: combining an opioid (e.g. morphine or buprenorphine) with a NSAID (e.g. meloxicam or ibuprofen), because of different sites of action- NSAIDs are synergistic (increased effectiveness) with opioids. Also, infiltrating the surgical area with a local anesthetic (e.g. bupivacaine) will block local nerves in the area of the surgical procedure. For example, a simple skin incision (to place a SC implant, etc.) could have bupivacaine injected into the SC space to block local nerves prior to making the skin incision or an articular (joint) procedure could have bupivacaine instilled into the joint (intraarticular injection).

6. Intraoperative Monitoring:

Monitor	Information obtained
Palpebral (eyelid) and pedal (foot/toe) reflexes	Depth of anesthesia
Respiratory rate and depth of chest excursion	Adequacy of ventilation
Oral mucous membrane color	Vasoconstriction or vasodilation, oxygenation
Blood volume/color at surgery site, color of intestines	Little blood/dark blood or pale intestines indicate low blood pressure
Capillary refill time (CRT)	Slow CRT indicates low blood pressure or cardiac output
Heart rate and rhythm	Identifies bradycardia or tachycardia, and dysrhythmias

- 6.1 Lubricate the eyes with ophthalmic ointment (as soon as the animal is anesthetized) to prevent drying of the eyes -which leads to corneal ulcers.
- 6.2 Assure adequate depth of anesthesia for the type of procedure to be performed. The most reliable depth indicator for painful procedures is the response to the toe pinch. If the animal moves or increases its rate of breathing in response to the pinch, it is inadequately anesthetized for a painful procedure (i.e. anything involving a skin incision).
- 6.3 Evaluate and support the physiological status of the anesthetized animal (see above chart). At the very least, the animal should be checked frequently to assure that it is still breathing regularly. The animal should be kept warm with a recirculating warm-water blanket, care must be taken with the use of heating pads or heat lamps to guard against thermal burns.
- 6.4 The administration of warm fluids such as **Lactated Ringers Solution (LRS)** is an essential part of anesthetized patient care. Adequate plasma volume is essential for maintaining cardiac output and tissue perfusion, especially because many of the anesthetic agents expand plasma volume capacity by vasodilation. "Third space" fluid loss by surgical handling of tissues and evaporation from organs produce a substantial loss of fluid. Isotonic (0.9%) saline is a **poor choice of fluid** as it will only increase intravascular volume by about one fifth of the volume infused and less than 20% of the infused volume is remaining in the intravascular space by 90 minutes following infusion, LRS is the best fluid choice. **IV** administration of fluids in rodents is usually not feasible in most surgical procedures, however, it is important to replace blood loss with a balanced electrolyte solution (LRS) at a rate of 2.5-3 times the volume of blood lost **during or immediately following surgery (SC or IP)**. **Warm the fluids** prior to administration (by holding the syringe under warm running tap water), room temperature or cold fluids can lower the body temperature and prolong recovery.

Recommended anesthetics listed in order of preference (1 = most preferred, 5 = least preferred).

Rodent Methods and Available Drugs (Ketamine or Telazol alone will not produce a state of surgical anesthesia)			
Anesthetic	Mouse Dosage	Rat Dosage	Route of Administration
1) Isoflurane (preferred anesthetic for rodents) -ability to adjust anesthetic delivery, rapid recovery	1-4% (to effect)	1-4% (to effect)	Inhalation (chamber induction then maintained using a nose cone)
2) Ketamine + xylazine + acepromazine (Rodent Cocktail) (xylazine has analgesic properties= a2 agonist, respiratory depressant)	0.04mg/g (ketamine) + 0.009mg/g (xylazine) + 0.001mg/g (acepromazine) Combine 2mls (ketamine 100mg/ml) with 0.4mls (xylazine 100mg/ml) and 0.5mls (acepromazine 10mg/ml) then dilute one part cocktail with 3 parts saline. Administer 0.07- 0.15ml/25g	3.5mg/100g (ketamine) + 0.6mg/100g (xylazine) + 0.1mg/100g (acepromazine) Combine 2mls (ketamine 100mg/ml) with 0.4mls (xylazine 100mg/ml) and 0.5mls (acepromazine 10mg/ml) Administer 0.07- 0.15ml/100g	IP
3) Ketamine + xylazine	0.1mg/g (ketamine) + 0.01mg/g (xylazine)	9mg/100g (ketamine) + 1mg/100g (xylazine)	IP

4) Tiletamine/zolazepam (Telazol) non-surgical procedures only	0.08 – 0.1mg/g	4mg/100g	IP (non-surgical procedures only , dose dependent duration of effect, 30-60 min.)
5) Pentobarbital (Nembutal) dose dependent duration of effect (narrow margin of safety, respiratory depressant, cardiac depressant)	0.05-0.09mg/g (diluted 1:9 in sterile saline)	4-5mg/100g (diluted 1:9 in sterile saline)	IP (20 min. surgical time) 120-240 minutes sleep time = very long recovery time

Analgesics should be administered for a minimum of 3 days following an invasive surgery (i.e. anything more than a skin incision).

Recommended analgesics listed in order of preferred use (1 and 2 = used prior to skin incision, 3 – 5 used post-operatively)

Analgesic	Mouse Dosage	Rat Dosage	Route of Administration and frequency
1) Bupivacaine 0.25% (local infiltration or nerve block)	0.01-0.1mls SC and IM	0.1-0.3mls SC and IM	Used anytime a skin incision is made. Use in and around the surgery site just prior to making the incision (IM+SC) Around nerve (for nerve block)
2) Butorphanol (torbugesic) Used prior to skin incision (preemptive). Due to its short duration of action this agent not recommended for post-op analgesia.	0.003-0.005mg/g (3 -5mg/kg)	0.2mg/100g (2mg/kg)	SC (rapid onset, short duration)
3) Buprenorphine (buprenex) + Meloxicam (metacam-NSAID) combining an opioid with an NSAID is more likely to relieve pain versus single agent use only	0.00005mg/g (buprenorphine) (0.05mg/kg) + 0.0002mg/g (meloxicam) (0.2mg/kg) see dilution below	0.003mg/ 100g (buprenorphine) (0.03mg/kg) + 0.02mg/100g (meloxicam) (0.2mg/kg) see dilution below	SC every 6-8 hours (buprenorphine) SC every 24 hours for 3 days post-op (meloxicam)
4) Buprenorphine (0.3mg/ml) Used as a single agent if NSAID cannot be used in study (scientific justification), or when pain is mild	0.00005mg/g (0.05mg/kg) Recommended range is 0.02 – 0.3mg/kg	0.003mg/100g (0.03mg/kg) Recommended range is 0.01 – 0.2mg/kg	SC every 3-5 hours (mouse) SC every 6-8 hours (rat)
5) Meloxicam (metacam) 5mg/ml (NSAID) Used as a single agent when pain is mild.	0.0002mg/g Dilute 0.014ml metacam into 0.9mls of saline = 0.006mg/0.1ml (0.1ml/30g)	0.02mg/100g Dilute 0.014ml (metacam) into 0.9mls of saline = 0.006mg/0.1ml (0.3ml/100g)	SC every 24 hours (SID) for 3 days post-op

END OF DOCUMENT

STANDARD OPERATING PROCEDURE

Surgical Proficiency Assurance

Written by: Michael Fallon DVM, VMO, AV
IACUC Approval: April 10, 2013
Last Revised: March 22, 2013
Approved by: Michael Fallon D.V.M. _____(initials)

1. Purpose:

- 1.1 To describe institutional policy in assuring proficiency when performing surgical procedures in laboratory animals.

2. Personnel:

- 2.1 Personnel approved to perform surgical procedures prior to January 1, 2013 are considered to be trained and qualified unless additional information suggests otherwise.
- 2.2 New personnel who request approval to perform surgical procedures after January 1, 2013 will be observed by our senior vet tech. This observation will be directed at ensuring proper sterile technique and proper prep and anesthetic practices. It will not be directed at evaluating the technical skill of the surgeon unless something is very obviously wrong with technique. The technical performance of the surgeries will be assessed objectively during post operative observations of animals by the vet tech.
- 2.3 Personnel approved prior to January 1, 2013 to do one type of surgery, who now wish to perform a different surgery, may be asked to arrange observation by the vet tech. Alternately, during IACUC review the IACUC and AV may decide that the skill level of the surgeon is well known, and no additional observation is needed.
- 2.4 The vet tech will document all observations using a standard observation form that has been developed for this purpose. This form will be reviewed by the IACUC during IACUC meetings. Based upon the results of the observation, an additional observation session by the vet tech and/or the AV is an option.

End of Document

STANDARD OPERATING PROCEDURE

Rodent Housing and Overcrowded Cage Policy

Written by: Sandra Meyer, RVT, LATG; Jim McNeill, BS, LATG

Date Approved: March 13, 2013

Last Revised: August 13, 2013

Approved by: Michael Fallon D.V.M. _____(initials)

Purpose: To describe species housing and cage densities for all rodents. The following standards must be followed to ensure compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Species housing: According to the “Guide for the Care and Use of Laboratory Animals” different animal species should not be housed in the same room. Rats and mice should be housed in separate rooms with a separate air supply/ventilation or separate cubicle (separate air supply) and not housed together in the same room or same cubicle UNLESS first approved by the IACUC as a departure from the recommendations in The Guide.

Rodent Housing Rooms in the VMU:

Conventional Mouse Rooms (non-sterile): 4A115, 4A120

Sterile Mouse Rooms (immune deficient mice): 4A110, 4A130 (BSLII/quarantine room), 4A133 (BSLII/quarantine room)

Quarantine Mouse Rooms: 4A111 (general quarantine and periodic BSLII work), 4A134 (PI lab designated as quarantine due to transfers from other institutions where rodents do not undergo routine 8 week quarantine and pathogen screening).

Conventional Rat room: 4A117, 4A119

Maximum Cage Densities for Rodents:

Rat: no more than 3 adult rats per cage or one breeding pair with one litter. Any rat weighing more than 500 grams must be housed 2 per cage.

Mouse: no more than 5 mice per cage and no more than one male with one female when breeding. Trio (one male, two females) breeding must be scientifically justified in appendix 10 and approved by the IACUC.

Breeding (& Timed Pregnancies):

1. Investigators interested in breeding rodents must obtain and complete the ACORP Appendix 10, DRAFT “Atlanta VAMC Rodent Breeding Appendix” and submit it to the IACUC for approval before breeding may begin.
2. Mouse matings may be established as pairs (one male, one female). If you wish to trio breed (one male, two female) you must scientifically justify the reason you need to trio breed in appendix 10 and obtain IACUC approval. Litters must be weaned between 21 and 28 days post partum unless scientific factors require a delayed weaning time. The cage tagging system is used by the animal care tech which documents the date of birth and the date the litter should be weaned. Litters should be weaned according to the date on the tag.
3. If the PI has indicated responsibility of all breeding in the ACORP Breeding Appendix, it is the responsibility of the investigator or research staff to periodically monitor cage density to ensure that maximum cage densities described above are not exceeded. If an animal care technician observes overcrowded cages, he/she will notify the investigator or his designated contact person of the situation. The cage will be flagged and an **Overcrowded Cage Report** noting location of the cage will be emailed to the investigator or contact person. Once notified, the investigator will be given 48 hours to correct the overcrowded cage.
4. If the overcrowded cage or cage past the wean date is not corrected within 48 hours the animal care technician will be responsible for correcting the cage. The technician will place rodents in separate cages to comply with density policies and will assign new cage cards to the cages with the following information: Investigator, protocol number, sex, number of rodents in cage, parent cage identification and date of birth.
5. The technician will note on the “Animal Health Report Form” and the “Technician Time Charge Form” that an overcrowded cage has been corrected. Upon notification by the VMO in writing that repeated problems requiring animal care staff intervention are occurring, investigators will be assessed a \$10.00 charge for technician time for each overcrowded cage that is corrected. The notification form on the next page will be sent by email to the investigator.

End of document

STANDARD OPERATING PROCEDURE

Rodent Breeding and Weaning

Written by: Sandra Meyer RVT, LATG ; Lisa Lefebvre ALAT
 IACUC Approval: March 13, 2013
 Last Revised: August 7, 2012
 Approved by: Michael Fallon D.V.M. _____(initials)

1. Purpose: To document and describe various techniques and husbandry used when breeding mice and rats.

2. Breeding Room

2.2 Room Temperature: 64°F - 75°F

2.3 Breeding Room Photoperiod: 14 hours of light and 10 hours of darkness.

2.4 Position the breeding rodent cages in a quiet area in the room with the least amount of noise and traffic, radios should not be used in breeding rooms (headsets only).

2.5 Do not place cage racks against the wall, vibrations from neighboring rooms can disturb the breeding rodents.

3. Breeding Cage

3.1 The person responsible for the breeding and weaning of the production colony (i.e. PI or PI staff) should check all the breeding cages daily for newborn pups. When a litter is born write the date the litter was born and the date the litter is due to be weaned on the litter/wean tag and place the tag on the cage.

3.2 Pregnant, litter/wean tagging system: if you know the date the female became pregnant you can use the purple “pregnant” tag to write the pregnant date and attach it to the cage cardholder. Using pregnant tags will help you know when to expect a litter to be born (21 days following pregnancy). Once a litter is born a yellow “litter/wean” tag is placed on the cage to indicate the date the litter was born and the date the litter is due to be weaned. Litters are weaned at 21 days old, however, some transgenic strains are too small at 21 days old and may not be ready for weaning (weanlings need to be able to reach food and water in overhead feeder). Allow additional days (typically 7 more days) with the parents before weaning when weanlings appear too small for weaning. Change the wean date on the litter/wean tag to indicate the new wean date when small weanlings need more time with parents before weaning.

3.3 ALL cages are required to have environmental enrichment provided to the animals, use nestlets in mouse cages and Enviro-dri in rat cages.

3.4 Do not change the cage more than twice per week. Changing the cage more than twice a week can disturb breeding rodents.

3.5 Always wear fresh gloves in between handling rodents from different cages, the smell of other rodents can disrupt breeding behavior.

3.6 The pups have the best chance for survival if the following guidelines are followed: Do not change the cage for 2 days before delivery or 3 days after delivery. Changing the cage will disrupt cage odor and the mother may neglect the pups. Reduce handling and observations to a minimum.

4. Physiological Data

MOUSE

Weight, adult-----25-40 g
 Weight, newborn-----1 g
 Life span-----1-3 years (depending on genotype)
 Sexual maturity-----40-60 days
 Estrous cycle frequency-----4 -5 days
 Duration of estrus-----10 hours
 Gestation period-----19-21 days
 Average litter size-----6-12 (strains vary)
 Begins eating dry food-----12-14 days
 Age at weaning-----21 days
 Breeding life-----8 months

RAT

Weight, adult male-----300-500 g
 Weight, adult female-----200-400 g
 Life span-----2.5-3.5 years
 Sexual maturity-----65-110 days
 Estrous cycle frequency-----4-5 days
 Duration of estrus-----13-15 hours
 Gestation period-----20-22 days
 Average litter size-----7-11
 Begins eating dry food-----10-12 days
 Age at weaning-----21 days
 Breeding life-----1.5 years

5. Mating Systems

5.1 Monogamous Mating: One male and one female are selected and paired together for the duration of their breeding life.

5.2 Harem/Trio Mating (**DUE TO FLOOR SPACE REQUIREMENTS TRIO BREEDING MUST BE SCIENTIFICALLY JUSTIFIED AND APPROVED BY THE IACUC –RAT AND MOUSE, OR LARGE MOUSE CAGES CAN BE USED BECAUSE THEY MEET FLOOR SPACE REQUIREMENTS**): One male is housed with two or more females. This results in the largest number of young from the least number of breeder animals. Harem mating is the most economical method of animal production. This system can be used only when it is not critical to know which female is the mother. When the Harem breeding method is used cages can easily become overcrowded when two litters are born and reach weaning age together. It is important to always wean cages of weanlings on the wean due date (unless too small) to prevent cage overcrowding. The animal facility is an accredited facility, part of the accreditation is that we follow the guidelines set forth in the “Guide for the Care and Use of Laboratory Animals,” overcrowded cages do not comply with the guidelines in the Guide and therefore, overcrowded cages must be avoided.

5.3 Separate Housing (**SEPARATE HOUSING MUST BE SCIENTIFICALLY JUSTIFIED AND APPROVED BY THE IACUC**): The male and female may be housed separately and brought together only for breeding. This system reduces the number of breeding animals needed, however, labor costs are high. If males are known to kill the young, or if males and females are aggressive toward each other, this is the system of choice.

5.4 Intensive and Nonintensive breeding: The “intensive” system requires that the male remain with the female or females continuously. The male will be present for post-parturient estrus 24 hours following parturition. In a “nonintensive” system the male and female are housed separately while the female is pregnant and the female is not permitted to mate again until the litter is weaned.

6. Detection of Pregnancy

6.1 Plug detection: The ejaculate from the males’ accessory sex glands forms a short-lived, white to yellowish plug in the vagina of the female. Presence of a vaginal plug is often used to determine if copulation occurred between mice. By checking female breeding mice each morning, the presence of a vaginal plug allows you to estimate the approximate time of mating as the middle of the preceding night. **The presence of a plug does not guarantee pregnancy.** 15% or more of plugged females are either not pregnant or never gestate due to reabsorption of fetuses. To check for the plug, restrain the mouse by the scruff or allow the mouse to grasp the wire bar lid with the front limbs, with a gloved hand use your fingers to gently separate the vulva and look for the plug in the opening of the vulva.

6.2 Palpation: at (or about) 14 days, detection of what feels like a string of pearls may be palpated.

7. Weaning

7.1 Pups are weaned at 21 days of age. If pups appear to be small at 21 days old, allow 3-4 more days with the parents (change the wean date on the wean tag). While weaning pups, place moistened food on the bottom of the cage. Weaning the pups on time is very important --pups will breed back to litter mates or the mother if not separated. If a new litter is present the chance of the new litter surviving is reduced if the older litter remains in the cage.

7.2 Sexing the pups: The anogenital distance (the distance between the anus and the genitals) is the method used to sex mice. The anogenital distance is greater in males than in females. Sexing mice takes practice, always have an experienced person check weanlings until the person doing the weaning is competent.

7.3 Sex the pups at weaning and place males and females into separate cages. Do not place more than 5 weanlings into one cage. Try to keep weanlings socially housed with 2 or more per cage following weaning. Recheck the sex of the pups after one week following weaning to verify that males are not placed with females --as pup’s age it becomes easier to sex them.

8. Fostering Pups

Newborn litters are sometimes fostered onto nursing surrogate mothers for a variety of reasons (e.g. the natural mother neglects the pups or the natural mother is not lactating properly). Certain mouse strains or genotypes are more susceptible to stress than other strains and may react by neglecting the litter or may fail to lactate. The decision to foster pups should be made within 24 hours following birth if a “milk spot” is not visible. The “milk spot” is a stomach full of milk and can be seen through the thin skin of the pup in the abdominal area within the first 24 hours if the mother is lactating. Another

alternative to dealing with delicate breeders is by removing the male from the breeding cage **BEFORE** the birth of the litter. Removing the male can help to reduce stress in the female, however, the male **must not** be removed **after** the birth of the litter or this can cause stress in the female.

8.1 The first step is to select a suitable foster mother. Try to choose a mother that has recently and successfully weaned a litter, has pups of a different coat color than the foster pups (easy identification at weaning), and has pups the same age (+ or - 2 days) as the pups to be fostered.

8.2 Remove as many of the “natural pups” as you wish to replace with foster pups. It is critical to have the foster litter size equivalent to the natural litter size, the milk supply of the foster mother can be affected if more pups are fostered than that of her natural litter size. Euthanize any natural pups removed from the foster mother (see SOP #06-001 Rodent Euthanasia).

8.2.1 It is best to keep the foster mother in her cage and transfer the foster litter to her cage. Before placing the foster litter into the cage, rub the foster pups into the nestlet material of the natural litter. If possible have the male or female urinate on the foster pups before placing them into the nest (allow for a good scent transfer to the new foster pups). An alternative approach to transferring a litter is to remove the entire nest containing the foster mother’s natural litter, place the nest under a heat lamp or some source of heat. Next place the foster litter in the nest and gently mingle pups from the natural and foster litters together to spread scent. Rub feces from the foster mother on the backs of the foster pups, when the foster mother cleans the pups, she will most likely accept the pups as her own.

9. Retiring and Replacing

9.1 Mouse: Retire breeders over 6-8 months old or after 5-6 litters.

Rat: Retire breeders over 1 year old.

9.2 Replace any breeders (male and female) that have produced 2 consecutive poor litters (quality and quantity).

9.3 Replace males that have not produced a positive pregnancy (in the presence of a receptive and fertile female) after 3-6 weeks with a different male.

End of document

STANDARD OPERATING PROCEDURE

Cleaning Oxymax Metabolic Caging and Accessories

Written by: Simon Musyoka Mwangi, Ph.D.
IACUC Approval: March 13, 2013
Last Revised: 7/10/12

1. Purpose: To document and describe procedure for cleaning Oxymax Comprehensive Lab Animal Monitoring System (CLAMS) metabolic cages.

PPE (Personal Protective Equipment): Gloves and Eye Protection
(gloves are under sink and eye protection is on shelf above sink).

2. Cleaning Water Bottles:

The water bottles that come with the chamber are made of mixed materials and should be cleaned (especially tip) prior to each experiment. These can be cleaned using either of the methods below:

➤ Soak in 10% bleach for 15 min and hand wash with clean water at temperatures less than 45°C (use the large pre-marked container, the bleach and bleach measuring container under sink for soaking bottles, use the same water to wash the chambers).

➤ Hand wash with soapy water at temperatures less than 45°C

DO rinse thoroughly.

WARNING:

DO NOT machine-wash

DO NOT expose to temperatures above 45°C

3. Cleaning CLAMS Cages:

The Oxymax chambers contain cages made of polycarbonate plastic. These cages can be cleaned as follows:

➤ machine-or hand-washed at temperatures below 90°C;

➤ handwashed with dilute (10%) bleach solution (use the same bleach water used to soak bottles –after bottles have been removed from container)

➤ **WARNING:**

➤ DO NOT employ ammonia or alcohol

➤ DO NOT expose to temperatures above 90°C

➤ DO NOT employ petroleum based cleaners

STANDARD OPERATING PROCEDURE

Animal Facility Emergency and Disaster Procedures

Written by: Michael Fallon DVM; Sandra Meyer, RVT, LATG ; Jim McNeill, B.S., LATG
IACUC Approval: March 13, 2013
Last Revised: July 16, 2012
Approved by: Michael Fallon D.V.M. _____(initials)

1. Purpose: To document and describe the proper procedure in the event of an emergency situation or inclement weather condition affecting the VMU.

2. Mechanical System Breakdown

2.1 In case of any mechanical breakdown or loss of air conditioning (including mechanical problems anywhere in the animal facility) or electricity, call Engineering Service:

Ext. 6100 Monday – Friday, 8AM – 4 PM.

After Hours/weekend: Ext. 6090. .

2.2 Explain the problem in detail including room numbers. Let them know that it is an emergency and must be taken care of promptly, stress the effects on the animals in that room (move the animals to another room if necessary).

2.3 Air conditioning or heating problems resulting in animal room temps above 85 degrees or below 45 degrees is an EMERGENCY. Animals must be removed immediately from the housing room and placed into the clean-side hallway (corridor), move the racks with cages in place. Any rodents removed from the room that were on automatic watering must be transferred into a cage with a wire bar lid and provided with a hydropac water pouch and food. Bowls of water can be placed into rabbit cages that were on automatic watering.

2.4 Notify the VMU Supervisor or Chief Veterinarian of the situation. They will advise you as to what else needs to be done. **Communication is the most important thing. Let SOMEONE know if there is a problem.**

Dr. Fallon: Ext. 7644, Cell: 404-732-5471 or 770-500-2191

Jim McNeill: Ext. 6162 or (C) 404-861-5290, (H) 678-482-1246 or Pager: 404-674-9459

2.5 Other emergency situations would include the following:
(Contact Jim McNeill as well as calling the specified extension)

2.5.a Water leak resulting in animal rooms/labs or offices being flooded. (6100)

2.5.b Smoke or fire- DIAL 55

2.5.c Medical emergency to yourself or someone in the animal facility- DIAL 33

2.5.d Security problem- DIAL 4911

2.5.e Hazardous spill- DIAL 55 and report the location and nature of spill (close off the area before leaving to call for help).**HazMat Coordinator:** x 6115 **Safety Officer:** x:2752 **Research Biosafety Officer** X3078

- 2.5.f Any emergency where the health of an animal or a person is in jeopardy (3078 / OHS personnel or 6162 for animal emergency).**
- 2.5.g Automatic watering system failure** resulting in animals unable to get water. (X6162) or (H) 678-482-1246.
Rodents: transfer to wire bar caging and place the animals on hydropac water pouches.
Rabbits: place water bowl in each cage and fill bowl with water from hydropac pouches by cutting the pouches open and pouring the water into the bowl.
Dogs: place water bowl in each cage and fill bowl with water from hydropac pouches by cutting the pouches open and pouring the water into the bowl.
Pigs: place water bowl in each cage and fill bowl with water from hydropac pouches by cutting the pouches open and pouring the water into the bowl.
- 2.5.h Any veterinary emergency-** Call Dr. Fallon Ext. 7644 or Cell: 404-732-5471 or Emory weekend “on –call” veterinarian at 404-726-6816

Call or page the animal facility management person (Jim, Sandy or Lisa) that is on duty that weekend if you are unsure of what to do in a situation occurring on the weekend. Look on the calendar (on cork board near Jims’ office) in the clean side hallway to see which person is on duty that weekend. Employee contact numbers are posted on the wall in the clean side hallway (near the calendar) and Dr. Fallon’s door (4A106).

3. Extended Loss of Power in Animal Facility

Engineering Service: Ext. 6100 Monday – Friday, 8AM – 4 PM
After Hours/weekend: Ext. 6090

Should there be an extended loss of power due to a storm, the primary concerns are that the animals receive food and water and be kept comfortable. You should do your very best to report to work, but **DO NOT PUT YOURSELF OR OTHERS IN DANGER**. If you cannot report for work, call Jim McNeill at 678-482-1246, Dr. Fallon at 404-732-5471, or Sandy Yurevich at 404-513-9360. If your phone is dead, keep trying every 30 minutes.

If you are able to make it into work:

- 3.1 Immediately call Jim McNeill at 678-482-1246, Dr. Fallon at 404-732-5471, or Sandy Yurevich at 404-513-9360 to let someone know you are at the animal facility, then check all animals to make sure they have food and water. Emergency power provided by a large generator should be on. If not, call Engineering Service at extension 6100 (Monday – Friday, 8AM – 4 PM). After Hours/weekend, call extension 6090.
- 3.2 Open the doors to all non-animal rooms on the north side of the building to increase the amount of light available during the daytime.
- 3.3 Dog and pig runs may be hosed down without the need for electrical power if you can see well enough to do so.

- 3.4 Check rodent cages and change out cages that are very soiled and a threat to the health of the animals. This includes transgenic mice in sterile caging, even if the hoods are not working. [Should document if hoods / microisolators not working and use appropriate PPE (gowns, gloves, respirator)]
- 3.5 If the temperature in animal rooms is comfortable, leave all doors to animal rooms closed. If the temperature is too hot or too cold, open all the doors to animal rooms except the doors to any BSL-3 animal room.
- 3.6 Continue to stay with the animals until you are relieved by another person.

4. Animal Housing Room Temperature Over-Heat Emergency Plan

Tridium Vykon Alarm computer system. Atlanta VA's HVAC control System.

- 4.1 Animal rooms equipped with over heating alarms: rooms 4a110, 4a111, 4a115, 4a117, 4a119, 4a120, 4a121, 4a123, 4a124, 4a130, 4a133, 4a128, 4a134B, 4A133, 4a104A, 4A134A and 4A115A, 4A117A, 4A115A.
- 4.2 Once an over heating alarms comes in to the tridium system, the system will sound an alarm to boiler plant computer.
- 4.3 An automatic text message will be sent to cell phones in the form of a text message to the list below:

Boiler plant operator @ 404-376-7087, **ATT**
A/C shop on duty personal @ 404-539-1685, **ATT**
Dr. Mike Fallon Veterinarian @ - 404-732-5471, **ATT**
Jim McNeill @ 404-861-5290 **ATT**
Tony Laracuate Director Research Operations @ 678-699-7444, **Verizon**
VA Police 404-538-4260, **ATT**

Jim McNeill VMU Supervisor @ 678-482-1246 or 404-861-5290

- 4.4 First response in the first 5 min. of the alarm boiler plant operator to call on site personnel: A/C Shop, Area Maintenance unit, Project Team to response to alarm.
- 4.5 Boiler plant initiates VMU Emergency Cascade callback
- 4.6 A/C shop personnel to respond with VA Police.
- 4.7 Fallon/Laracuate will call Boiler Plant Cell Phone and/or A/C Cell phone to verify alarm and problem.
- 4.8 What to check:
 - A) If more than one room in alarm.
 - B) Check Air Handler unit 4A AH 16 Supply air **temperature**
 - C) Check Chiller supplying 4A AH 16.
 - D) If only one room in alarm.
 - E) Check supply air **temperature** to room.
 - F) The RANCO room **temperature over heating alarm should have closed the heating supply valve if this has not happened closed the manual supply valve at the TU Box.**
 - G) **If the animal housing room temperature exceeds 85 degrees, remove the Animals to the clean side hallway outside the room.**

5. Entire Animal Facility Overheat

In the event the entire animal facility exceeds 85 degrees: all animals will be removed and transported down the service elevators by animal care staff and VA police and placed in the basement level hallways next to loading dock B until the facility temperature is corrected. The facility supervisor should initiate an effort to make local arrangements for animal housing in the event the animal facility temperature cannot be corrected within a few

hours. All BSLII animals will be removed and euthanized in the event BSLII housing cannot be obtained through local arrangements.

6. Animal Facility Fire

In the event of a fire: all efforts will be made by animal care staff, firemen and VA police to remove animals from the animal housing room when it does not endanger human life to do so. The animals will be placed under the sprinkler heads on the clean side hallway of the animal facility and will not be removed from the building due to the risk of using service elevators during a fire.

7. Animal Facility Water Shortage

In the event of a water shortage: the animal facility will maintain a minimum backup supply of 1200 pre-filled and ultra-filtered disposable water pouches (hydropac water pouches) to provide water to all the animals in the animal facility for up to three weeks.

8. Wild Rodent Infestation/Intrusion

In the event that a wild rodent has been found inside an animal research facility or where there are reports of wild rodents in the facility interstitial spaces or adjacencies, the following actions should be taken:

- 8.1 Notify the pest control technician (Kevin McGruder at ext. 6065) if they are not aware of the circumstances.
- 8.2 Clear out any rooms where intrusion has been noted for inspection, trapping, exclusion and decontamination.
- 8.3 Discard any opened or broken bags of animal feed. Unbroken bags should be removed and surface sterilized using the MSKCC protocol (Thurlow, Ralph W.; Arriola, Raquel; Soll, Clifford E.; Lipman, Neil S. Evaluation of a Flash Disinfection Process for Surface Decontamination of Gamma-irradiated Feed Packaging. Before storage elsewhere.
- 8.4 Health Surveillance of trapped rodents:
- 8.5 Remove traps with live rodents noting the place where the trap was with a tape mark.
- 8.6 Transport rodent on glue trap to necropsy in filter top cage.
- 8.7 Signalment and basic necropsy observations should include species ID of the subject where possible, noting the approximate age (e.g. adult, juvenile, recent weanling), the precise location where trapped, gender and recording body weight. In the case of females note whether it was lactating and the condition of the uterus.
- 8.8 Specimens to collect include serum (for UGA diagnostic serology testing), tissues for histopathology, tissues for freezing, examination of the pelage for ectoparasites (particularly fleas and *Ornithonyssus bacoti*), anal tape collection for pinworms, and GI tract direct examination and fecal floatation for the presence of helminthes and cestodes.
- 8.9 Necropsy on down draft table using ABSLII precautions.

9. Inclement Weather

General Precautions: Whenever a weather situation is expected to make travel to the VA for all personnel very difficult, arrangements will be made prior to the bad weather for VMU personnel to stay overnight in a location designated by hospital leadership and as directed by Dr. Fallon. In the event of non-anticipated inclement weather, employees can receive updated instructions by Direct Dial 404-327-4970 or 404-321-6111 Press 1 and extension 4970 or 800-944-9726 Press 1 and extension 4970. When inclement weather conditions such as snow and ice leave roadways impassable, the medical center will provide ground transportation for mission essential employees. Employees must contact their supervisors to be placed on a prioritization listing that's maintained by management in the Emergency Operations Center EOC.

10. Structural Damage to Animal Facility

- 10.1 In the event of structural damage to the animal facility, all BSLII animals will be segregated into separate lab or VMU space, if such space is available. If no space is available, BSLII animals will be

ethanized according to IACUC guidelines.

- 10.2 Sticky traps will be used to catch escaped rodents. Existing door sweeps throughout the entire facility will contain escaped rodents until they are trapped on sticky traps.
- 10.3 During tornado season, a 30 day supply of animal food will be placed into waterproof containers and stored inside the walk in food cooler.

End of Document

STANDARD OPERATING PROCEDURE

Rodent Euthanasia Using CO2

Written by: Sandy Meyer, RVT, LATG ; Lisa Lefebvre, ALAT; Jim Mcneill, B.S., LATG
IACUC Approval: November 13, 2013
Last Revised: November 6, 2013
Approved by: Michael Fallon D.V.M. _____(initials)

Purpose: To document and describe the proper technique used to euthanize rodents with CO2.

Rodents should be euthanized as described in your IACUC approved protocol (See "Method of Euthanasia" in your protocol)

DO NOT PLACE ADULT ANIMALS THAT HAVE NOT BEEN HOUSED TOGETHER INTO THE CHAMBER TOGETHER

1. MICE:

- a) place the mouse cage into the chamber and secure the chamber lid to ensure the gas will not leak out. If the cage does not fit into the chamber place paper towels on the chamber floor to protect the floor from urine and feces.
- b) Turn the main valve on the CO2 tank counter clock-wise (your left) two turns.
- c) Set the flow meter to 32 (do not set it any higher than 32).
- d) Once the mouse becomes unconscious turn the flow meter off, leave the mouse in the chamber for 3 minutes to confirm death. If you remove the mouse before 3 minutes you will need to cut the chest open to confirm death by creating a pneumothorax. Neonates/ Pinkies should be placed into a zip lock bag (label the zip-lock bag with PI name), place the CO2 hose into the bag and zip up the bag flush to the hose. Slowly inflate the bag with CO2 and quickly remove the hose and zip the bag closed. The zip lock bag should remain inflated and the pups should remain in the zip lock bag for a minimum of 30 minutes (could take up to 50 minutes). Confirm the pinkies are dead prior to disposal (the skin is purple or blue and cold).

2. RATS:

- a) Place paper towels on the chamber floor to protect the floor from urine and feces, place the rat(s) into the chamber and secure the chamber lid to ensure the gas will not leak out.
- b) Turn the main valve on the CO2 tank counter clock-wise (your left) two turns.
- c) Set the flow meter to 32 (do not set it any higher than 32).
- d) Once the rat becomes unconscious turn the flow meter off, leave the rat in the chamber for 6 minutes to confirm death. If you remove the rat before 6 minutes you will need to cut the chest open to confirm death by creating a pneumothorax. Neonates and pups must remain in the chamber for 10-12 minutes (after 5 minutes turn on the flow meter to refill the chamber and allow 5 more minutes).

IF RODENT WAS PLACED ON PAPER TOWELS INSIDE THE CHAMBER PLEASE SPRAY AND WIPE OUT THE CHAMBER WITH QUATRICIDE

3. Confirm the rodent(s) is not breathing and the heart has stopped.
4. Place the animal into a red biohazard bag/carcass bag or a zip lock bag and place the bag into a yellow or red barrel (not the large grey barrel) inside the walk-in carcass cooler.
5. If you are saving the rodent for the investigator, place the rodent into a zip-lock bag. Label the bag with the PI name, date of death and the room number the animal was housed in. Place the bag on the shelf in the carcass cooler.

STANDARD OPERATING PROCEDURE

BioSafety Level II (BSLII) Laboratory

Written by: Sandra Meyer RVT, LATG ; Jim McNeill, B.S., LATG
 IACUC Approval: March 13, 2013
 Last Revised: February 8, 2011
 Approved by: Michael Fallon DVM, PhD _____ (initials)

1. **Purpose:** To document and describe standard microbiological practices, safety equipment, and containment techniques used when working with infectious, hazardous or toxic agents.
2. **Personnel:** These procedures apply to **all research staff utilizing hazardous agents in the animal facility and animal husbandry staff who care for animals exposed to such agents.**
3. **Standard Microbiological Practices for Use with Infectious or Toxic Agents at the BSLII Level**

BSLII Cabinet: When infectious agents are used in animals, all animal manipulations, infectious injections and post infection cage and animal handling during the quarantine period will be performed wearing the proper PPE and using the BSLII cabinet.

Fume Cabinet: When toxic agents (cell toxins) are used in animals, all mixing of agents will be performed inside a fume cabinet while wearing the proper PPE. The administration of these toxins to animals may be performed in a low traffic area of your animal procedure lab while wearing the proper PPE.

3.1 **Personal Protective Equipment (PPE) BSLII**

- Latex or nitrile gloves •Safety glasses with sideshields •Surgical Mask •Disposable Gown •Shoe Covers

Wear PPE while handling animals and administering BSLII agents to animals. PPE can be found in any of the two stainless steel cabinets located in the clean-side hallway of the animal facility.

- a. Access to the laboratory is limited or restricted when experiments are in progress and **a sign is placed on the outside of the room door indicating “Experiment In Progress.”**
 - b. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
 - c. Eating, drinking, handling contact lenses, and applying cosmetics are not permitted in the work areas.
 - d. Mouth pipetting is prohibited: mechanical pipetting devices are used.
 - e. Only needle-locking (luer-lock) syringes or disposable syringe-needle units are used for injection or aspiration of infectious or hazardous materials. Used disposable needles must not be bent, sheared, broken, recapped, or otherwise manipulated by hand before disposal.
 - f. Used needles or sharps are carefully disposed of in sharps containers. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Full sharps containers are sealed and placed in the walk-in carcass cooler (from there they will be autoclaved and disposed of).
 - g. Spills and accidents that result in overt exposures to infectious or hazardous materials are immediately reported to the facility supervisor (Jim McNeill, Ext. 6162).
 - h. **ALL work surfaces are decontaminated on completion of work. Use one part bleach to three parts water freshly mixed solution for infectious material, a spray bottle containing the fresh mixture should be maintained within the lab.** Use the manufacturer recommended decontamination procedure for any cell toxins (see Jim McNeill or Sandy Yurevich for MSDS if necessary).
4. Once you have completed your work with any BSLII agent use an ungloved hand to remove your protective eyewear, then replace with fresh gloves and wash the protective eyewear with hot soapy water.
- 4.1 While wearing a fresh pair of gloves and only touching the outside of the biohazard bag, remove

the bag from the trashcan, tape it closed and place it into the red (biohazard) trash bin (located in the hallway near the service elevators). It is not necessary to autoclave the red bag containing your PPE, all biohazard bags placed into the red trash bin will be autoclaved by the hospital prior to disposal (be sure the bag is taped closed with autoclave tape).

- 4.1 “Infectious agents”** dirty rodent cages are placed into **red autoclavable biohazard bags** and placed on the “dirty-side hallway” on a cart (all bags should be taped closed with autoclave tape and the room number where the infectious agent was administered written on the bag). Our cage wash technicians will autoclave the cages prior to washing.
- 4.2 “Toxic agents”** STZ (beta cell toxin) dirty rodent cages may be placed directly on the dirty side hallway once the 24 hour (following the last injection) quarantine has ended (quarantine period in toxins is based on pharmacokinetic half-life of the agent). **Chemotherapy agents** pharmacokinetic half-life of drug varies between agents (SEE MSDS), all empty dirty cages used in animals receiving chemotherapeutic agents must be placed into “**yellow chemo bags**” and placed into the carcass cooler until the pharmacokinetic half-life of the agent has passed; once passed the cages are placed on the dirty side hallway (by VMU staff) for washing (SEE SOP#11-005 Chemotherapy in Small Animals for more details).

5. Chemotherapy Drugs

5.1 Personal Protective Equipment (PPE)

•**Double nitrile gloves** •**Splash goggles** •**Water-proof Gown** •**Shoe Covers** •**Head Cover (when indicated)**

5.2 Personnel: High-risk individuals (i.e. immunosuppressed or pregnant) should be informed of the possible consequences of handling chemotherapeutic agents and given the option of avoiding any possible exposure by not working with animals that have received such agents.

5.3 Storage: Chemotherapeutic agents should be stored in a secure area. A locking refrigerator or cabinet is best. The manufacture’s instructions should be followed carefully regarding storage temperature.

5.4 Dilution: Toxic drugs should be mixed in a **vertical** laminar flow hood (fume cabinet). Hands should be washed before and after mixing chemicals. Gloves are not substitute for hand-washing. Always wear double nitrile gloves, or thicker gloves designed specifically for chemotherapy when handling any of these agents. Use luer-lock needles only during dilution. If a flow hood is not available, a respirator mask should be worn, respirator mask should be “fit tested” prior to use.

5.5 Spills: (See MSDS in room 4A125) Spill kits should be kept on hand. An accidental spill should be dealt with promptly and carefully (**use 10% dilute bleach solution to neutralize adriamycin/doxorubicin**). If your skin has been contaminated by the spill, wash thoroughly with soap and water, rinse well. If a drug has splashed into your eyes. Use an eye wash continuously for 15 minutes and report to employee health immediately.

5.6 Special Concerns

Vial Pressures: The dilution of some agents (like cisplatin) causes a significant pressure to be built up in the vial. The use of a venting device (chemo dispensing pin) should be considered unless all air is to be taken out. The venting device inserts into the rubber top of the drug vial. As diluent is added to the vial, pressure is released through a 0.2 micron, hydrophobic, air-venting filter on the side of the pin.

5.6.1 Adriamycin and Heparin: Heparin has been shown to precipitate adriamycin, the combination should not be used.

5.6.2 Cisplatin and aluminum: Cisplatin is inactivated in the presence of aluminum. Only plastic-hubbed needles should be used with this drug.

5.6.3 Administration: both administrator and animal restrainer should wear PPE during administration of any chemotherapeutic agent. An intravenous catheter should be securely in place prior to administration, perivascular injection will result in tissue necrosis. Use luer-lock needles and do not recap used needles. Oral chemotherapy tablets or capsules should not be cut or opened.

5.7 Disposal: See SOP#11-005 “Chemotherapy in small animals” for cage handling procedures following chemotherapy in animals. Chemotherapeutic waste should be placed into **yellow chemo bags** and placed into the chemotherapy drug container (labeled “BIOHAZARD Chemotherapy Drugs”) inside the walk-in carcass cooler (4th floor). Needles and other sharps should be placed into a yellow “chemotherapy drugs sharps container” and placed into the walk-in carcass cooler when container is full.

5.8 Chemotherapy Drug Considerations

5.8.1 Adriamycin (Doxorubicin): Will cause severe tissue necrosis if it escapes from blood vessels during injection. It can also cause a severe anaphylactic-like reaction in some patients. Pretreatment with antihistamines and corticosteroid may be recommended by the OHSP physician if prior sensitivity is known. Do not flush catheter with heparin. **Administer slowly over at least 10 to 15 minutes.**

5.8.2 L-asparaginase: Subcutaneous administration is less painful than IM or IP administration. Can also cause an anaphylactic reaction in some patients. Pretreatment with antihistamines and corticosteroid may also be recommended. Do not administer intravenously.

5.8.3 Cisplatin and 5-fluorouracil: are fatal to cats.

End of document

STANDARD OPERATING PROCEDURE

Animal BioSafety Level 2 (ABSL 2), Toxic Chemical, Radioactive, and rDNA Use in Rodents Species: Mouse and Rat

Written by: Sandra Meyer RVT, LATG ; Jim McNeill, B.S., LATG
IACUC Approval: November 6, 2013
Last Revised: September 23, 2013
Approved by: Michael Fallon D.V.M, Ph.D. _____(initials)

1. Purpose:

To describe animal care and BSLII procedures when using BSLII, toxic chemicals, and radioactive agents, or rDNA in a mouse or rat model that requires ABSL2 containment--this includes both competent and incompetent viruses unless otherwise specified.

For specific details using Chemotherapeutic agents in Small Animals see SOP #11-005

2. Personnel:

The procedures apply to **all investigators and research staff administering BSLII agents, toxic chemicals and radioactive agents. or handling animals and caging following administration. The procedures also apply to all VMU animal care staff.**

3. LIST OF AGENTS APPROVED TO USE IN RODENTS HOUSED IN VMU AS OF NOV. 2013

BSLII INFECTIOUS AGENTS:

HIV –human infected monocytes
Adenovirus—non-replicating viral vector
Salmonella
Cryptosporidium
Citrobacter rodentium (non-zoonotic)
rDNA

TOXIC CHEMICAL AGENTS:

Streptozotocin (STZ)
Tamoxifen (dispose of PPE into Yellow bag and place into necropsy walk-in cooler for incineration)
Cyclosporin A

3.1 Personal Protective Equipment (PPE) FOR BSLII INFECTIOUS AGENTS and rDNA work that must be done at ABSL-2 per the IBC:

BSLII CABINET: used during infectious agent handling, administration to animals and post-infection cage changing.

PPE: Disposable gown, double gloves, eye protection with side shields, mask.

Following administration of “Infectious Agents” ALL rodents will be manipulated using long forceps unless the rodent is anesthetized.

3.2 Personal Protective Equipment (PPE) FOR TOXIC CHEMICAL AGENTS

RESPIRATOR OR FUME CABINET: used during mixing of toxic agents.

PPE= Disposable gown, double gloves, mask and safety glasses used during mixing, administration and cage changing.

RADIOACTIVE AGENTS:

[¹⁴C]-oleate

Personal Protective Equipment (PPE) (NO LAB COATS) RADIOACTIVE AGENTS:

WORK AREA: plastic back absorbable pads placed on work bench and on the floor (beneath work area).

PPE: Personal radiation dosimeters (film badges), disposable gown, disposable gloves, disposable face and mask shield combo, disposable shoe covers. Wear PPE while handling radioactive materials, animals, and tissues.

4. Housing, Quarantine and Husbandry:

Housing: Housing of ABSLII animals is determined based on the agent used. Following IACUC approval please consult with the animal facility supervisory staff before using BSLII agents in animals. **The PI must provide the VMU with an MSDS for the use of infectious, toxic chemical and radioactive agents in animals and the agent must be included in this SOP before any work can begin.**

4.1 Quarantine: The cage or cubicle is quarantined, and a **quarantine sign is placed on the cubicle or rack**. If it is a temporary quarantine the quarantine sign will indicate when the quarantine starts and ends. Anyone entering the cubicle or removing cages from the rack is required to wear the specified Personal Protective Equipment (PPE). During quarantine for infectious agents all animal manipulations will take place in the BSLII cabinet wearing the proper PPE. ALL quarantined cages will be **flagged** using a biohazard card (temporary quarantine) or a biohazard sticker (permanent quarantine/infection).

4.2 STANDARD QUARANTINE PERIOD FOR THE FOLLOWING AGENTS:

Chemotherapy agent: see SOP#11-005 **Chemotherapy in Animals**

Recombinant DNA or other nucleic acids as required by the IBC: per IBC approval

Streptozotocin (STZ – beta cell toxin-chemical): 24 hours following the last injection –biohazard card on the cage.

Cyclosporin A (toxin/ immunosuppressant) 48 hours following the last administration –biohazard card on the cage.

Tomoxifen (Carcinogen): 72 hours (3 days following the last administration –biohazard card on the cage)

Adenovirus (viral vector): 72 hours (3 days following the last injection – biohazard card on the cage)

HIV: Permanent quarantine following infection (biohazard sticker on cage card)

A. baumannii: Permanent quarantine following infection (biohazard sticker on cage card)

Klebsiella pneumoniae: Permanent quarantine following infection (biohazard sticker on cage card)

Salmonella: Permanent quarantine following infection (biohazard sticker on cage card)

Cryptosporidium: Permanent quarantine following infection (biohazard sticker on cage card)

[¹⁴C]-oleate: Permanent quarantine following administration of radioactive agent. Animals that will be euthanized the same day the agent is administered will remain in the lab following administration and will not return to the animal housing room

.*Cryptococcus neoformans*: Permanent quarantine following infection (biohazard sticker on cage card).

***Citrobacter rodentium*:** Permanent quarantine following infection (biohazard sticker on cage card).

5. Cage handling/changing during and following the quarantine period:

Prior to the initiation of any study using BSLII agents it should be determined who will be responsible for changing out the cages following quarantine. The investigator may request the animal caretaker (responsible for the care of the room housing the quarantined mouse) to change out the cages following quarantine, otherwise the investigator/investigator staff is responsible for changing out the cages following quarantine. Cages should be changed out as follows:

5.1 BSLII infectious Agents and rDNA molecules: While wearing the proper PPE all cages containing infected/ABSLII animals must be changed out in a BSLII cabinet. **Use long forceps to transfer rodents into a clean cage.** All dirty cage bottoms are placed into autoclavable red bags and the bag is taped closed using autoclave tape (Do not place more than 2 stacks of cages per bag and no more than 8 cages per stack). Using a permanent marker write the room number and BSLII agent used on the bag or autoclave tape and place the bag on the dirty side hallway. After euthanizing the animal, Hydropac pouches and cage components should remain in the cage. The cage lid is taped to the cage using autoclave tape and the name of the BSLII agent is written on the autoclave tape and the cage is placed on the dirty side hallway.

5.2 STZ (chemical toxin): Write the date the STZ was administered on all cage cards and place a biohazard card on each cage containing rodents that have received STZ. The quarantine period for STZ is 24 hours following the last STZ injection. Post the quarantine sign indicating the duration of quarantine on the rack containing the cages. Once quarantine has ended the cage may be placed on the dirty-side hallway -there is NO special cage handling following quarantine due to the pharmacokinetic half-life of STZ (5-15 minutes).

5.3 Cyclosporin A (chemical toxin/ immunosuppressant): Write the date or the date range the Cyclosporin is to be administered (MM/DD/YY- MM/DD/YY) on all cage cards of cages receiving Cyclosporin and place a biohazard card on each cage containing rodents that have received Cyclosporin. The quarantine period for Cyclosporin is 48 hours following the last injection/administration. Post the quarantine sign indicating the duration of quarantine on the rack containing the cages. Once quarantine has ended the cage may be placed on the dirty-side hallway -there is NO special cage handling following quarantine due to the pharmacokinetic half-life of 19 hrs.

If cages must be changed during the quarantine period (cage flood): Cages will be changed while wearing PPE (described above). Cages are placed into red biohazard bags, tape the bag closed and tape a sign on the bag that includes the following: Cyclosporin A, the date and “cages may be removed from the bag after 48 hours (cage quarantine period), the cages no longer require special handling and may be washed after removal from the bag”.

5.4 RADIOACTIVE AGENTS (animal euthanized the same day): Prior to administration— all corncob bedding is removed from the animal cage and replaced with absorbable pads with plastic backing. **Rat:** remove the red tube from the cage and replace with a fresh supply of brown paper/nesting material (this will provide the animal with something to do—otherwise it may chew on the absorbable pad). Remove the wire bar from the cage and place a few pellets

Continued Next Page- →

of food inside the cage and a small piece of gel pack (disposable –used as water supply during animal transport). Place a filtered cage lid on the cage. **Mouse:** Remove the mouse shack and place a fresh nestlet inside the cage (this will encourage mouse to chew nestlet instead of absorbable pad). Remove the food/water container and place a few pellets of food inside the cage and a small piece of gel pack. Place a cage lid on the cage. All cages that contained radioactive animals will remain in the animal procedure lab until the cages are wiped and tested for radioactive material.

Call Sean Riggin at extension 2543 or 404-862-1744 for wipe testing of room/workbench and cages for radioactive material detection after each experiment involving the agent. Once the cages have been tested and are free of radioactive material they may be placed on the dirty side hallway for washing. Freeze the animal carcass in a yellow radioactive bag and keep the animal frozen until pick up. Animal tissues removed from radioactive animals should be disposed of in the same manner as the animal carcass.

5.5

Tamoxifen (Toxin): Tamoxifen will be mixed/ constituted inside a fume cabinet or over the down draft table (necropsy room) and will be administered using an absorbent pad on the benchtop workspace. Absorbent pads should be disposed of after each use into a yellow bag (tape the bag closed and place into the carcass cooler for pick up). All cage changes should be done in a change out station using care to contain dirty bedding inside dirty cage. The cage is changed **72 hours following the last Tamoxifen injection (when quarantine has ended)**. The bedding is considered contaminated and requires special handling. Wearing PPE, transfer the animal to clean cages. Remove the Tamoxifen biohazard card and place the cages into Yellow chemo bags. **NOTE:** If the administrations will continue transfer the Tamoxifen biohazard card to the new cage. Tape the Yellow biohazard bag closed and place on the dirty side cage wash. Wearing the proper PPE and following the Yellow Bagged cage instructions (on the wall to the right of the dump station) cage wash technicians will place a yellow bag into the dump station and the dirty bedding from the yellow bagged cages will be dumped into the yellow bag.. The bag is taped closed and placed into the walk in cooler for pick up and incineration.

6. Glassware

Broken glassware used in infectious animals: broken glassware or laboratory instruments must not be handled directly by hand and must be removed by mechanical means such as a brush and dustpan, tongs, or forceps and placed into a **sharps container (not a trash can)**. Secure lids when sharps container is full and place the container into the carcass cooler for disposal.

7. Radioactive Gavage needles:

Store gavage needles in containers with lids in between each use and label the container as “Radioactive.” Dispose of the gavage needles at the end of the study by placing the gavage needles into a yellow radioactive bag/bucket, close the bag and place the lid on the bucket.

8. Work surfaces are decontaminated on completion of work as follows:

- A) **Adenovirus (viral vector) or rDNA:** Freshly mix bleach --one part bleach with three parts water-- in a labeled spray bottle--spray work surface and allow 30 minutes of contact time before wiping. Place a sign on the work surface indicating the time you sprayed the surface and when it can be wiped with paper towels.
- B) **STZ (toxin/chemical):** Wash chemical away with water.

- C) **Cyclosporin (toxin –immunosuppressant):** Ethanol
- D) **Cryptococcus Neoformans:** Decontaminate the inside of the BSLII cabinet using 1% bleach, allow 3-4 minutes of contact time before wiping the cabinet out.
- E) **HIV:** Bleach solution --one part bleach with three parts water-- in a spray bottle labeled “bleach solution”--spray work surface and allow 30 minutes of contact time before wiping. Place a sign on the work surface indicating the time you sprayed the surface and when it can be wiped with paper towels. **The bleach solution should be discarded and replaced once a week.**
- F) **RADIOACTIVE AGENT: Following administration:** place all sharps into sharps containers and label container as “Radioactive,” place gavage syringes into a yellow radioactive bag. **Following euthanasia:** Remove all absorbable pads, gel packs, remaining food, and PPE and place into yellow radioactive bag/bucket, close the bag and place the lid on the bucket. All cages that contained radioactive animals will remain in the animal procedure lab until the cages are wiped and tested for radioactive material. Call Sean Riggan at extension 2543 or 404-862-1744 for wipe testing of room/workbench and cages for radioactive material detection after each experiment involving the agent.

Once the cages have been tested and are free of radioactive material they may be placed on the dirty side hallway for washing. Freeze the animal carcass in a yellow radioactive bag and keep the animal frozen until pick up. Animal tissues removed from radioactive animals should be disposed of in the same manner as the animal carcass.

- G) **TAMOXIFEN:** Place PPE and absorbent pad used on benchtop into a yellow bag, tape bag closed and place into walk-in carcass cooler for incineration.

9. Removal of rodent carcass from cage during quarantine:

9.1 Animal injected with BSLII infectious agents or rDNA: Wearing the indicated PPE place the cage into the BSLII cabinet to remove the rodent carcass from the cage during the quarantine period (unless otherwise requested to leave the deceased rodent in the cage for the investigator to remove). Place the rodent into a biohazard bag, label the bag with the PI name, date, room and cubicle/rack number the animal was removed from, using a Sharpie (permanent marker) write the name of the infectious agent or RDNA the rodent is infected with on the bag (e.g. HIV, Adenovirus, Salmonella, Cryptosporidium, Klebsiella, A. baumannii, etc). Save the carcass on the shelf in the walk-in carcass cooler (clean-side of facility) and notify investigator.

9.2 Animal injected with chemical toxin: Wear the indicated PPE to remove the rodent carcass from the cage during the quarantine period (unless otherwise requested to leave the deceased rodent in the cage for the investigator to remove). Place the rodent into a zip lock bag, label the bag with the PI name, date, room and cubicle number the animal was removed from and using a Sharpie (permanent marker) write the name of the Toxic agent the rodent was injected with on the outside of the bag (e.g. Chemo drug, STZ). Place a biohazard sticker on the zip lock bag. Save the carcass on the shelf in the walk-in carcass cooler (clean-side of facility) and notify investigator, Animal Care Technician should report any deceased rodent on “Animal health report form” located on the door of the animal room (PI staff should also report any deceased rodent on Animal Health Report form).

End of document

Atlanta VAMC Veterinary Medical Unit

STANDARD OPERATING PROCEDURE

Animal BioSafety Level 2 (ABSL 2), and Chemotherapeutic Agents

Species: Rabbit

Written by: Sandy Yurevich RVT, LATg ; Jim McNeill, B.S., LATG

IACUC Approval: INACTIVE

Last Revised: June 27, 2007

Approved by: Michael Fallon DVM., PhD _____(initials)

1. Purpose: To describe animal care and biosafety procedures when materials classified as a Biosafety Level 2 (BSL 2) or chemotherapeutic (cancer-fighting) drugs are used in rabbits. This SOP covers the following:

- 1.1 Infection with a virus classified at the BSL2 level such as adenovirus, including non-replicating virus types.
- 1.2 Administration of chemotherapeutic drugs such as adriamycin (doxorubicin) that are known to be toxic to animals or humans and require special precautions to prevent accidental exposure.

2. Personnel: These procedures apply to **all research staff utilizing hazardous agents in the animal facility and animal husbandry staff who care for animals exposed to such agents.**

3. INFECTIOUS/ VIRAL VECTORS

3.1 Personal Protective Equipment (PPE)

•Latex or nitrile gloves •Safety glasses with sideshields •Surgical Mask •Disposable Gown •Shoe Covers •Head Cover

3.2 Housing: Standard rabbit caging.

3.3 Quarantine period: 72 hours post-injection with a viral or chemotherapeutic agent. During this time, the entire cubicle is quarantined, and a **quarantine sign is placed on the cubicle**, the sign should include the quarantine start date and the date the quarantine ends. Anyone entering the cubicle is required to wear the Personal Protective Equipment (PPE) listed above. Unless exceptional circumstances prevent it, quarantined rabbits should not be housed in cubicles with non-exposed rabbits. Following quarantine, pans of exposed rabbits will be changed out and autoclaved (for viral agents) prior to disposal in a red biohazard bag, or dumped in a yellow chemotherapy disposal bag (for chemotherapeutic agents). The cubicle will remain in quarantine until all pans of exposed rabbits have been changed out after 72 hours have passed.

3.4 Animal Husbandry (Work with infected rabbits last).

- 3.4.1 Work surfaces are decontaminated on completion of work. Use quatricide (2oz or 60mls/gallon of water) for **infectious agents** (all animal rooms have a spray bottle with quatricide).
- 3.4.2 Sweep the cubicle after the quarantine period. Any materials swept from the cubicle floor must be placed in red biohazard bags (for viral agents).
- 3.4.3 Mop the cubicle with Quatricide after each cage change.
- 3.4.4 After the quarantine period, change out all contaminated rabbit pans.
- 3.4.5 Label contaminated rabbit pans as a “biohazard” and notify the cage wash technician.
- 3.4.6 On completion, place disposable gown, mask, gloves and shoe covers into a red biohazard bag. With an ungloved hand, remove protective eyewear and wash with hot soapy water.
- 3.4.7 Wear a fresh pair of gloves and only touch the outside of the red biohazard bag, remove the bag from the trashcan, tape it closed and place it into the large trash bin (located in the hallway near the service elevators).

3.5 Dirty-Side Cage Wash Technician

The cage wash technician will wear PPE and immediately scrape the contents of the pans into red biohazard bags (place biohazard bags in the disposal station and scrape pans there) and autoclave the contents in the bag. The pans are sprayed with quatricide (**viral agents only**) and allowed to sit for 10 minutes before placing them into the cage washer.

4. CHEMOTHERAPY DRUGS

4.1 Personal Protective Equipment (PPE)

•**Double nitrile gloves** •**Splash goggles** •**Water-proof Gown** •**Shoe Covers** •**Head Cover**

4.2 Housing: Standard rabbit caging with thick trash bags used to line the catch pans.

4.3 Quarantine period: Minimum 72 hours or up to 5 days, depending on recommendations of Biosafety Officer or Industrial Hygienist.

4.3.1 During quarantine, the entire cubicle is quarantined, a **quarantine sign is placed on the cubicle**, the sign should include the quarantine start date and the date the quarantine ends. Anyone entering the cubicle is required to wear Personal Protective Equipment (PPE). Treated rabbits may be quarantined in cubicles with untreated rabbits if needed. Following quarantine, only pans with bags will need to be handled as a biohazard. The cubicle will remain in quarantine until all bags have been removed.

4.4 Animal Husbandry

- 4.4.1. All hoppers are filled to capacity with rabbit food on the quarantine start date.
- 4.4.2 Rabbit pans are completely lined/covered with thick trash bags for urine and feces collection, the corncob bedding is placed in the pan on top of the bags.
- 4.4.3 Following the quarantine period (while wearing PPE), all bags are removed from pans and placed into a yellow chemo bag. Yellow bags are then placed into containers (labeled “BIOHAZARD chemotherapy drugs”) inside the walk-in carcass cooler for BFI to pick up.
- 4.4.4 If a rabbit pan becomes exposed/contaminated with feces and urine during the quarantine period you will need to scrape the feces or urine into a yellow bag and clean the pan with a **disposable towel and 10% dilute bleach solution**. After cleaning the pan, place the dirty towel into the yellow chemo bag with the feces and urine.
- 4.4.5 Mop the cubicle with 10% dilute bleach solution after each cage change, the dilute bleach is a neutralizing/deactivating agent for adriamycin (see MSDS in room 4A125 for additional information). Do not sweep the cubicle until it has been mopped, the cubicle may be swept after the floor has dried following mopping.
- 4.4.6 On completion, place disposable waterproof gown, mask, gloves and shoe covers into a clean yellow chemo bag. With an ungloved hand, remove safety glasses and wash with hot soapy water. Wear a fresh pair of gloves and only touch the outside of the yellow bags, remove the bag from the trashcan, tape it closed and place it into the chemotherapy drug container in the cooler.
- 4.4.7 The rabbit rack is placed on the dirty side hallway and each grate is sprayed with 10% diluter bleach solution to deactivate any drug that may be on the grate due to urine.
- 4.4.8 If a rabbit is found dead or has to be euthanized during the quarantine period, wear PPE and use a disposable absorbent pad under the rabbit to catch any urine during (and following) the euthanasia. Place the rabbit into a yellow bag and place the bag into the labeled chemo barrel in the cooler.

4.4.9 Technical Notes - Chemotherapy Administration: An indwelling intravenous catheter will usually be placed into the marginal ear vein of rabbits to deliver the chemotherapy, Adriamycin is a tissue toxic material and perivascular injection (leakage of agent out of the blood vessel into the surrounding tissue) will result in severe tissue necrosis. Heparin should not be used to flush catheters when using adriamycin (doxorubicin). SEE SOP # 10-001 (BSL 2 Laboratory) for information in biosafety storage and handling of chemotherapy drugs.

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Atlanta VAMC Veterinary Medical Unit

STANDARD OPERATING PROCEDURE

Animal Biosafety Level 2 (ABSL 2)

Species: Swine

Written by: Sandra Meyer RVT, LATG; Jim Mcneill, B.S., LATg
IACUC Approval: March 13, 2013
Last Revised: June 18, 2007
Approved by: Michael Fallon D.V.M. _____ (initials)

1. Purpose: To document and describe animal care and biosafety procedures when swine have been injected/infected with a viral vector; this includes non-replicating virus types unless otherwise specified.

2. Personnel: These procedures apply to **all research staff utilizing hazardous agents in the animal facility and animal husbandry staff who care for animals exposed to such agents.**

2.1 Responsible Personnel:

Prior to the initiation of any study using BSL 2 vectors or agents, it should be determined who will be responsible for cleaning the cages during quarantine. The investigator may request the animal caretaker (responsible for the care of the room housing the quarantined swine) to clean the cages during quarantine, otherwise the investigator/investigator staff is responsible for cleaning the cages during quarantine and will follow the approved cleaning procedure listed here.

3. INFECTIOUS/VIRAL VECTORS AND CELL TOXINS

3.1 Personal Protective Equipment (PPE)

•Latex or nitrile gloves •Face shield •Surgical Mask •Disposable Gown •Shoe Covers •Head Cover

3.2 Housing: Standard swine caging (elevated grate type stainless steel caging/animal runs).

3.3 Quarantine period:

Adenovirus (vector): 72 hours (PPE required during administration).

Streptozotocin (STZ-cell toxin): 24 hours following the last injection (PPE required during administration)

3.3.1 Quarantine: The entire animal housing room is quarantined (this will also apply when only one pig has been infected), and a **quarantine sign is placed on the room doors (clean side hallway door and dirty side hallway door)**. Anyone entering the room is required to wear Personal Protective Equipment (PPE). During quarantine all animal manipulations will take place inside the animal room whenever possible; if manipulations must take place outside the animal room, room 4A122 (recovery room) should be scheduled for use (see calendar schedule on room door). While performing animal manipulations in room 4A122 during the quarantine period, only key personnel (wearing PPE) should enter the room. All tabletops and counter tops in room 4A122 will be disinfected with quatricide and the floor will be mopped with quatricide immediately following the animal manipulation. The mop water will be poured down the floor drain (room 4A122) immediately following use.

3.4 Cage Disinfection: PPE required during cage cleaning. All items in the cage are considered potentially contaminated (food bowls, toys, floor grate, floor under grate, urine and feces). The entire run is sprayed with quatricide- including the food bowls and toys using a spray bottle filled with a pre-diluted concentration of quatricide (large barrel of pre-diluted quatricide for bottle refill in room 4A122).

3.5 Hosing/Rinsing Cage: Be sure quatricide siphon tube is inside the quatricide container; turn the hot water off (this will prevent mist aerosolization of particles that might occur if hot water is used during hosing). Hose away

any fecal matter or urine from the cage (and under the cage) into the drain behind the cages, be sure to rinse away all the quatricide previously sprayed on the cage, food bowls and toys. Place the food bowls into an **autoclavable** biohazard bag then seal the bag closed with autoclave tape, place the dirty bowl on the dirty side of the hallway for autoclaving.

4. Any (potentially contaminated) broken glassware used during the infection procedure must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Sharps containers of contaminated needles are autoclaved before placing the container into the cooler for pick –up (use autoclave tape to indicate that the sharps container has been autoclaved).

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STANDARD OPERATING PROCEDURE

Chemotherapy in Animals

Written by: Sandra Meyer, RVT, LATG
IACUC Approval: March 13, 2013
Last Revised: February 8, 2011
Approved by: Michael Fallon D.V.M. _____(initials)

1. Purpose: To describe chemotherapy safety procedures when chemotherapeutic (cancer-fighting) drugs are used in small animals. This SOP covers the following: administration, storage, dilution, disposal, spills, and post therapy animal care when chemotherapeutic drugs (that are known to be toxic to animals or humans and require special precautions to prevent accidental exposure) are used in animals.

2. Personnel: The following procedures apply to **all research staff utilizing chemotherapeutic drugs in the animal facility and animal husbandry staff caring for animals exposed to these agents. Any investigator using chemotherapeutic agents will provide the animal facility (Jim Mcneill 4A125) with amd MSDS for the agent.**

3. CHEMOTHERAPY DRUGS

Personal Protective Equipment (PPE) to be worn during administration and post therapy care of animals: double nitrile gloves, or double Talc-free gloves or gloves made specifically for chemotherapy administration , splash goggles (or face shield) water-proof gown •shoe covers •head cover

4. Personnel: High-risk individuals (i.e. immunosuppressed or pregnant) should be informed of the possible consequences of handling chemotherapeutic agents and given the option of avoiding any possible exposure by not working with animals that have received such agents.

5. Storage: Chemotherapeutic agents should be stored in a secure area in a separate location from other drugs. A locking refrigerator or cabinet is best. The manufacture's instructions should be followed carefully regarding storage temperature.

6. Spills: (See MSDS in room 4A125) Spill kits should be kept on hand. An accidental spill should be dealt with promptly and carefully (**use 10% dilute bleach solution to neutralize adriamycin/doxorubicin**). If your skin has been contaminated by the spill, wash thoroughly with soap and water, rinse well. If a drug has splashed into your eyes. Use an eye wash continuously for 15 minutes and report to employee health immediately. One person should be responsible for cleaning the area of the spill, **PPE should be used**. Paper toweling should be used to absorb fluid and the area should be washed with soap and water. All waste should be enclosed in a large heavy duty ziplock bag and disposed of appropriately (see disposal). Laundering contaminated clothing can expose staff members to chemotherapy agents.

7. **Special Concerns**

Vial Pressures: The dilution of some agents (like cisplatin) causes a significant pressure to be built up in the vial. The use of a venting device (chemo dispensing pin) should be considered unless all air is to be taken out. The venting device inserts into the rubber top of the drug vial. As diluent is added to the vial, pressure is released through a 0.2 micron, hydrophobic, air-venting filter on the side of the pin.

8. Dilution: Toxic drugs should be mixed in a fume hood or on top of the downdraft table in the necropsy room (4A132). Hands should be washed before and after mixing chemicals. Gloves are not a substitute for hand-washing. Always wear double nitrile gloves, or thicker gloves designed specifically for chemotherapy when handling any of these agents. Use luer-lock needles during dilution, do not fill syringes more than 2/3 full to avoid having the plunger separate from the barrel. If a fume hood is not available, drugs should be mixed on an absorbent plastic backed sheet in a low-traffic, draft free, well-ventilated area, and a respirator mask should be worn, respirator mask should be “fit tested” prior to use.

8.1 Adriamycin and Heparin: Heparin has been shown to precipitate adriamycin, the combination should not be used.

8.2 Cisplatin and aluminum: Cisplatin is inactivated in the presence of aluminum. Only plastic-hubbed needles should be used with this drug.

8.3 Oral chemotherapy tablets or capsules should not be cut or opened.

8.4 Administration:

8.4.1 Ensuring the correct dose is vital. It is important to check your math, a misplaced decimal point can result in a fatal overdose. Problems can also arise when the body surface area is derived from body weight in pounds instead of kilograms, or if the drug is reconstituted to the wrong concentration.

Dog: Doxorubicin $30\text{mg}/\text{m}^2 \times \text{_____m}^2 = \text{_____mg}$

Cat and Rabbit: Doxorubicin $20\text{mg}/\text{m}^2 \times \text{_____m}^2 = \text{_____mg}$

8.4.2 Both the administrator and animal restrainer should wear PPE during administration of any chemotherapeutic agent.

8.4.3 An intravenous catheter should be securely in place prior to administration, perivascular injection will result in severe tissue necrosis when administering vincristine sulfate and adriamycin. The catheter must be placed cleanly on the first stick, if not move to the next vein. Record the vein used for each chemotherapy administration in the animal record. Use luer-lock needles only and do not recap used needles, flush the catheter with **saline only** prior to drug administration and following administration to ensure that no drug remains within the catheter.

8.5 Adriamycin (Doxorubicin): Will cause severe tissue necrosis if it escapes from blood vessels during injection. It can also cause a severe anaphylactic-like reaction in some patients. Pretreatment with antihistamines (SC 15 minutes prior to treatment) is recommended if prior sensitivity is known. Do not flush catheter with heparin. **Administer over 20 minutes.**

8.5.1 **Emergency procedures:**

1. Stop drug infusion immediately.
2. Begin saline infusion at 20ml/kg/hr.
3. Administer Solu-Delta_Cortef 10mg/kg IV bolus.
4. Administer diphenhydramine 1mg/kg IV over 10 minutes.
5. Prepare epinephrine (1:1000) 0.5mg/m² IV

8.6 L-asparaginase: Subcutaneous administration is less painful than IM or IP administration. Can also cause an anaphylactic reaction in some patients. Pretreatment with antihistamines and corticosteroid may also be recommended. Do not administer intravenously.

8.7 Cisplatin and 5-fluorouracil: are fatal to cats.

- 9. Disposal:** To be environmentally responsible, chemotherapeutic waste should have a separate disposal area from routine trash or biohazardous waste. That includes empty vials, fluid lines, catheters, syringes, needles, gloves, or any item that could have residue of the toxic chemical. A puncture-proof container with a tightly-fitting lid and appropriately labeled can be used in the absence of a yellow (chemo) sharps container. Chemo sharps containers are stored inside the walk-in carcass cooler in the animal facility in between each use (not in your lab). Once the container is full, using a permanent marker write the word “full” on the container and leave it in the walk-in carcass cooler for disposal.

Chemotherapeutic waste should be placed into yellow chemo bags, **taped closed** and placed into the chemotherapy barrel/ container (labeled “BIOHAZARD Chemotherapy”) inside the walk-in carcass cooler (4th floor). Needles and other sharps should be placed into a yellow chemotherapy sharps container, label the container “full” and leave it in the carcass cooler for BFI to pick it up.

- 10. Post treatment animal care:** waste (feces, urine, rodent cage with bedding) from the animal is biohazardous for the first **72 hours following treatment.**

Place a biohazard card on each animal cage that has been treated with chemo agent (using the cage card holder) and place a “quarantine” sign on the cubicle. The quarantine sign should include the following:

- 1) The agent administered and a description of the toxic effects to humans.
- 2) The duration of the quarantine (e.g. the start time and date and end time and date).
- 3) The required PPE to enter the cubicle.
- 4) The method of handling the waste and animal carcass (i.e. placed into yellow bag, taped closed and placed on the shelf in the carcass cooler for 72 hours).
- 5) PI and staff contact information (e. g. name of person and office and cell phone number).
- 6) The name of the person responsible for cage handling or placing the cage into a yellow bag following quarantine.

Wear PPE when cleaning the animals’ soiled cage or entering a cubicle.

Wipe or mop soiled runs (dog or pig) with soap and water, do not hose runs, hosing can aerosolize metabolites in the urine or feces.

Soiled bedding and waste should be disposed of as chemotherapy waste in yellow bags and placed into the carcass cooler. Rodent cages: the cage with bedding (no animal carcass) is placed into a yellow chemo bag and placed on the shelf in the carcass cooler for 72 hours. Wearing PPE and after 72 hours the cage wash technician will dump the dirty bedding into a yellow chemo bag and wash the cages in the tunnel washer. The PI and PI staff are responsible for placing the rodent cage into a yellow bag and writing the date on the bag using a permanent marker following the end of the quarantine.

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STANDARD OPERATING PROCEDURE

Using Radioactive Tracers in Animals

Written by: Sandra Meyer, RVT, LATG ; Sean Riggins (Radiation Safety Officer)

IACUC Approval: March 13, 2013

Last Revised: February 8, 2011

Approved by: Michael Fallon D.V.M. _____(initials)

Animal research involving radioactive tracers is common in the biomedical research community. Each experiment brings its own difficulties and radiation safety challenges, but there are many common elements. We will review those elements in this section. There are four stages of animal studies - each with its own radiation safety concerns:

Injection

Housing

Euthanating

Clean-up and Waste Disposal

Injection

Special care and precautions need to be taken during this phase of animal studies – some unique to the use of radioactive materials, some not. Minimum PPE for this evolution will be protective gloves, lab coat or apron, and eye splash protection (preferably a face shield).

Syringes containing certain radioisotopes (e.g.; P-32, Fe-59, Tc-99) will be significant radiation sources. Syringe shields, dry-run practice, and using the smallest dose necessary are methods to lower the radiation dose that the extremities will receive. Ring badges will be worn on the syringe hand or hand expected to receive the greatest dose. As with any chemical used in animal studies, it is not a good idea to inject yourself! With radioisotopes this will result in an internal as well as an external exposure. It will also cause an exposure to a wound, which is not desirable. Extra care should be taken to prevent needle sticks. The researcher should be familiar with injecting animals with non-radioactive substances before attempting an injection using radioisotope. Proper absorbent material must be laid out before injection to capture any radioactive spills, blood drops, or urine/feces spills that may occur. When clearing the needle, DO NOT spray the isotope into the air! If this needs to be performed, liquid will be directed into an absorbent or appropriate receptacle. Radioactive trash receptacles for dry, biological, and sharps waste should be positioned as close to the injection area as possible. These should be labeled with appropriate

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radioactive and biohazard labels (as applicable) and prepared to receive waste. Proper container inventories will be maintained to ensure the radioisotope and quantity of radioisotope in the container is recorded. **DO NOT OVERFILL CONTAINERS – ESPECIALLY SHARPS CONTAINERS!**

If the radioisotope is volatile, or likely to become airborne, approved ventilation will be used. Consideration should be made and preparatory actions taken if the radioisotope will be exhaled by the animal in significant quantities. The room should be properly posted and controlled for radioactive material work. Spill clean-up supplies should be readily available and their location known by all personnel involved. The area should be isolated and cages available to ensure no radioactive animal can escape and become a moving spill!

Housing

From a radiation safety standpoint this is probably the riskiest time during an animal study. There is a radioactive source that can be mobile, that may be able to inject (through biting) radioactive material into researchers, that can contaminate areas outside of contamination boundaries with urine, feces, or saliva, and may even be creating radioactive airborne contamination just by breathing! Obviously, precautions have to be taken. Minimum PPE for this evolution will be protective gloves, lab coat or apron. The animals will be isolated from the general population room and housed in a cubicle and the cubicle will be posted with a "Radioactive Materials" label and will be locked or guarded at all times.

The cages will be labeled with radioactive material labeling tape and the radioisotope and amount shall be posted on the cage. Cages will be lined with absorbent material and will be constructed such that material cannot be spilled from the cage by the animal. Bedding, food, urine, and feces that fall from the cage will be considered radioactively contaminated. If the cages themselves cannot ensure containment, then the area surrounding the cages will need to be controlled and covered with appropriate absorbent material. Distinct borders will be established and marked with radioactive material tape to denote potentially contaminated areas. Appropriate anti-contamination clothing will need to be worn when coming in contact with these areas (e.g. gloves, shoe covers, lab coats, etc.).

Spill clean-up supplies should be readily available and their location known by all personnel involved. Controlled ventilation will be evaluated if the possibility exists for airborne radioactive contamination. An example of this evaluation would be if the airborne radioactivity is in the form of exhaled CO₂. Prior to work, the room would be verified to be at negative pressure with respect to the outside areas, the ventilation rates for the room would be determined, the expired activity estimated, and, finally, the air concentrations calculated and compared to allowable limits for personnel exposure and discharge to the environment. This evaluation would determine if controlled ventilation is required or not. Only personnel who have radiation safety training specific to the activity they will perform will be allowed to handle animals that have been administered radioactive materials or the cages or bedding for these animals.

If the bedding or water pouch is to be changed during housing of the animals, it will be packaged as radioactive material and kept for Radiation Safety disposal. No potentially contaminated material (cages, feed trays, wirebars etc.) may leave the controlled area/cubicle without first being surveyed to ensure it is free from radioactive contamination. This survey will be performed by Radiation Safety personnel.

Euthanatizing

It is sometimes necessary to sacrifice animals at the end of, or as part of, an experiment. If these animals contain radioactive material they will need to be controlled and disposed of properly.

Minimum PPE will be protective gloves, lab coat or apron. All radioactively contaminated samples taken from the animal will be controlled under the rules and regulations regarding radioactive material. Radioactive carcasses and biological material will be bagged or packaged in leak-proof packaging that is labeled with radioactive material marking tape, labels, or tags. The radioisotope and the quantity of the radioactive material will be noted on the package.

Radioactive carcasses and biological material will be frozen and lime should be added to bags containing carcasses or biological material that is awaiting disposal, especially if the possibility exists for the material to thaw before disposal (loss of power with no back-up, aging freezer, etc.).

Clean-Up and Waste Disposal

Everyone's favorite part of an experiment is clean-up! All through the experiment you anxiously await that special time where you get to scrub work benches, decontaminate cages and instruments, and package various smelly items. So, are there any radiation safety considerations for this stage of animal studies? You bet there are. . . Minimum PPE for this evolution will be protective gloves, lab coat or apron, and eye splash protection (preferably a face shield). More protection may be needed. Evaluate PPE thoroughly prior to beginning clean-up. If in doubt, contact the Radiation Safety Officer for assistance with protective equipment selection.

As stated above, radioactive carcasses and biological material will be bagged or packaged in leak-proof packaging that is labeled with radioactive material marking tape, labels, or tags. The radioisotope and the quantity of the radioactive material will be noted on the package. Radioactive carcasses and biological material will be frozen while awaiting disposal. Lime should be added to bags containing carcasses or biological material, especially if the possibility exists for the material to thaw before disposal (loss of power with no back-up, aging freezer, etc.). Bedding will be carefully poured into radioactive waste bags and the bags sealed for pick-up by radiation safety.

Cages will be wiped down with damp rags and mild detergent. All cleaning waste will be bagged as radioactive. Radioactive labeling will not be removed until radiation safety has surveyed the cages and verified that they are clean (free of radioactive contamination). Absorbent paper will be carefully folded into itself and disposed of as dry, radioactive waste. Sharps containers will be closed and labeled with the name of the isotope and an estimate of radioactive material quantity. All instruments should be wiped down with mild soap on damp rags similar to the decontamination of the cages. Special care should be taken to avoid injury if sharp instruments will be decontaminated. If the instruments are to be released from radiological controls, they should be set aside to await radiation safety surveys. If they are to be kept as radioactive material use only instruments, they should be marked with radioactive material tape and stored in a controlled area. Once all waste is packaged and all areas wiped-down, post-work wipe surveys should be taken. These surveys should be entered in the laboratory's Radiation Safety Log Book. Areas found to be contaminated should be decontaminated with mild soap. If decontamination is unsuccessful, bag the item or cover the area and notify Radiation Safety. Update survey records, inventory records, and waste records. Once surveys are complete, schedule a waste pick-up and a time to release equipment with Radiation Safety.

RADIOACTIVE TRACERS IN RODENTS: SEE VMU SOP #11-006 FOR SPECIFIC ANIMAL AND CAGE HANDLING PROCEDURES

END OF DOCUMENT

Atlanta VAMC Veterinary Medical Unit
STANDARD OPERATING PROCEDURE
Transfer of Laboratory Animals

Written by: Sandra Meyer RVT, LATG
 IACUC Approval: November 6, 2013
 Last Revised: June 5, 2013
 Approved by: Michael Fallon D.V.M. _____(initials)

- 1. Purpose:** To document and describe consistent and safe techniques for the transfer of laboratory animals between the VAMC Veterinary Medical Unit and other institutions.
- 2. Commercial Animal Transport Services**
 - 2.1** The VMU uses Marken and AirNet for shipping animals. Marken is used during optimal outdoor temperatures 50° - 75°F and AirNet is used when temperatures are below 50°F or above 75°F. Temperature consideration is given to both the sending institution outdoor temperature and the receiving institutions outdoor temperature. Institutions sending animals to the VA must also use AirNet to send animals whenever outdoor temperatures are not optimal.
- 3. Procedures for transferring animals out of the VA**
 - 3.1** The process of an animal transfer must be initiated by submitting an Outgoing Animal Transfer Form to the VMU transfer coordinator. Request a transfer form from the VMU veterinary staff or go online to the AREF website at <http://www.atlaref.org/index.cfm> select "Research Submissions" and click on "Veterinary Medical Unit" then click "Policies and Procedures." Failure to fill out the form completely may result in delays.
 - 3.2** Before submitting this form to the VMU, please determine (with the receiving party) who will pay the cost of shipping. The VMU has an account with Marken (commercial shipping service) and prefers to use Marken in situations where the VMU can recover the cost of shipping from the VA PI (billed to the VA PI protocol). Billable shipping charges include processing, local transportation, commercial shipping charges, shipping crates and optional crating by the VMU Veterinary examinations of animals and preparation of health certificates are not billed.
 - 3.3** Receiving party is paying the cost of shipping: receiving party should arrange for the VMU to use the receiving parties account with a commercial shipping service. Please note: if a commercial shipper cannot provide a vehicle equipped to handle environmental extremes (equipped with heating and air-conditioning), animals will only be permitted to transfer to other facilities during optimal/acceptable outdoor temperatures. The acceptable/optimal outdoor temperature range is 50°-75° F, this includes the Atlanta temperature and temperatures that will be encountered in the vehicle along the entire traveling distance to the final destination. To prevent delays in transferring animals receiving parties paying for the cost of shipping should use a commercial shipping service that provides a vehicle equipped with heat and air-conditioning (Marken 1-800 932-6755 or AirNet 1888-888-8463 dial 1).
 - 3.4** The cages containing the animals to be shipped should be clearly marked/identified (bright piece of tape or marker, etc.) so that the veterinary staff can find, observe and , if necessary, crate them.
 - 3.5** When the VMU has received a completed Transfer Form, the animals will be examined by the veterinary staff and a health report will be prepared (allow 48-72 hours).
 - 3.6** The VMU transfer coordinator will contact the veterinary staff at the receiving institution to inform them of the health status of the animals, ensure that the receiving institution is prepared for the transfer, and the receiving investigator is approved to use them (allow an additional 24-48 hours).
 - 3.7** Once the receiving institution has approved the transfer, the VMU will coordinate shipment with the receiving institution. Non-rodent animals must be crated by VMU staff or Emory drivers. Rodents may be crated by the VMU upon request (for a fee) or by the investigator or their designee.

Continued next page→

4. Species Specific Shipping Crate Contents:

4.1 Rodents: Use a plastic Jackson or Taconic filtered crates and secure crates with packing tape, provide corncob bedding or shavings, gel pack and rodent chow inside the crate.

4.2 VMU Veterinary Staff must crate the following species for transport:

Rabbits, Cats, Dogs, Pigs: Provide bedding/newspaper if transport time (or total time in crate) will exceed 4 hours, provide water if transport time (or total time in crate) exceeds 12 hours, provide food if transport time (or total time in crate) exceeds 24 hours. Ask for and follow any written instructions provided by the company transporting the animals.

Rabbits: One rabbit per crate, use a filtered rabbit crate (Covance) secured well with packing tape.

Cats: One cat per crate, use plastic pet carrier and ensure all locking wings and the door(s) are all secure. Do not use carriers if hardware (locking wings, etc.) is missing or the cat could escape.

Dogs: One dog per crate, use medium to large plastic kennel cab and ensure all kennel hardware is secure. Place newspaper or shavings in the kennel, dogs tend to urinate even if it is a short trip.

Pigs: One – two pigs per crate (pigs must be able to stand and turn around freely in crate. Use the large or extra large kennel cab and ensure all kennel hardware is secure.

5. Animal Identification Required for Transfer:

5.1 Rodent: Rats and mice can be identified through ear notching, ear tagging or by removing each cage card from the cage and attaching the card to the outside of the filtered crate (directly over the compartment the rodent has been placed in). The date of birth should be included on each cage card. Permanent individual identification of rodents is recommended when animals of different genotypes are to be shipped.

5.2 Rabbit: Upon request rabbits receive an ear tattoo from the vendor prior to shipment to the VA.

5.3 Feline: Cats must have a permanent method of identification before transporting. Cats receive an ear tattoo from the vendor prior to shipment to the VA. The VMU can tattoo the ID number on the ventral side of the right ear for any cat that may not have received a tattoo from the vendor.

5.4 Swine: Pigs arrive from the vendor with an ID number on the ear tag.

5.5 Canine: Dogs must have a permanent method of identification before transporting. Dogs typically arrive from our current vendor with a USDA tag on their collar and have been assigned an ID number (written on the paperwork). Following arrival, the VMU removes the collar and tattoos the ID number on the ventral side of the right ear.

6. Paperwork:

6.1 Medical Record: A copy (not original) of any existing animal medical record is provided to the driver of the transportation vehicle. The driver will give the copy of medical record to the facility in which the animal is to be transferred.

6.2 USDA /Record of Disposition of Dogs and Cats: A completed USDA form must be provided to the driver. USDA forms are completed by veterinary staff upon receiving the completed “Animal Transfer Form.”

7. Anesthetics, Medications or Special Diets:

7.1 Any animal medications or special diets should be given to the VMU veterinary staff with written dosage or feeding instructions (scheduled drugs are prohibited from transport with the animal). The VMU veterinary staff will give the medications and/or special diets to the driver and the driver will give the medications and instructions to the veterinary staff within the facility in which the animal is to be transferred. VMU veterinary staff will call the facility prior to the transfer and make arrangements for medications to be administered by veterinary staff.

7.2 The PI and VMU veterinary staff initiating the transport will notify receiving staff if an animal received anesthetic or tranquilizing drugs prior to transport.

8. Emory DAR Vehicle Usage:

- 8.1 There is no transportation/shipping cost to transfer animals from the VA to Emory using the Emory DAR vehicle.
- 8.2 Upon request (by the VMU), the Emory DAR vehicle can provide transportation of animals to Yerkes Primate Center. There is an Emory transportation fee for service from the VA to Yerkes, this fee is initially paid by the VMU and billed back to the VA PI's protocol. The Emory DAR vehicle is not available for the transport of animals from the VA to Yerkes on Monday or Tuesday of each week.

9. Personal Vehicle Usage for Rodent Transportation to Yerkes:

- 9.1 IACUC approval and ACORP documentation is required to use personal vehicles to transport rodents to Yerkes. Include the intention to use personal vehicles for transport when writing protocols for IACUC approval or submit a modification to an existing protocol for this purposes.
- 9.2. Following IACUC approval, PI's may transport rodents in personal vehicles under the following conditions:
 - 9.2.1 The animals are crated appropriately (see above) and discreetly (covered with yellow gown) transported down the service elevators (back 4th floor hallway) and loaded into vehicles near the loading docks on the "Basement" level.
 - 9.2.2 The gown is used to cover the seat in the vehicle and the animal crate is placed inside the vehicle on top of the gown. Do not place rodents into the trunk of the vehicle. Rodents should be transported in ambient temperatures inside the vehicle –this may include the use of heating or air-conditioning inside the vehicle. Do not stack crates on top of each other unless the crate has filtered sides.
 - 9.2.3 Coordinate/schedule the rodent arrival time through the Yerkes contact person. Do not just "show up" with rodents.
 - 9.2.4 Use the Yerkes entrance near the MRI facilities unless otherwise specified by the Yerkes contact person. The contact person should meet you at the door. Use the log in book in the MRI facilities to sign in, include protocol number and animal species.

10. Procedure for transferring animals into the VA from other institutions (non-vendors)

- 10.1 Request an Incoming Animal Transfer Form from Sandra Meyer at sandra.meyer@va.gov
- 10.2 Email the form to the PI planning to send the animals with instructions for the PI to complete the form and email it to Sandra Meyer AND the transfer coordinator at the institution sending the animals.
- 10.3 The transfer coordinator or veterinarian will email the requested health information to Sandra Meyer for review.
- 10.4 If there has been any virus or parasite reported in the transferring facility in the last 12 months the animals will need to be tested for the virus or parasite and test negative before the transfer can be approved.

End of Document

SOP # 13-001 (Page 1 of 1)
Atlanta VAMC Veterinary Medical Unit

STANDARD OPERATING PROCEDURE
Rodent Tail Biopsy/Snipping

Written by: Sandra Meyer RVT, LATG
IACUC Approval: March 13, 2013
Last Revised: June 18, 2007
Approved by: Michael Fallon D.V.M. _____(initials)

1. **Purpose of Tail Biopsy:** For the production of genetically altered rodents, it is often necessary to sample tissue for DNA analysis. Usually, the end of the tail is sampled. The following policy will ensure the humane sampling of tissue for biopsy from rodents. Use PCR vs. Southern blot for genotyping, whenever possible, this reduces the tissue requirement and permits using ear punches and saliva for genotyping.
2. In the mouse, the terminal tail ossifies between 2 and 4 weeks of age. Thus, tail sampling is recommended in mice less than three weeks of age. In mice < 12 days the distal tail biopsy may be performed without anesthesia. Animals >12 days must be anesthetized with a short acting anesthetic (e.g., isoflurane or local anesthetic/tail numbing).
3. Sampling must be performed using sharp, sterile scalpel blades. If tail biopsies are performed on multiple mice, instruments must be disinfected appropriately between animals, and it is recommended that the use of each blade be limited to no more than 5 times. The smallest possible section of tail should be removed and adequate hemostasis must be achieved via a styptic (e.g., silver nitrate, cautery, tissue adhesive, etc.). **It is recommended that tail samples be limited to no more than 0.5 cm of tissue.**

4. **Alternatives:**

The following alternatives to tail biopsies/snipping should be considered:

- 4.1 Tissue can be obtained by ear punching which can also serve as identification tissue in many genotyping kits. Sigma-Aldrich have a PCR kit called XNAT-1kt and reagents may be replaced/reordered and does not require you to order the entire kit.
- 4.2 Small quantities of blood from distal veins (e.g., saphenous vein) may be used for analysis.
- 4.3 PCR analyses using saliva and hair have also been described among others. Genra Systems and Sigma each have DNA kits for whole blood; Genra Systems has a salivary DNA kit.

5. **References:**

Irwin, M.H.; Mofatt, R.J.; Pinkert, C.A. Identification of Transgenic Mice by PCR Analysis of Saliva. *Nature Biotechnology* (1996) 14, 1146-1148.

Schmitteckert, E.M.; Prokop, C.; Hedrich, H.J. DNA Detection in Hair of Transgenic Mice—A Simple Technique Minimizing the Distress on the Animals. *Laboratory Animals* (1999) 33(4), 385-389.

Zimmermann, K; Schwarz, H.P.; Turecek, P.L. Deoxyribonucleic Acid Preparation in Polymerase Chain Reaction Genotyping of Transgenic Mice. *Comparative Medicine* (2000) 50(3), 314-316.

Pinkert, CA. Transgenic Animal Technology: Alternative in Genotyping and Phenotyping. *Comparative Medicine* (2003) 53(2): 126-139.

End of Document

STANDARD OPERATING PROCEDURE

Waste Disposal

Written by: Brent Swenson DVM; Jim McNeill, B.S., RLATG
IACUC Approval: March 12, 2013
Last Revised: June 6, 2012
Approved by: Michael Fallon D.V.M. _____(initials)

Purpose: To document and describe animal facility waste disposal procedures.

- 1. Background:** Accumulated waste from the animal facility requires proper collection and disposal so as to maintain a sanitary environment for the animals, while minimizing exposure of personnel or the community to potentially hazardous materials. Animal facility waste will be categorized according to risk and disposal methods and each risk category will be described.
- 2. Responsible Personnel:** All research and support personnel who generate waste in the animal facility are responsible for proper disposal of waste they have generated. Disposal of waste generated in animal husbandry will be the responsibility of animal care personnel assigned to the area, with supervisory oversight assigned to the facility manager or his designee.
- 3. Waste Classification:** Waste in the VAMC animal facility will be classified according to level of hazard. Unless otherwise instructed, in writing, all waste from a given animal room will be treated as the highest risk category for any waste used in that room. For example, if one cubicle generates Category 3 waste, waste from all cubicles in the room will be treated as Category 3 waste.
 - 3.1 Category 1** waste is non-hazardous, recyclable waste, such as white paper, plastics or aluminum cans.
 - 3.2 Category 2** waste is non-hazardous, non-recyclable waste and includes unconsumed feed, feces, used bedding that is NOT contaminated with hazardous chemicals, radioactive materials or Biosafety Level (BSL) 2 or 3 agents. The majority of waste generated in the animal facility is expected to be Category 2 waste. Category 2 sharps will constitute a subcategory requiring different handling.
 - 3.3 Category 3** waste is potentially hazardous waste generated from animal rooms maintained under BSL 2 or greater conditions, including contaminated carcasses. Category 3 sharps will constitute a subcategory requiring different handling.
 - 3.4 Category 4** waste is contaminated or uncontaminated animal carcasses.
 - 3.5 Category 5** waste comprises waste contaminated or potentially contaminated with hazardous chemicals, radiation, or infectious agents classified at BSL 2 or above, and/or for which special precautions have been imposed by the Medical Center Health and Safety Personnel.
- 4. Waste Identification Procedures:**
 - 4.1** Category 1 (recyclable) waste will be labeled as prescribed by Medical Center policies.

- 4.2 The default category for waste will be Category 2. Except for animal carcasses and unless posted otherwise, waste from all rooms may be handled as Category 2 waste.
- 4.3 All rooms maintained under BSL 2 or greater conditions will be labeled as such on the door or cubicle using universal symbols and standard signage, to include the name of the hazardous agent, the species in which it is being used and the names and phone numbers of persons who may be contacted in case of questions. Waste from **all** such areas will be classified as Category 3 waste.
- 4.4 Any room in which Category 5 waste is generated will be labeled as such on the door or cubicle in block print at least 1" high. In addition, specific precautions to be observed will be posted on the door, typed in English, and will include the names and phone numbers of persons who may be contacted for instructions in case of questions. **All** waste from such rooms will be handled as Category 5 waste. Category 5 waste will be placed in labeled boxes provided by stericycle. Appropriate labeling such as yellow bags, chemotherapy waste labels, and chemotherapy waste bar code labels will be placed on boxes.
- 4.5 Gray plastic containers (TB02) will be provided for Category 3 and 4 waste. A "Regulated Medical Waste" bar code and yellow "Incineration Only" sticker must be affixed to the outside of each gray plastic container. This will route the containers for incineration at the contractor's stericycle facility. Medical waste will be placed in DOT (Department of Transportation) approved red biohazard bags and sealed with tape. Plastic ties are secured on both sides of the gray (TB02) container.

5. Collection Procedures:

- 5.1 Category 1 waste will be collected into receptacles and by means prescribed by current Medical Center policy.
- 5.2 Category 2 waste will be collected into clear plastic bags. Used bedding will be dumped into clear plastic bags in the dirty-side of the cagewash area, using one of the ventilated dump stations. Category 2 sharps will be deposited in puncture-resistant containers designed for sharps disposal immediately after use in the area where they were used. Under no circumstances should sharps be discarded into plastic bags.
- 5.3 Category 3 waste will be collected into **doubled red or orange** bags labeled with the universal biohazard symbol and the bag will be secured at the open end. The bags will be placed in red plastic Medical Center provided red container carts for autoclaving by the Medical Center and then disposed of by the Medical Center Waste Management, Inc. (waste contractor).
 - 5.3.1 Waste originating from a BSL 3 facility will be autoclaved using the BSL3 facility autoclave. We currently have no BSL3 facility.
 - 5.3.2 Waste originating from BSL 2 rooms or cubicles will be autoclaved in the Medical Center autoclave in the basement after being transported in VAMC red plastic containers.

5.3.3 Category 3 sharps will be deposited in puncture-resistant containers designed for sharps disposal. When filled, these containers will be sealed and autoclaved (using an appropriate autoclave as designated above) prior to transport.

5.4 Category 4 waste will be placed into the approved DOT biohazard bags and sealed with tape. Prior to transport for disposal, the bags will be placed in gray plastic containers (TB #2) specifically designated for biohazard disposal. Carcasses weighing more than 150 pounds (per DOT regulations) will be dismembered, bagged and deposited in multiple containers as necessary.

5.5 Category 5 waste will be collected in yellow bags boxed, labeled and picked up by Waste Management Inc. for disposal. Yellow plastic barrels containing the yellow plastic bags and sharp containers will be held in the walk-in cooler until pick up by Waste Management, Inc (Medical Center vendor)

6. Disposal Procedures:

6.1 Category 1 waste will be transported to central collection receptacles designated for recycling by the Medical Center.

6.2 Category 2 waste will be transported using the service elevator to the Medical Center basement and deposited in the Sani-Pak compactor. Containers holding Category 2 sharps will be sealed when filled, transported to the Medical Center Sani-Pak where they will be autoclaved and disposed as Category 3 waste.

6.3 Category 3 waste will be transported in red container carts to the Medical Center basement where they are autoclaved before pick-up by a Waste Management Inc. (disposal contractor).

6.4 Category 4 waste will be stored in the walk-in cooler (4A132A) until pick-up.

6.4.1 Pick-up is Tuesday mornings. Gray TB02 containers must be loaded before the end of the workday prior to pick-up (Monday), and taken to the loading dock by 08:30 the morning of the scheduled pick-up (Tuesday). **Pick-up is scheduled every 4 weeks on Tuesday.**

6.4.2 The animal facility supervisor or associate supervisor must check each gray container (TB02) for proper packaging.

6.4.3 The animal facility supervisor or associate supervisor must sign for each pick-up at loading dock B, or a signed hazardous material document form may be taped to the side of the gray containers.

6.4.4 A hazardous material shipping document receipt for each pick-up will be given to the supervisor or assoc. supervisor by the disposal contractor. These forms must be returned to the animal facility supervisor or associate supervisor. A copy of each receipt is stored in a binder in 4A125 and available to Mr. Jeff Jones, Industrial Hygienist, Safety Office, ext 6115, pager 404-628-1719

6.5 Supplies including containers. Bags, labels, and tape may be obtained from Stericycle at (404) 462-9090

STANDARD OPERATING PROCEDURE

IACUC Policy for Non-Pharmaceutical Grade Drugs

Written by: Sandra Meyer RVT, LATG
IACUC Approval: August 14, 2013
Last Revised: August 5, 2013
Approved by: Michael Fallon D.V.M. _____(initials)

1. Policy:

The Atlanta VA Medical Center follows guidance provided by the PHS Office of Laboratory Animal Welfare (OLAW) which states:

OLAW and USDA consider that the use of non-pharmaceutical grade compounds should be based on : scientific necessity: no availability of an acceptable veterinary or human pharmaceutical-grade compound: and specific review and approval by the IACUC.

Investigators are expected to use pharmaceutical-grade medications whenever they are available, even in non-survival procedures. Non-pharmaceutical grade chemical compounds, whether for experimental or veterinary use, may only be used after specific review and approval by the IACUC, based upon the combination of scientific necessity an non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings alone do not adequately justify the use of non-pharmaceutical-grade compounds in animal.

2. Definitions of Key Terms Specific to the Policy

- a) Pharmaceutical grade refers to a standard or level of purity suitable for the production of medicine within the pharmaceutical community.
- b) Non-pharmaceutical grade agents refer to chemical compounds that have not been formulated for production of medicine.

3. Applicability

This policy applies to all VA Research related animal activities that fall under the IACUC's jurisdiction.

End of Document

STANDARD OPERATING PROCEDURE**IACUC Policy for the Use of Injectable Multidose Drugs**

Written by: Sandra Meyer RVT, LATG
IACUC Approval: Pending
Last Revised: March 11, 2014
Approved by: Michael Fallon D.V.M. _____(initials)

1. **PURPOSE:** To describe the IACUC's policy, responsibilities and procedures for the use of injectable multidose (combined) drugs.
2. **POLICY:** The proper reconstitution, storage and use of pharmaceutical and non-pharmaceutical agents used in animals are essential to the maintenance of animal welfare. The VA IACUC requires adherence to the guidelines outlined below to ensure compliance with both government and manufacturer recommendations while addressing the realities of drug use in animal research. Deviations from or modifications to these guidelines must be requested of, and approved by, the IACUC.
3. **Definitions of Key Terms Specific to this Policy:**
 - a. Pharmaceutical grade is a term referring to a standard or level of purity suitable for the production of medicine within the pharmaceutical community.
 - b. Non-pharmaceutical grade agents refer to chemical compounds that have not been formulated for production of medicine.
 - c. USP (United States Pharmacopeia) is an official public standards-setting authority for all prescription and over-the-counter medicines and other health care products manufactured or sold in the United States.
4. **Applicability:** This policy applies to all Atlanta VA research related animal activities that fall under the IACUC's jurisdiction.
5. **Guidelines:**
 - A. **General**
 1. Multiple-dose injectable drugs vials and injectable fluids bags should be examined prior to use for evidence of physical or chemical contamination.
 2. Vials or fluids bags that have the following characteristics are considered contaminated and are not to be used:
 - Contain particulate matter, precipitates, turbidity, or discoloration
 - Mislabeled
 - Noticeable coring (damage to the rubber stopper)
 3. Drugs must be stored according to manufacturer's recommendations.
 4. For refrigerated drugs, after the dose to be used is removed from the vial/bag, it should be allowed to reach room temperature prior to injection.

B. Withdrawal of drug from vials and fluids bags

1. The top of the vial or the injection port for fluids bags should be swabbed with an alcohol prep pad prior to each use.
2. The desired quantity should be withdrawn using a sterile needle and syringe.
3. At no time will the vial be reentered with a needle/syringe which has been previously used.

C. Transferring a drug to another container

1. If a drug is to be transferred from the original vial and stored in another vial (e.g. diluting or mixing with another drug):
 - All needles/syringes, vials/containers and fluids used for dilution must be sterile.
 - If the drug is to be diluted and stored for more than 24 hours, it must be diluted with a sterile fluid **containing a preservative (e.g., bacteriostatic water).**
 - The vial must be labeled with:
 - a. Name of the drug
 - b. Concentration of the drug
 - c. Date of expiration (copied from the original vial)
 - d. Initials of the person who transferred the drug
 - In the case where two or more drugs are combined with different expiration dates, the earliest expiration date will be used.
 - All containers that the drug will be transferred to must have sealed rubber stoppers.
 - If the original container does not have a rubber stopper, the transfer must be done in a laminar flow hood or biosafety cabinet to ensure sterility.

D. Expiration

1. Uncontaminated multi-dose vials must be stored according to manufacturer's recommendations and can be used up to the manufacturer's expiration date provided that:
 - They show no signs of contamination
2. For reconstituted pharmaceutical grade drugs, the drug must be discarded at the time recommended by the manufacturer.
 - The vial must be labeled with:
 - a. Drug name, concentration or activity, and expiration date**
 - b. Initials of the person who reconstituted the drug
 - c. Date of reconstitution
3. For reconstituted non-pharmaceutical grade drugs, the following guidelines should be followed:
 - a. Must be prepared to United States Pharmacopeia standards for sterility.**
 - b. Initials of the person who reconstituted the drug
 - c. Date of reconstitution
 - d. Solutions derived from non-sterile components must be filtered into sterile, sealed containers. A very viscous product may require a 0.45 µm filter, but this increases the chance of improper sterilization and may require verification of sterility.
 - e. Date of expiration: Unless indisputable efficacy and quality assurance data can be provided that substantiates a more generous expiration date, the following requirements apply:

- The drug may be given for up to 24 hours after reconstitution for any of the approved routes of administration of the drug- i.e. intravenously (IV), intraperitoneally (IP), subcutaneously (SC), intramuscularly (IM) or intracranially (IC)
 - The drug may be stored in a refrigerator or freezer for up to 30 days if the vial is sterile and has a sealed rubber stopper. Stored drug may only be used for SC injection. It cannot be used for IP, IV, IM or IC injection. **It may not be used for immunocompromised animals after 24 hours.**
4. For single-dose drug vials (without preservatives), the following guidelines for expiration should be followed:
- The drug may be given for up to 24 hours after opening for any of the approved routes of administration of the drug - i.e. IV, IP, SC, IC or IM
 - Providing it has been labeled with the date the container was first used, the drug may be stored in a refrigerator for up to 30 days if the vial has a sealed rubber stopper or is sterilely transferred to a vial with a sealed rubber stopper. This drug may only be used for SC injection. It cannot be used for IP, IV, IM or IC injection. It may not be used for immunocompromised animals after 24 hours.
5. For injectable fluid bags (Lactated Ringers, Sodium Chloride, etc.), the following guidelines for expiration should be followed:
- The fluids may be given for up to 24 hours, after first use, for any approved route of administration.
 - Providing it has been labeled with the date the bag was first used, the fluid bag may be stored in a refrigerator for up to 30 days if it has a sealed rubber stopper. This drug may only be used for SC injection. It cannot be used for IP, IV, IM or IC injection. It may not be used for immunocompromised animals after 24 hours. However, we recommend strongly to not keep fluid bags more than seven days.

Note: Terminal procedures under anesthesia may be considered for exception to these guidelines

E. Suggestions:

- Please note that control drugs must be disposed according to VMU Controlled Substance Policy (SOP 16-002).
- Please, consult the IACUC policy for use of nonpharmaceutical grade drugs (SOP 16-001)
- The use of smaller injectable fluids bags appropriate to the immediate need is recommended to facilitate the implementation of this policy.
- Consider using the “MicroClave Vial Adapter” from Abbott (<http://www.abbottanimalhealth.com/>) which allows needle free aspiration or mixing of drug solutions in drug vials. Some benefits of that system are:
 - Reduces risk of contamination to vial and drug due to repeat needle sticks to the seal.
 - Prevents leaking due to repeat needle sticks to the seal, reducing loss of medications.
 - Reduces risk of accidental needle stick exposure to staff.

End of document

VMU Radio Policy

According to the Guide for the Care and Use of Laboratory Animals, Radios, alarms, and other sound generators should not be used in animal rooms unless they are parts of an approved protocol or an enrichment program. Therefore, radios or any sound making device is not allowed in animal housing rooms unless approved by the IACUC as a part of an animal protocol or enrichment program. Anyone that would like to listen to music inside the animal room can do so using ear buds connected to a hand held audio player.

In addition, music played in rooms not housing animals must be at a volume that does not disturb animals in other rooms or the concentration of other people working in the area.