UNIVERSITY of WASHINGTON

Animal Use Training Session
Mouse Lab Handout*

*This document is updated on an annual basis. In the interest of your research please contact the AUTS program to ensure you have a current handout.

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ANIMAL USE TRAINING SESSION: MOUSE

1. Introduction:

This handout is designed as a supplement to the information provided in the Mouse Animal Use Training Session. Remember that in order to perform any of the techniques described in this handout all personnel and the experimental procedures being done must be on an approved Institutional Animal Care and Use Committee (IACUC) protocol. Where noted, some of the techniques in this handout require certification by a qualified individual designated by the Attending Veterinarian (AV). Please be aware, other training might be required. Contact the Animal Use Training Session (AUTS) Program for training requirements.

1.1 Practicing Techniques:

You are not allowed to train/practice on your own animals unless 1) you have training approved in your protocol or 2) the animals are directly being used in an experiment. Example: You are NOT allowed to practice on animals because you are culling your colony unless it is in your protocol. Utilize the training program for your training needs. If you are trained in your lab, it must be documented. If it is a certifiable technique, the training must be performed by an individual designated by the AV.

1.2 Preparing for Class and Laboratory Work:

It is important to prepare yourself, work space, and animals before you start working. Laboratory work can be stressful and you are often on your feet. Wear comfortable closed toe shoes, lightweight comfortable clothing, eat a healthy meal, and stay hydrated. Have your work space set up to limit repetitive and uncomfortable movement. Ensure your animals are healthy before you start work and know how to contact Veterinary Services if you have any issues. Always give yourself extra time to finish your work; rushing can lead to mistakes and injured animals.

1.2.1 Preparing for your Hands-on Class:

1. Read the handouts included with the reminder email before coming to class.
2. Know what specific techniques (if any) you need to learn.
3. Wear lightweight comfortable clothing because you will be wearing a surgical gown.
4. Wear comfortable shoes because you will be standing for 1 or more hours.
5. There may be a potential for exposure to animal allergens, so come to class prepared if you have allergies (e.g., bring a mask).
6. Eat a meal and drink water before coming to class. Sometimes class can be stressful, particularly if you are new to working with lab animals or are not comfortable with needles and get squeamish around blood. During times of stress, one can get light headed and faint. Eating a meal will help prevent fainting during a stressful situation.

1.3 Course Objectives:

- Performance of gender determinations.
- Proper handling and restraint of mice for routine procedures (e.g., physical examination, administration of medication, and venipuncture).
- Observation of mice for recognition of normal and abnormal physical and behavioral changes (e.g., anorexia, poor grooming, diarrhea, fight wounds, etc.) and how to report abnormal findings to appropriate veterinary service personnel.
- Demonstration of identification methods.
- Demonstration of methods of venipuncture and exsanguination (to include techniques, sites, restraint, volumes, and frequency). Individuals wishing to be certified to perform the retro-orbital bleed technique must demonstrate competency at performing this method of blood collection.
- Demonstration of sites and techniques for administration of medication by the following routes:

  - Oral
  - Subcutaneous
  - Intraperitoneal
  - Intravenous
  - Retro-orbital
- Discussion of appropriate methods of anesthesia (to include agent, route, and monitoring).
- Discussion of appropriate methods of euthanasia. Individuals wishing to be certified for use of cervical dislocation must demonstrate competency at performing euthanasia by this method.

1.4 Why Mice are Used in Research:

- Mammal of small size
- Relatively short life span
- Proficient reproductive capabilities
- Susceptibility to microbiological and chemical agents
- Much baseline data on embryology, genetics, gerontology
- Immunologic response similar to humans
- Ease of maintenance

1.5 Strains of Mice and Genetics:

- Random or outbred stock - Animals derived from mating of unrelated individuals, genetically heterogeneous (e.g., Swiss Webster).
- Inbred strains - A strain is regarded as inbred when it has been mated brother x sister for 20 or more consecutive generations.
  - Offer a high degree of genetic uniformity.
  - Advantage of inbred mice - low level of within-strain genetic variability.
  - Disadvantages of inbred mice - small litters, fewer litters, more neonatal deaths (inbreeding depression).
  - Usually designated by capital letters (e.g., A/J; AKR; BALB/c; CBA; C57BL/6J; C3H; SBA; SJL).
- F1 hybrids - Crosses between two different inbred strains, have hybrid vigor (e.g., Af x C57BL/6J = AB6F1).
- Congenic inbred strains - Strains that differ by a mutant gene; mutation occurs and it is maintained by repeated backcrosses onto an inbred strain and is designated by the full or abbreviated symbol of the background strain. Capital letter = dominant, lower case letter = recessive.
  - Example: jaundice (ja) mutation occurred in an outbred strain and was introduced into two parental strains.
    - ABB6F1 - ja/ja = homozygous state (manifests mutation)
    - WBB6F1 - ja/+ = heterozygous state (does not manifest mutation)
    - WBB6F1 - +/+ = wild type (normal)
- Mouse Knockout and Mutation Database: http://www.informatics.jax.org/

1.6 Physiologic Data:

Body temperature:..... 35.8-37.6ºC (96.4-99.7°F)
Heart beats/min:..... 600 (328-780)
Breathing rate/min:..... 163 (84-230)
Weight, adult male:..... 25-40 grams
Weight, adult female:..... 25-40 grams
Birth weight:..... 1 gram
Sexual maturity:..... 5-8 weeks

Estrous cycle frequency: ..... 4-5 days
Duration of estrus: ..... 10 hours
Time of ovulation: ..... 2-3 hours after estrus onset (spontaneous)
Rebreed after parturition: ..... postpartum estrus
Gestation period: ..... 19-21 days
Litter size: 6-12
Begins eating dry food: ..... 12-14 days
Weaning age: ..... 21 days
Breeding life: ..... 8 months
Mating: ..... pair (monogamous) or
..... harem (polygamous); one male, 2 females
Life span: ..... up to 2.5 years

1.7 Mouse Anatomy:

http://www.informatics.jax.org/cookbook/

1.8 Genetic Monitoring:

- Drift occurs in inbred strains; important to monitor in some way or prevent (e.g., cryopreservation).
- Breeding colony – Obtain advice on arranging a colony, best breeding schema, etc. to maximize production for research needs and minimize chances for genetic contamination and drift.
- Also see Rodent Genotyping, UW IACUC Approved Animal Use Policies website: https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies

1.9 Intercurrent Disease:

- Important to purchase good quality mice from reputable vendors as intercurrent disease or latent viruses could have an effect on research. A list of approved animal vendors is available on the Animal Purchasing website: https://depts.washington.edu/compmed/animal/.
  - Infectious agents that cause clinical illness or deaths are detrimental to research program (e.g., data retrieved from a sick or dying mouse is not likely to be valid).
  - Vendors will supply Health Surveillance information.
  - Sub-clinical, persistent infections can have equally profound effects on biological responses.
  - Many rodent viruses have been isolated from transplantable neoplasms and may modify tumor growth kinetics. The influence of viral infections on the biology of chemical carcinogenesis is not well understood, but is potentially substantial. Contact the Rodent Health Monitoring Program (rhmp@uw.edu) for information on having tissues and cell lines tested.

1.10 Viral Infections in Mice:

- The majority of rodent virus infections are sub-clinical in nature, but can have profound effects on research results.
- Acute - Characterized by short incubation, short course of active infection that may be clinically apparent or asymptomatic followed by a brief period of virus excretion and death or recovery of the host.
- Persistent - Characterized by a long course, can be chronic or latent. May be clinical or asymptomatic, but virus is commonly excreted or detectable in host tissues through the course of infection.

  Note: The pathogenicity of chronic persistent infections such as Mouse Hepatitis Virus (MHV) can also be enhanced by immunosuppression, stress, or concomitant infection by another agent.

1.11 Movement of Infectious Agents:

- Infectious agents can travel from room to room carried by personnel and equipment. Following these Specific Pathogen-Free (SPF) procedures can dramatically limit pathogen transfer:
  - SPF garments (booties, gloves, bonnets, gowns, etc.) should be worn only in the room in which they are supplied. Do not exit the animal room wearing these items.
  - Coordinate any movement of animals with the Facility Supervisor and the Rodent Health Monitoring Program (RHMP).

1.12 Quality Assurance Program:

- Faculty member responsible at UW: Dr. Susan Dowling (206-221-3933 or sdowling@uw.edu).
- All mice received at UW must be ordered through Department of Comparative Medicine Animal Purchasing, which ensures some control of the pathogens coming into the facility. Quality Assurance data is obtained from each vendor and mice are periodically screened for rodent viruses.
- In-house colonies are screened quarterly and evaluated by the Quality Assurance Program.
  - Mouse viruses screened: mouse hepatitis virus (MHV), rotaviruses, and minute virus of mice (MVM).
  - Endoparasites and ectoparasites screened: pinworms and mites.

1.13 Purchasing Mice:

- Animal Purchasing (email: animals@uw.edu, phone: 206-543-0640, web: https://depts.washington.edu/compmed/animal) can supply information on mouse vendors. Major vendors have web addresses and can supply information about their stock and health surveillance of their colonies. JAX, Taconic, and Charles River generally supply specific pathogen free (SPF) mice. Certain lines of transgenic and knockout mice are available through JAX and Taconic.
  - The Jackson Laboratory - https://www.jax.org/
  - Charles River Laboratories - http://www.criver.com
  - ENVIGO (formerly Harlan Laboratories) - http://www.envigo.com/
  - Taconic Biosciences - http://www.taconic.com/
  - DCM Shared Rodent Service - https://catalyst.uw.edu/gopost/board/animals/8509/
- Bringing Mice into a UW Facility - All mice must be ordered through Animal Purchasing and cleared with RHMP. Mice coming from any non-approved source (e.g., vendor, institution, etc.) must go through a monitored quarantine for approximately 8 weeks.
- Rodent classification:
  - Germfree (or gnotobiotic) - Cesarean-derived.
  - Specific Pathogen Free (SPF) - Free of specific pathogens known to cause disease in some strains of mice or certain immunodeficient mice.
    - All Comparative Medicine rodent facilities are SPF housing (K-wing, T-wing, 6th Floor, Foege, ARC, Brotman, SLU 3.1, HR&T, Roosevelt, Guthrie, and CHDD).
  - Conventional - Non-SPF housing harbor certain mouse pathogens but may not cause overt disease
    - There are no conventional housing facilities available in the Health Sciences Complex, however, there are some lab-managed facilities that can support conventional animals.

2. Laboratory Outline:

2.1 Health monitoring:

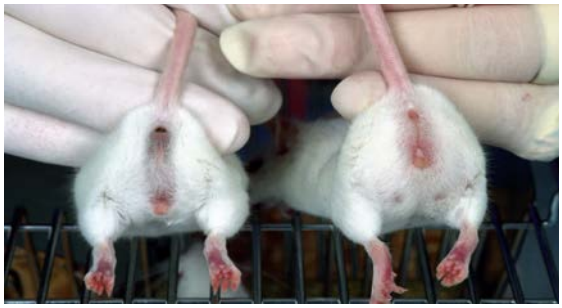
- It is important to be familiar with the characteristics of a healthy mouse and normal rodent behavior in order to recognize an unhealthy animal. Normal mice should be active, have a smooth and shiny

haircoat, bright clear eyes, and good body mass.


Sick mouse: hunched, rough haircoat

- Signs of disease or discomfort: huddles in corner, hunched posture, scruffy hair coat (due to dehydration or lack of grooming), feels cool to the touch, avoids cage mates, diarrhea (i.e., sticky feces), pale appearance to eyes, nasal/ocular discharge, coughing, sneezing, chattering, vocalizing, decreased appetite, weight loss (see Appendix C: Body Condition Scoring), respiration increased or labored.
- If severe signs are seen or when more than one of these signs is noted, waiting for possible improvement without immediate medical intervention or euthanasia often results in needless suffering and spontaneous death.
- Submit a Sick Animal Report to Veterinary Services: A laboratory animal veterinarian or veterinary technician will assist you in determining if noted illness is experimentally induced or spontaneous disease. IF MICE ARE DYING IN YOUR COLONY, IT COULD BE AN INFECTIOUS AGENT. CONTACT VETERINARY SERVICES.
- Use caution when handling sick mice as the stress can be sufficient to cause them to expire.

2.2 Gender Determination and Breeding:


MALE FEMALE

- Compare anogenital distance of males versus females (distance between anus and genital papilla is ~ 2 times longer in males).
- Perform when weaned (approximately 21 days of age). Accuracy may be difficult if younger than 3 weeks (determine through comparison).
- Mice are sexually mature at 5 – 7 weeks. Improper gender determination may result in unwanted pregnancies.
- Some females will cannibalize their pups (multiple causes) – minimize handling or disturbing females with newborn pups. If it is necessary to change a cage with newborn pups, transfer the nestlet material with the pups.
- More information on timed mating is available here: (https://depts.washington.edu/compmed/veterinary/files/MouseFact_sheet.pdf).

2.3 Weaning:

Generally, rodents are weaned at 21 days of age. Weaning is when a litter is removed from the cage were the female is housed.

1. Place animals into fresh new cages. DO NOT mix males who are from different litters. Mixing males can cause fighting.
2. Place 1-2 tablespoons of gel in a dish on the cage floor.
3. Check for enrichment (nestlet) in cage. If not present, place in cage
4. Place fresh food in one side of the clean wire top so it is flush with the surface. Do not over or under fill the food.
5. Check water source. If working in a facility that uses water bottle, check to see water bottle is full, not leaking and the sipper tube on the water bottle is down. If working in a facility with an automatic watering system, toggle water valve.
6. Put on a lid with a cage card holder.
7. Put on new cage card with all the required information.

2.4. Cage Cards:

Cage cards must be legible and include:

1. PI Name *(required by The Guide)*
2. Protocol Number *(required by The Guide)*
3. Budget Number *(needed for DCM to bill efficiently)*
4. Contact Name and Number *(required by The Guide; needed in order to reach someone regarding animal care issues with that cage)*
5. Birth Date or Date of Arrival (age/wt = when ordered) *(required by The Guide)*
6. Source (vendor; sire and dam if breed in house) *(required by The Guide)*
7. Strain/Stock *(required by The Guide)*
8. Sex *(required by DCM)*
9. Release # (DCM cage cards only) *(used for tracking purposes by DCM)*

Note: cage cards can be printed at no cost by DCM staff (breeding or colony cards)

2.5 Cage Density and Breeding Schemes:

Animals must not be overcrowded. Below are the cage density limits.

1. 5 adult mice.
2. Standard (pair) breeding: one standard mouse cage can house one adult male, one adult female, and one litter until weaning.
3. Trio breeding (two females and one male):
    a. Can be used only for inbred strains or genetically modified mice where small litters (average 6 pups or less per litter) or poor breeding efficiency is seen.
    b. If unusually large litters (more than 12 pups, 2 females and 1 male) are born, one of the two females with her litter must be separated to a new cage before the pups reach two weeks of age.
    c. If a third litter is born prior to weaning of the previous litters (thus new pups in the cage with older pups present), older pups must be weaned immediately (i.e., when two litters belonging to one female are present in the cage, the older litter from this female should be weaned immediately).
    d. When pups of very different ages (e.g., 19-20 days old versus newborn) are present in the cage, the older pups must be either weaned or separated with the female.
4. Three females and one male: Females need to be separated after pregnancy is detected, prior to delivery. If pregnancy is not detected, then females with litters must be separated before the pups are 10 days old (typically non-lactating females are removed from the cage soon after the litter is noted).
5. Animals will be weaned by the research group on or before 21 days of age unless an IACUC variance is approved.

The IACUC policy regarding the number of mice in a breeding cage can be found at https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies. Variances to the policy must be approved by the IACUC and posted.

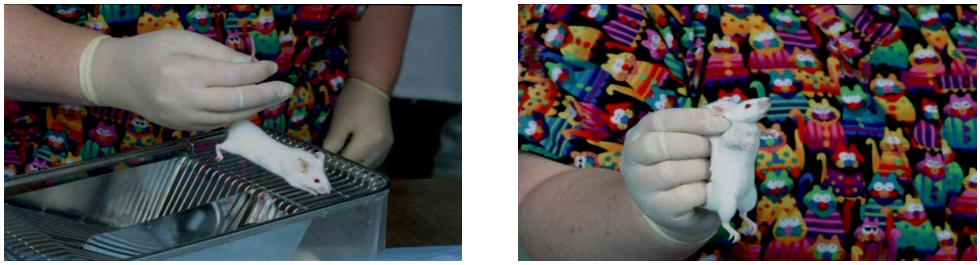
See Appendix E for more information on how to prevent overcrowding when trio breeding.

2.6 Restraint and Handling:

1. Pick the mouse up by the base of the tail and place it on the wire cage-top.
2. Position fingers at the base of the mouse's ears and skull. When fingers are correctly positioned ears should be pinched slightly together during restraint.
3. Utilize one-hand versus two-hand restraint techniques; the restraint technique selected depends on handler preference and the procedure to be performed.

4. Excessive traction during restraint can easily choke the mouse. If held too tightly the eyes may protrude, the mouse may turn bluish (cyanotic), or begin gasping which can indicate restriction of respiration (it is possible to kill a mouse by holding it too tightly).
5. DO NOT spin or twirl the mouse by the tail.

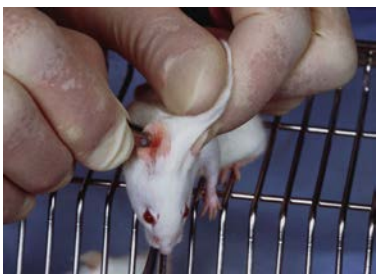
NOTE: If bitten, it is inappropriate to throw or drop the mouse. Be ready to overcome this response by reviewing/rehearsing the proper steps to take when a mouse bites. When bitten, place hand on cage top - mouse will release bite; return mouse safely to cage. Wash bite wound with soap and water. Tetanus immunization should be up to date (within the past 5 years). The Employee Health Clinic is located at Hall Health Center (206-685-1026), please contact them if the wound is severe. Report all accidents/incidents to Environmental Health and Safety using the Online Accident Reporting System (OARS) at: http://www.ehs.washington.edu/ohsoars/index.shtm



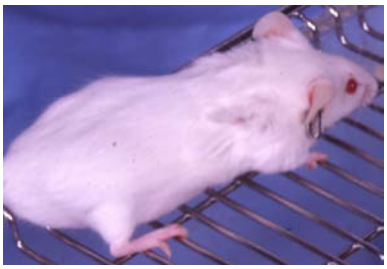
Standard one-handed mouse restraint

2.7 Methods of Identification:

- Temporary - Tail or haircoat marking with a non-toxic permanent marker, such as a Sharpie marker (will be removed during normal grooming, usually within 24-48 hours).
- Permanent - Ear punch (see Appendix A), ear tag, implanted microchip, tattooing (see Vendor List). NOTE: amputation of toes requires strong justification and approval from IACUC



Ear Punch



Ear Tag

2.8 Injection Techniques:

A 25 gauge (g) or smaller needle is recommended. (Larger numbers indicate smaller gauge needles.)

2.8.1 Subcutaneous:

Site: Dorsal shoulders/neck (in juveniles), inguinal or flank regions (in adults).

Volume: Recommended maximum 0.25 ml. It can depend on the drug.

Restraint: Standard one-hand or two-hand (with the help of an assistant) technique.

Subcutaneous Injection

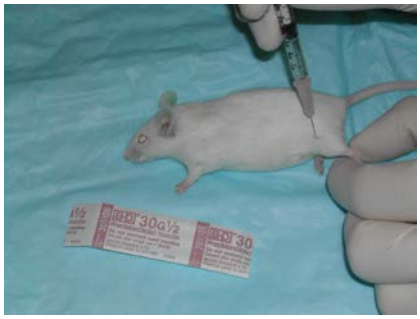
2.8.2 Intramuscular:

This technique is difficult to perform, painful for the mouse (they have a very small muscle mass), and not recommended if avoidable.

Site: Use quadriceps muscle located on dorsal surface of femur. Avoid use of hamstring on ventral surface of femur as a high risk of sciatic nerve paralysis is possible.

Volume: Recommended maximum 0.05 ml. Use of 30 g needle is preferable.

Restraint: Anesthesia or sedation is preferred. Conscious animals may be restrained by an assistant (difficult to access and hold quadriceps still while also restraining mouse), skill and practice are needed for this technique.



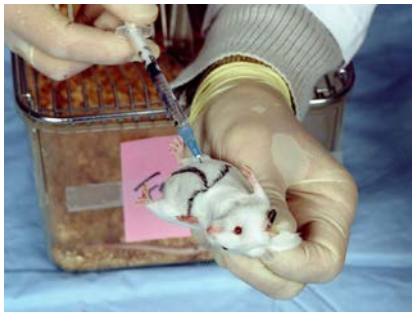
Intramuscular Injection

2.8.3 Intraperitoneal:

Site: Lower right or left quadrant and lateral to umbilicus.

Volume: 10µl/g. Recommended maximum 0.5 ml. Diluting solutions with sterile saline improves absorption.

Restraint: Standard one-hand or two-hand techniques. Positioning mouse head down will cause viscera to fall towards diaphragm (away from injection site).



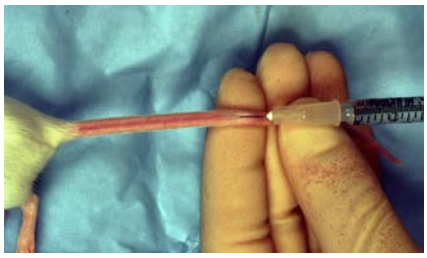
Intraperitoneal Injection

2.8.4 Intravenous:

Site: Lateral tail veins.

Volume: 10µl/g. Recommended maximum (bolus) 0.125 ml. Recommended maximum for a slow infusion is 0.3 ml. Use of 27-30 g needle is preferable.

Restraint: Restraint tubes or sedation required. Improve visibility of vessels (vasodilation) by cautiously warming entire mouse (heat lamp over cage) or by placing tail in warm water. Aspiration of blood to ensure correct needle placement is not effective.



Intravenous Injection

2.8.5 Oral Gavage:


Oral Gavage

Generally, gavage must only be performed on awake animals.

Site: Placement of liquid directly into stomach via a tube through the mouth and esophagus (see Vendor List). In order to avoid inadvertently placing the liquid into the respiratory system, it is critical that the gavage needle be both the correct length (distance between the corner of the mouth and last rib, usually a 22 g x 1.5" gavage needle is used) and be advanced gently without resistance.
Volume: 10µl/g. Recommended maximum 0.25 ml.
Restraint: Standard one-hand technique.

2.8.6 Retro-Orbital:

Certification is required.

Tools: Use of 26-30 g needle is preferable and syringe.
Site: Orbital sinus behind the eye. Needle is positioned at the medial canthus (corner).
Volume: Recommended maximum 0.125 ml (bolus). Recommended maximum for slow infusion is 0.3 ml.
Restraint: Anesthesia is required.

2.8.7 Intranasal:

Tools: Pipetter with a P20 pipette tip.
Site: Nostril
Volume: Standard recommended maximum volume = 20uL.
Restraint: Chemical restraint standard one-handed technique

Key Concepts:

- For unanesthetized animals, a solid restraint is necessary to ensure that the head is immobilized. Anesthesia may help prevent sneezing and twitching.
- If anesthetized with Isoflurane, the mouse should have a slow respiratory rate before removing from the induction box or nosecone. Breathing should never be labored or agonal.
- Position of the animal will affect where the substance will absorb. Absorption in the nasal canal – place mouse on its back/horizontal with the belly facing up and the head lying naturally. Absorption in to the lungs – While administering, place mouse horizontally with belly up. After administration, hold mouse upright or an anesthetized mouse can be placed back against a vertical board while being hung using a filament from the top incisors.
- Administration must be slow (about 1 min depending on volume) to ensure mouse does not suffocate.
- Hold pipet tip just above one nostril and slowly let one drop of the substance land on the nostril. Place one drop at a time. Let the mouse breath in the substance before you continue administration.
- Substance my run down the middle of the nose onto the teeth the into the mouth if administered too quickly.
- No bubbles should form.
- Split the volume between each nostril if feasible and consistent with the scientific objective.

- The respiratory pattern may change but there should be no signs of respiratory distress.
- If anesthetized with Isoflurane, exposing the animal to isoflurane after dosing may help prevent sneezing.

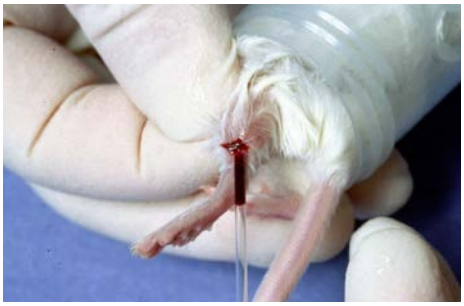
2.9 Blood Collection Techniques:

Site selection is dependent upon the volume required and whether collection is to be repeated or if collection is terminal (exsanguination). Serial/repeated sample collection: orbital sinus, submandibular, submental, or saphenous vein (hind leg) techniques.

2.9.1 Lateral Saphenous Vein:

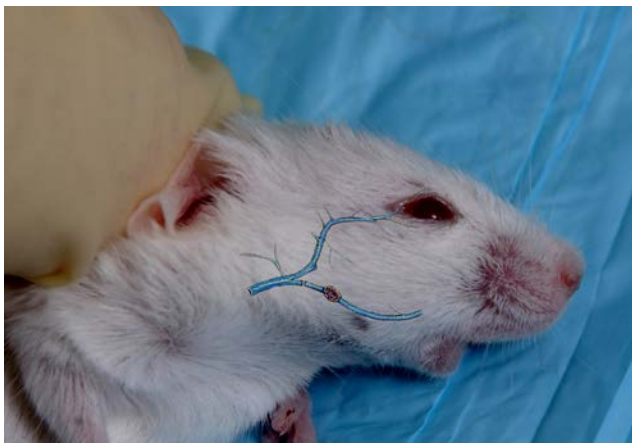
Tools: External restraint like a 50 cc plastic centrifuge tube with holes in tube. Bland ophthalmic ointment. 23-25 g needle. Capillary tube or blood collection tube. Gauze sponge.
Site: Lateral or Medial Saphenous Vein
Restraint: Restraint devise, one handed for medial, or chemical restraint.
Key Concepts:

- Place mouse in tube headfirst. Gently grasp one rear leg and hold it extended from the tube.
- Slick down the hair on the lateral (outside) surface of the leg with bland ophthalmic ointment.
  - Allows visualization of the lateral saphenous vein.
  - Keeps blood from soaking into fur.
- Lance vein in the area where it crosses between the stifle and hock, using the 25 g needle. Enter at 45 degree angle and perpendicular to the vessel.
- Collect the blood with a capillary tube as it appears from the site.
- When the sample has been collected, apply gentle pressure to site to stop the bleeding.

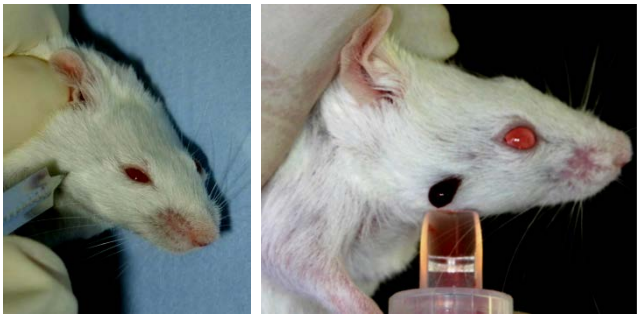

Lateral Saphenous Vein Bleed

2.9.2 Submandibular:

- A small vascular bundle at the back of the jaw characterizes the cheek of mice (see photo). This is where the orbital veins, the submandibular vein, and other facial veins join at the beginning of the jugular. With practice an individual can become proficient and collect as much as 0.2 ml of blood rapidly.
- Tools:
  - 4-5 mm lancet
  - Blood collection vessel (e.g., Microtainer® tube)
  - Gauze sponge
- Restrain mouse using the one-handed technique.


Orbital and Submandibular veins
(http://www.medipoint.com/html/animal_lancets.html)

- The puncture point is at the back of the jaw, slightly behind the hinge of the jawbone and toward the ear. Avoid the jawbone, and instead puncture just behind the point at which the upper and lower jawbones meet.
- The puncture should be done with enough force to quickly insert the lancet point to the hilt.
- Quickly grab a collection vessel, because the blood can flow rapidly at first, and then slows in a very short time.
- When the sample has been collected, apply gentle pressure to the site with a gauze sponge to stop the bleeding and loosen the scruff.



Puncture location
(http://www.medipoint.com/html/animal_lancets.html)

2.9.3 Submental (Chin) Bleed

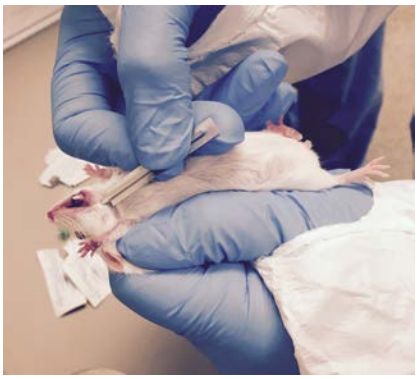
- This method is similar to the Submandibular bleed described above, but the location of the collection site differs. Instead of puncturing along the jaw line, the submental vessels are located ventrally under the chin area and can be visible.
- Tools:
  - 3-5 mm lancets
  - Blood collection tubes
- It is especially important to have a secure hold on the mouse so that the head is held in line with the body and any loose skin around the chin is retracted.
- Locate the star-shaped bundle of vessels under the chin of the mouse (labeled "Landmark Whorl" in photos below) and clearly visible as the lighter area on the Black 6 mouse in the photo on the right.

Submental Bleed Puncture Locations

- The puncture points are on either side of the midline (labeled "Puncture Site" in photos above), and visible as darker areas on the Black 6 mouse in the photo on the right.
- Using a 3-5mm lancet (e.g., Medipoint, Inc) quickly insert the lancet up to the hilt. Be sure to

have the collection container close by since the blood comes quickly.

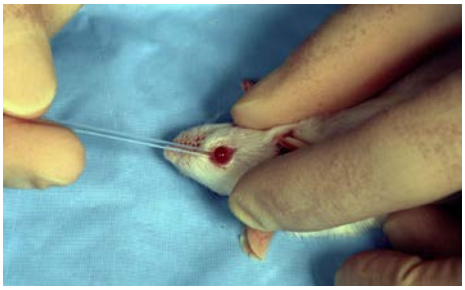
- Once the desired amount of blood has been collected (see 2.10 below for guidelines), release the animal.
- It is not necessary to sponge off or stop the bleeding as it will automatically cease once the animal is released.
- To the right is a photo of a submental bleed in progress. Note the mouse restraint and angle of penetration of the lancet.
- Advantages of this method include:
    - Small target area for increased accuracy
    - No anesthesia required
    - Ease of collecting maximum volume of blood



Submental Bleed in Progress

2.9.4 Retro-Orbital Bleed:

- Bleeding from the retro-orbital sinus should be done with the mouse under anesthesia. The Institutional Animal Care and Use Committee (IACUC) will allow this technique without anesthesia. Certification to perform retro-orbital bleeding for both awake and anesthetized mice is required by the IACUC. Such certification may be obtained in a Mouse AUTS class through the Training Instructor, or through a qualified individual designated by the Attending Veterinarian. See IACUC policy "Blood Collection in Laboratory Animals": https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies
- Position a microhematocrit tube or narrow pipette in medial or lateral canthus (corner) of eye.
- In order to achieve hemostasis, remove pipette quickly after sample collection and apply immediate gentle pressure over closed eyelids with a gauze sponge. Hemostasis is important to minimize scar tissue and damage to eye.
- If anesthesia is used, place sterile ophthalmic lubricant in eyes after sample collection.
- Microhematocrit (PCV) tubes hold 0.07-0.125 ml of blood (a 25 gram mouse may have up to 2 microhematocrit tubes of blood taken every two weeks - based on withdrawal of up to 1% body weight).



Retro-Orbital Bleed

2.9.5 Tail Vessel Lancing and Tail Tip Amputation:

Lancing the tail vein or artery with a blade/needle or amputating the tip of the tail to collect blood is discouraged. If a group needs to obtain blood with this technique, scientific justification is required and will need IACUC approval.

2.9.6 Tail Prick:

Tools: 3mm lancet or 25g needle, collection tube or glucose strip.
Site: 2-3mm from tip of tail.
Volume: 2-3 drops.
Restraint: Holding the tail while animal explored the cage or wire top.
Key Concepts:

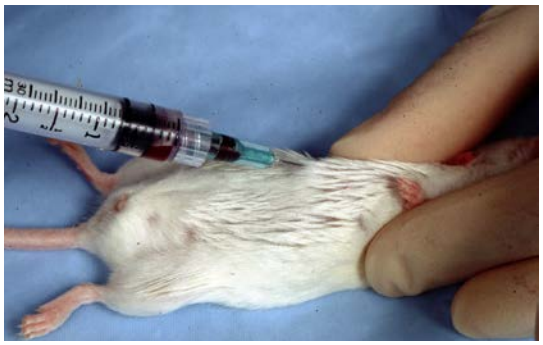
- Hold the tail while the animal is allowed to explore the cage or wire top. It helps if they can grip this wire top.

- Hold the tail below the prick site. About 5mm from the tip of the tail.
- Prick site is about 2-3mm from tip of tail.
- Approach the prick site with the lancet or needle(needle is bevel up) at a very shallow angle to the tail, with the lancet/needle facing inward toward the mouse.
- Quickly and gently insert about 1-2mm of the needle/lancet into the tail and immediately pull out and a drop of blood with form.
- You can obtain slightly more blood by gently milking the tail.

2.9.7 Terminal Sample Collection (Exsanguination):

Anesthesia required for all exsanguination sites/methods. Sites: cardiac puncture or orbital sinus.

- Cardiac Puncture: Using a 23 g needle and 3 ml syringe, approach the heart either from under the sternum (as illustrated) or from the lateral left thoracic wall near the point of the flexed elbow. Once the needle is in the heart (preferably a ventricle), stabilize the hub and gently apply negative pressure to the plunger (the heart chamber will collapse if the negative pressure is too high). Do not repeatedly probe with the needle. Be prepared to euthanize the mouse once the maximum blood volume has been collected.



Cardiac Puncture

- Orbital Sinus: Place mouse on parafilm. Using a Pasteur pipette with negative pressure on its aspiration bulb, enter the orbital sinus at medial canthus of the eye to collect sample. If blood escapes around the pipette, aspirate off of parafilm. Can also use microhematocrit tubes to enter orbital sinus and obtain blood samples. Be prepared to euthanize the mouse once the maximum blood volume has been collected.

2.10 Blood Collection Volumes:

- 1-3-6% Rule for Venipuncture:
  - 1% of body weight = maximum volume per collection every 2 weeks
  - 3% of body weight = amount expected at exsanguination
  - 6% of body weight = approximate total blood volume
- Example: 25 gram mouse
  - 1% = 0.25 ml every 2 weeks
  - 3% = 0.75 ml at exsanguination
  - 6% = 1.50 ml approximate blood volume

2.11 Anesthesia:

Be extremely careful when preparing anesthetics for mice as small errors can result in death of the animal. WEIGH mice before dosing. Before using anesthesia in mice, always check your IACUC protocol for approved agents.

2.11.1 Anesthesia for Mice:

- KETAMINE/XYLAZINE - Tranquilizer/dissociative agent, useful drug combination due to ease of administration and relative safety. Can be used for brief, minor surgical procedures (e.g., SQ implantation of minipump). Will provide approximately 25-30 minutes of moderate level anesthesia. Apply sterile ophthalmic ointment to keep corneas from drying. Use front foot toe pinch to assess level of anesthesia. Do not use hind foot toe pinch because under

Ketamine/Xylazine the hind foot reflex is not suppressed as it is under other anesthetics.

Note: Ketamine is a controlled substance and requires a DEA license.

Prepare: 0.65 ml Ketamine (100 mg/ml) +
0.22 ml Xylazine (20 mg/ml) +
9.13 ml of sterile saline
Yielding solution strength = 6.9 mg/ml (1% solution)
Beyond-use date is 1 month after mixing
Dosage: 0.02 ml/g body weight IP
Administer: 130 mg/kg Ketamine, 8.8 mg/kg Xylazine

- TRIBROMOETHANOL (Avertin) - Requires preparation of stock solution and then a working solution prior to use. Provides anesthesia for approximately 30-45 mins. Complications: peritonitis has been reported following IP administration of this agent.

  Prepare: 2.5% working solution
  25 mg/ml
  Beyond-use date is 1 month after mixing
  Dosage: 0.020-0.026 ml/g body weight IP
  Administer: 0.5 – 0.65 mg/g, 500 – 650 mg/kg

  See Appendix B for ordering and preparation of Tribromoethanol (Brand name: Avertin)

- PENTOBARBITAL (Nembutal®) (barbiturate) - Provides deep anesthesia and analgesia. Mice of different ages and strains differ markedly in their responses to single injections of barbiturates. Work with this barbiturate to establish best and safest dosage for mice being used. Dose may vary with age and strain of mice. Pentobarbital also has a narrow margin of safety and the difference between the surgical anesthesia dose and an overdose may be very small. Commercial solutions of Pentobarbital (Nembutal®) must be diluted (sterile saline) before they can be administered.

  Note: Pentobarbital is a controlled substance and requires a DEA license.

  Prepare: 2.00 ml of 50 mg/ml Pentobarbital (Nembutal)
  8.00 ml of sterile saline
  Yielding solution strength = 10.0 mg/ml (1% solution)
  Beyond-use date is 1 month after mixing
  Dosage: 0.04-0.09 grams/kg = 40-90 mg/kg, body weight given IP
  Example: 25 g mouse at 0.04 mg/ml would receive 0.1 ml given IP

- ISOFLURANE or SEVOFLURANE
  - Can only be used in a calibrated vaporizer.
  - Delivers lethal concentrations when used in an "open", non-calibrated method (bell-jar or nose cone).
  - With all inhalant agents, the mouse should not be allowed to come in direct contact with the agents because they are drying and irritating to the mucous membranes.
  - Must be used in a fume hood or the vaporizing system must be fitted with scavenging devices.

- Induction and recovery is rapid.
- Longer duration of anesthesia is possible.
- Be sure to prevent hypothermia.
- Does not provide any post-operative analgesia. Use of analgesia pre-operatively is recommended for painful procedures.

2.11.2 Anesthesia Assessment:

- Pain reflex tests - Ear, toe, and tail pinch. Withdrawal of foot or flicking of ear/tail indicate insufficient plane of anesthesia for surgical procedure (except with Ketamine/Xylazine where hind foot toe pinch will still cause reflex regardless of anesthetic depth).
- Palpebral reflex - Slight movement or closing of eyelids when eyelashes or the medial canthus of the eye are touched indicates insufficient plane of anesthesia for surgical procedure. This technique may be unreliable because mice have poor palpebral reflex.
- Respiratory/Cardiac rates - Increased rates associated with painful stimulus indicate insufficient plane of anesthesia for surgical procedures.

2.12 Pain Assessment and Postoperative Care:

Remember: Approach pain assessment with the view that if a procedure causes pain or discomfort in humans, then it will do the same in animals. Consult with Veterinary Services (206) 543-6257 for recommended analgesia doses and frequencies.

- Signs of pain/discomfort may include: Ruffled coat, hunched posture, pale eyes, decreased activity. Pain is particularly difficult to assess in small rodents. Signs of pain or discomfort are often difficult to distinguish from disease in rodents. Many times signs are evidenced during the night-time (dark cycle) when we do not observe the animals. Veterinary medical staff should be consulted to determine appropriate intervention methods (e.g., analgesia, medical treatment, or euthanasia).
- Postoperative care: Postoperative warming and monitoring is essential (warming light a safe distance from cage, circulating water heating pad). Personnel must monitor all animals until they return to consciousness. Place anesthetized mice on paper towel or a similar substrate (not on bedding such as corncob or shavings). Recover mice individually (not in a cage with awake mice). Allow animals an area to move away from the heat source.

2.13 Surgical Requirements:

- All personnel must be listed on the IACUC approved protocol.
- When planning to do a surgical procedure for the first time, obtain advice from a laboratory animal veterinarian or veterinary technician in Veterinary Services (206-543-6257) and ensure individuals are certified.
- Non-Survival Surgery
  - Animals are not allowed to recover from anesthesia.
- Survival Surgery
  - Animals are allowed to recover from anesthesia.
- Non-Aseptic Surgery
  - May be performed in a suitable laboratory and aseptic (sterile) technique is not required. Per the Guide for the Care and Use of Laboratory Animals, "at a minimum, the surgical site should be clipped, the surgeon should wear sterile gloves, and the instruments and surrounding area should be clean."
- Aseptic Surgery
  - Must be performed by an individual certified by a designee of the Attending Veterinarian. See policy here (https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies).

- Aseptic surgery on rodents may be performed in a laboratory that is easily sanitized and not used for any other purposes during the time of surgery. To prevent infections, STERILE INSTRUMENTS AND SURGICAL GLOVES MUST BE USED. ASEPTIC PROCEDURES MUST BE FOLLOWED, INCLUDING THE PREPARATION OF THE INCISION SITE (i.e., shaving and scrubbing with an appropriate disinfectant such as Betadine®).
- A new set of sterile surgical instruments must be used if surgeries are done on multiple animals. Initial sterilization of instruments must be by an approved method such as autoclaving or gas sterilization. Hot bead sterilizers can be used to re-sterilize instrument tips between animals.

Hot Bead Sterilizer

2.14 Euthanasia:

General Information: Animals communicate with sounds and smells humans cannot perceive. Do not expose animals to the death of others. Be sensitive to other animals and people in the vicinity. Ensure all equipment is in good working order. Species should be separated and never over crowded. Acceptable methods of euthanasia are injectable anesthetics, inhalation anesthetics, carbon dioxide, cervical dislocation*, and decapitation*. All methods must be approved on your IACUC protocol. Per the AVMA Guidelines on Euthanasia (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf), decapitation requires scientific justification and approval by the IACUC.

*NOTE: Individuals performing decapitation of unanesthetized mice older than 14 days and/or cervical dislocation of unanesthetized or anesthetized mice at the UW must be certified by a designee of the Attending Veterinarian.

FETUSES - Rodent fetuses do not have to be removed from the uterus and separately euthanized after the mother has been euthanized.

- Anesthetic Overdose
  - Inhalant - Expose to fumes of inhalant anesthetic by placing mouse in a small bell jar inside a fume hood (avoid any direct contact of mouse to agent) or place mouse in a closed receptacle and introduce anesthetic from a vaporizer. Continue exposure to fumes for 3-5 minutes after respiration has ceased (at least 5 minutes total).
  - Injectable - Calculate injectable agent dosage at 4-5 times normal anesthetic dosage.
- Carbon Dioxide (CO_2)
  - General Information
    - Always use the timer to ensure adequate length of exposure.
    - Place a dead animal tag on the bag with 1) P.I.'s name, and 2) Euthanasia date.
    - Do NOT euthanize in animal rooms or in front of living animals.
    - Do NOT leave the room with the CO_2 running.
    - Do NOT overcrowd the cages. All animals must have floor space.
    - Do NOT mix males from different cages or incompatible females together.
    - Do NOT expose animals to a cage that is "pre-filled" with CO_2.
    - Do NOT expose animals to a CO_2 flow rate higher than noted on the CO_2 tank. Over exposure can burn the mucous membrane.

- When removing dead mice from the cage, run your fingers through the bedding to be sure there is no one left behind in the cage.
- Clean up between cohorts of animals. This includes the hood, chamber, and work surface. Animals release pheromones when being euthanized that can induce stress in other animals.
- Neonatal rodents will NOT die with CO2 alone (i.e., animals that are 10 days of age or younger, whose eyes are not open and fur growth is incomplete).
- You will need to supply your own sharp scissors for decapitation.
- If you have questions regarding euthanasia, please contact Veterinary Services within your facility, the Facility Supervisor, the IACUC office or the Facility Director.

- Euthanizing Neonates/Pups
  - Age: 0-10 days old – CO2 exposure followed by decapitation.
    1. Turn CO2 on at the proper low flow rate posted next to source.
    2. Remove cage top.
    3. Put CO2 lid on cage.
    4. Maintain gas flow for at least 5 minutes for mice. Use the timer.
    5. Turn off CO2 tank.
    6. Pups should not be breathing when removed from the cage.
    7. Remove animals from CO2 cage and decapitate with sharp scissors designated for decapitation.
    8. Dispose of remains in plastic bag with dead animal tag, and then into designated refrigerator/freezer.
  - Age: 0-6 days old – hypothermia followed by decapitation.
    1. Place neonates onto a surface so they DO NOT come in direct contact with ice. For example, can be on several layers of paper towel or a petri dish.
    2. Once animals have stopped moving, you can check to see if animals are anesthetized by firmly pinching a toe. If they do not respond to a toe pinch, they are anesthetized.
    3. Once anesthetized, you can decapitate them with sharp scissors designated for decapitation.
    4. Place animals in a dead animal bag and place in designated refrigerator/freezer.

- Euthanizing Young Adults & Adults (over 10 days)
  1. Turn CO2 on at the proper low flow rate posted next to tank.
  2. Remove cage top.
  3. Put CO2 lid on cage.
  4. Maintain gas flow for at least 5 minutes for mice. Use the timer.
  5. Turn off CO2 tank.
  6. They should not be moving or breathing when removed from the cage.
  7. Remove dead animals from CO2 cage.
  8. Perform the approved secondary method. The University of Washington IACUC requires a secondary method of euthanasia to confirm death when euthanizing with CO2 and this method must be approved in your IACUC protocol. Please refer to IACUC policy "Euthanasia of Research and Teaching Animals": https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite/Policies
     - ✓ Cervical dislocation by UW certified individuals.
     - ✓ Exsanguination (remove blood from heart).
     - ✓ Thoracotomy (open up the chest to collapse the lungs).

- ✓ Decapitation.
- ✓ Anesthetic overdose.
- ✓ Placed in a bag full of CO2 for disposal.

9. Dispose of remains in plastic bag with dead animal tag and place bag into designated refrigerator.

- Cervical dislocation - Must be performed rapidly to achieve separation at the cervical (neck) region and NOT the back. Always check to be sure no heart beat is palpable before discarding the carcass. Certification of competency is required when performed on all mice regardless of age. Learn this technique by initially practicing on anesthetized mice with an experienced trainer; contact personnel in the Animal Use Training Program for assistance.
- Decapitation - Must be performed using clean, sharp surgical scissors or guillotine of the appropriate size for the animal being euthanized. The equipment used to perform decapitation must be maintained in good working order and records demonstrating service of the equipment must be maintained. See Appendix D: Decapitation Notes for more information.
  - NEONATES (0-14 days old) - Decapitation of neonates must be performed using clean, sharp surgical scissors. Neonates can be anesthetized using CO_2 prior to decapitation; this reduces movement of the mouse and makes decapitation easier. If you are unable to use CO_2, anesthesia can be achieved by inducing hypothermia. For information on how to create hypothermia, please contact the Animal Use Training Program. Certification of competency is not required for decapitation of neonates.
  - ADULT MICE- Mouse should be placed in a DecapiCone® prior to decapitation. Certification is required if the animal is to be decapitated while awake. Anesthetized decapitation does not require certification.

ONLINE RESOURCES

Animal Use Training Program	http://depts.washington.edu/auts/
AVMA Guidelines on Euthanasia	https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
DCM Animal Purchasing	http://depts.washington.edu/compmed/animal/index.html
DCM Shared Rodent Service-free	https://catalyst.uw.edu/gopost/board/animals/8509/
Ear Punch Numbering System	http://www.medicine.virginia.edu/research/cores/transgenic/all-mice-considered/ears-page
Environmental Health & Safety	http://www.ehs.washington.edu
Instrument Sharpening	http://auk.wanprc.org/services/
Mouse Anatomy	http://www.informatics.jax.org/cookbook/
Mouse Knockout and Mutation Database	http://www.informatics.jax.org/
Office of Animal Welfare	http://oaw.washington.edu/
Technique Videos	http://film.oslovet.veths.no

CONTACT INFORMATION

Department of Comparative Medicine

Veterinary Services
- *6th Floor, CHDD, Guthrie, Roosevelt, T-wing, and all Lab-Managed Facilities* 543-6257
  - *E-mail* ..... vs6floor@uw.edu
- *ARCF* 221-2250
  - *E-mail* ..... vsarcf@uw.edu
- *Brotman* 897-1508
  - *E-mail* ..... vsmercer@uw.edu
- *Foege* 221-4803
  - *E-mail* ..... vsfoege@uw.edu
- *Harborview* 897-5065
  - *E-mail* ..... vshrt@uw.edu
- *K-wing* 616-8716
  - *E-mail* ..... vskwing@uw.edu
- *SLU 3.1* 685-6605
  - *E-mail* ..... vsslu3@uw.edu

Central Animal Surgery 543-6150
Rodent Health Monitoring Program (RHMP) 221-2441
- RHMP E-mail ..... rhmp@uw.edu
- RHMP Web Page ..... https://depts.washington.edu/compmed/rhmp/index.html

Pathology Services 685-3040
Office of Animal Welfare (OAW) 685-7363
- OAW E-mail ..... oawrss@uw.edu
- OAW Web Page ..... http://oaw.washington.edu/

Animal Use Training Program 221-7709
- Training E-mail ..... auts@uw.edu
- Training Web Page ..... http://depts.washington.edu/auts/

Animal Purchasing 543-0640
- Purchasing E-mail ..... animals@uw.edu
- Purchasing Web Page ..... http://depts.washington.edu/compmed/animal/index.html

Drug Services

Ordering
- Website ..... http://depts.washington.edu/drugsvcs/home/
- Voicemail 598-6058
- Fax 598-3808
- Email ..... drugsvcs@uw.edu

Environmental Health and Safety

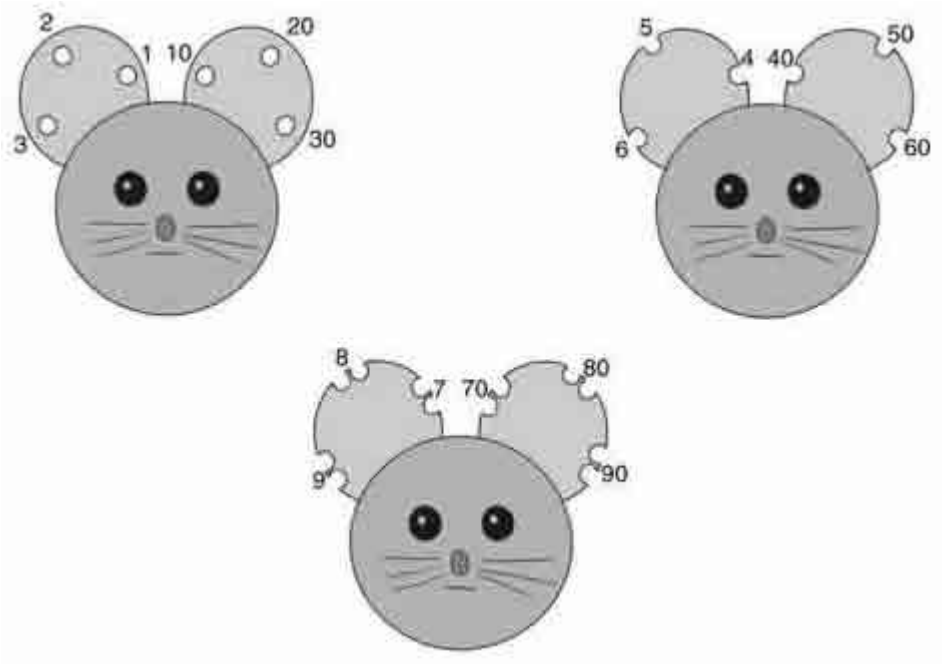
Occupational Health Nurse Consultant 221-3025
Occupational Health Clinic – Hall Health 685-1026
Environmental Health & Safety Web Page ..... http://www.ehs.washington.edu

VENDOR LIST

PRODUCT	VENDOR	PHONE	WEB SITE
anesthesia vaporizers and equipment	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
anesthesia vaporizers and equipment	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
anesthesia vaporizers and equipment	Summit Medical	800-877-8989	
autoclave bags and pouches	Fisher Scientific	800-766-7000	www.fishersci.com
drug storage cabinets	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
drugs and anesthetics	UWMC Drug Services	206-598-6058	
ear punches & ear tagging supplies	National Band and Tag Co.	606-261-2035	www.nationalband.com
ear punches & ear tagging supplies	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
ear punches	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
guillotine	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
guillotine & scissor sharpening	WaNPRC Machine Shop	206-543-1039	http://auk.wanprc.org/services/
heating apparatus	Physitemp Instruments Inc.	800-452-8510	www.physitemp.com
heating pad (recirculating warm water)	Gaymar Industries	800-828-7341	www.gaymar.com
heating pad (chemical)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
hot bead sterilizer	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
hot bead sterilizer	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
hot bead sterilizer	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
hot bead sterilizer	Inotech Biosystems International, Inc.	800-635-4070	www.inotechintl.com
hot bead sterilizer	Fine Science Tools	800-521-2109	www.finescience.com
micro-chip identification	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
micro-chip identification	Bio Medic Data Systems, Inc	800-526-2637	www.bmds.com
needle re-capper	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
oral gavage needles	Webster Veterinary Supply	800-225-7911	www.jawebster.com
oral gavage needles	Instech Laboratories, Inc..	800-443-4227	www.instechlabs.com
plastic rodent restraint	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
plastic rodent restraint	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
plastic rodent restraint	VWR Scientific Products	800-932-5000	www.vwrsp.com
plastic rodent restraint	Plas Labs, Inc.	800-866-7527	www.plas-labs.com
plastic rodent restraint (heated)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
plastic rodent restraint (heated)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
stockinet	Webster Veterinary Supply	800-225-7911	www.jawebster.com
surgeon supplies (glove, gown, etc.)	Fisher Scientific	800-766-7000	www.fishersci.com
surgeon supplies (glove, gown, etc.)	VWR Scientific Products	800-932-5000	www.vwrsp.com
surgeon supplies (glove, gown, etc.)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
surgical instruments	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
surgical instruments	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
surgical instruments	Fisher Scientific	800-766-7000	www.fishersci.com
surgical instruments	VWR Scientific Products	800-932-5000	www.vwrsp.com
surgical instruments	Health Care Logistics, Inc.	800-848-1633	www.healthcarelogistics.com
surgical instruments	Fine Science Tools	800-521-2109	www.finescience.com
surgical instruments (micro)	Roboz Surgical Instrument Co.	800-424-2984	www.roboz.com
surgical instruments (micro)	Fine Science Tools	800-521-2109	www.finescience.com
surgical instruments (micro)	Braintree Scientific Inc.	781-843-2202	www.braintreesci.com
surgical instruments (micro)	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
tattoo marking systems	Harvard Apparatus	800-272-2775	www.harvardapparatus.com
tattoo marking systems	Ketchum Manufacturing Inc.	613-722-3457	www.ketchum.on.ca

Appendix A:
Ear Punch Codes

Pattern for ear punch numbering system



Drawn by William Ober (2001)

From page 86, Figure 31 in Manipulating the Mouse Embryo, A Laboratory Manual, 1986, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Appendix B:
Avertin Chart

AVERTIN

2.2.2-TRIBROMOETHANOL (AVERTIN)	ALDRICH CAT#T4,840-2	25G	$26.35
TERT-AMYL ALCOHOL	ALDRICH CAT #24,048-6	100ML	$16.20

AVERTIN STOCK SOLUTION:

GOOD FOR SEVERAL MONTHS. KEEP IN DARK CUPBOARD. ADD 25 ML TERT-AMYL ALCOHOL TO 25 GM BOTTLE OF AVERTIN. USE THE BOTTLE IT COMES IN. AVERTIN DISSOLVES SLOWLY. SHAKE EVERY FEW MINUTES. THIS PROCESS MAY TAKE A HALF HOUR OR MORE. YOU CAN SPEED UP THE PROCESS BY PUTTING IT INTO A WARM WATER BATH.

AVERTIN WORKING SOLUTION

BEYOND-USE DATE IS 1 MONTH AFTER MIXING. KEEP IN REFRIGERATOR. MIX 2.5 ML OF AVERTIN STOCK SOLUTION INTO 97.5 ML OF STERILE SALINE. PUT INTO DARK BOTTLE OR WRAP BOTTLE IN FOIL. BE SURE TO LABEL YOUR BOTTLE AND DATE. (2.5% SOLUTION)

USAGE

WARM YOUR WORKING SOLUTION TO ROOM TEMPERATURE BEFORE USING AND SHAKE SEVERAL TIMES WHILE WARMING.

INJECTIONS OF AVERTIN ARE GIVEN IP (INTRA PERITONEAL).

DOSE: 0.020-0/.026 ML/GM BODY WT.

IF DESIRABLE PLANE OF ANESTHESIA IS NOT APPARENT IN 5-8 MIN. YOU MAY GIVE MORE ANESTHESIA UNTIL DESIRED PLANE OF ANESTHESIA IS REACHED--GO UP IN INCREMENTS OF 0.002 ML/GM

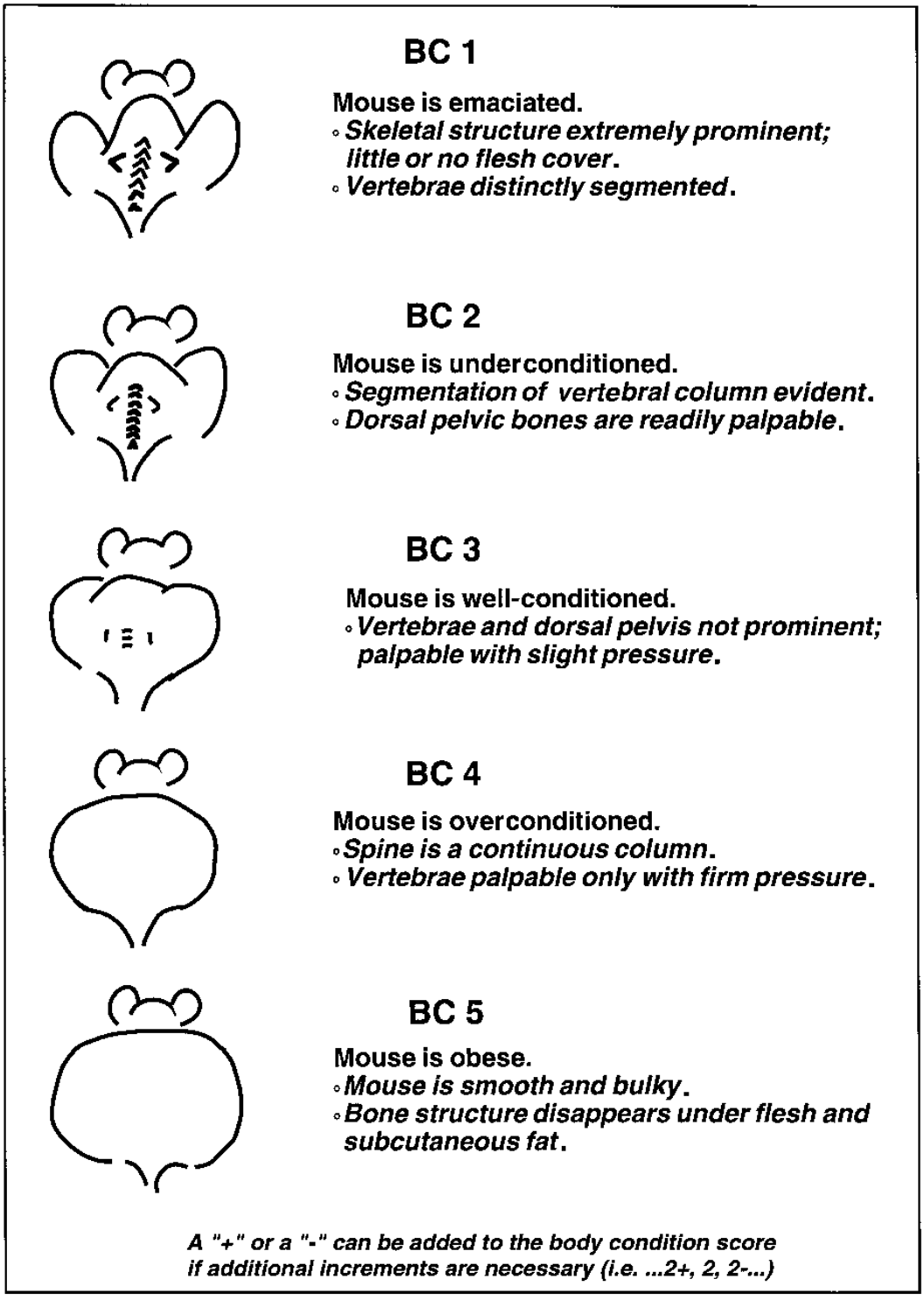
NOTE: pH OF THE SOLUTION SHOULD BE ABOVE 5. IF NOT, MAKE UP NEW SOLUTION. EFFECTS MAY VARY WITH AGE OF SOLUTION.

References:

Lieggi CC, Artwohl JE, Leszczynski JK, Rodriguez NA, Fickbohm BL, Fortman JD. Efficacy and safety of stored and newly prepared tribromoethanol in ICR mice.Contemp Top Lab Anim Sci. 2005 Jan;44(1):17-22.

Lieggi CC, Fortman JD, Kleps RA, Sethi V, Anderson JA, Brown CE, Artwohl JE. An evaluation of preparation methods and storage conditions of tribromoethanol.
Contemp Top Lab Anim Sci. 2005 Jan;44(1):11-6.

Appendix C:
Body-condition (BC) scoring



From "Body Condition Scoring: A Rapid and Accurate method for Assessing Health Status in Mice" Laboratory Animal Science, June 1999.

Appendix D:
Decapitation Notes

Some text borrowed from: http://iacuc.ufl.edu/Policy%20for%20Decapitation%20of%20Rodents.pdf.

Important:

DO NOT perform this procedure unless properly trained and authorized.

DO NOT perform this procedure unless approved as part of an IACUC protocol.

DO NOT depress the guillotine lever unless the rodent's head is **fully engaged in the guillotine**.

DO NOT depress the guillotine lever unless the rodent's head is **immobile**.

DO NOT depress the guillotine lever unless **your finger**s are **out the way.**

DO NOT depress the guillotine lever unless you are confident that the rodent's head will be removed in one clean stroke.

DO NOT allow any distractions in the room during this procedure.

Decapitation Procedure:
1) Procedure should be performed in a room that is isolated from all other rodents.

2) The guillotine will be placed upon a clean and stable benchtop or other stable surface, and the sharpness and smooth operation of the guillotine will be verified before introducing any rodent.

3) The rodent will be removed from its home cage, or experimental environment, and carried to the guillotine. The researcher will make every effort to adjust the transport of the rodent until it appears calm (note that the affective state of the animal may be determined by the experimental conditions). Although not required, use of de-capi-cones is suggested.

4) The researcher will hold the rodent securely, and place the rodent on the stage at the entrance to the guillotine, then gently and assertively move the head forward until the neck is directly above and below the upper and lower blades.

5) When the head is in position, pause momentarily and **verify the head is completely through the opening** of the guillotine, and that your hand and **fingers are clear of the blade path**.

6) Smoothly, quickly, and assertively depress the guillotine lever, decapitating the rodent.

Comments: Sometimes – depending on the method of restraint, rodents will put their arms/paws through the opening with the head – this does not matter. It is better to minimize stress to the animal-- just proceed with the decapitation, don't spend any time trying to get the paws out.

Another comment: Some people who have performed this procedure before recommend gently swinging or swaying the animal to disorient it prior to decapitation. I do not recommend this.

Care of the Guillotine:
1) After each decapitation, rinse and/or wipe down the guillotine and surrounding area to remove all blood and tissues.

2) At the end of each day of use, thoroughly wash the guillotine with detergent and water, and dry it. After drying, oil the moving parts with light machine oil (e.g. 3-in-1 oil), and run the blade up and down several times to spread the oil.

3) Sharpen or replace blades whenever they are dull. Contact either the vendor of the guillotine for specific instructions, or the WaNPRC Machine Shop on the main UW campus will perform this.

Also see UW IACUC Policy: Euthanasia of Research and Teaching Animals

Contact the WaNPRC Machine Shop at UW:
(206) 543-1039
grega@uw.edu
http://auk.wanprc.org/services/

Appendix E:

Preventing Overcrowded Cages when **Trio Breeding**