Laboratory Checklist for Animal Research

This is a list of basic requirements of the University of Virginia Animal Care and Use Committee (ACUC) for performing animal research. Review the requirements to ensure compliance in preparation for laboratory audits, ACUC semi-annual inspections, or AAALAC site visits. These requirements are based on ACUC policies, PHS *Policy on Humane Care and Use of Laboratory Animals*, *Guide for the Care and Use of Laboratory Animals*, USDA *Animal Welfare Act*, and AVMA *Guidelines for the Euthanasia of Animals* – links are at the end of the document as a reference.

	PROTOCOL and PERSONNEL
	The animal use protocol must be **approved** and accurately describe *all of the procedures* performed on animals.
	A current version of the approved **protocol must be available** as a reference for all animal handlers. A hard copy must be in the laboratory **or** animal handlers must know how to *easily* access the protocol online (https://researchcompliance.web.virginia.edu/acuc/).
	Animal handlers must **read the approved protocol** and be aware of the procedures listed and understand that those are the *only procedures* that can be conducted without a protocol modification.
	Personnel should be aware of the roles of the ACUC (protocol modifications/approval), OAW (animal handler training/compliance/policies/controlled substances), and CCM (animal procurement/housing/husbandry/technical support).
	Personnel are responsible for adhering to all **ACUC Policies** and have an understanding of where to locate them (https://researchcompliance.web.virginia.edu/acuc/pi/policy/iacuc_policies.html).
	Report observed or suspected animal abuse, mistreatment, or non-compliance with approved protocols, University policy, local, state, or federal regulations. Concerns can also be made anonymously. Concerns should be reported to OAW, ACUC, or CCM (https://research.virginia.edu/office-animal-welfare/reporting-animal-welfare-concerns).
	Personnel must be listed on an approved protocol as an **animal handler before working with animals**. Unapproved personnel are not permitted to touch animals or enter the vivarium unless accompanied by an approved animal handler.
	New animal handlers **must attend** the Animal Research Orientation Seminar (two-part requirement) and **complete** all online training modules specific to their protocol.
	Animal handlers must be thoroughly **trained** in **each procedure** they will be performing, and be able to demonstrate proficiency upon request.
	Animal handlers must enroll in the **Occupational Health Medical Surveillance Program** and be currently "OK FOR WORK".
	Ensure strict adherence to occupational health and safety practices when animal use occurs in the laboratory and **educate individuals with indirect exposure (non-animal handlers) of the potential risks** - *allergens* associated with indirect exposure to animals or animal products; *hazardous chemicals* associated with anesthetic gasses and disinfectants; *infectious disease* transmissible between animals and humans (zoonoses). (https://research.virginia.edu/sites/vpr/files/2019-07/ohindirectexposure.pdf)
	Individuals who directly participate in an animal use activity, but who by the nature of their employment status or involvement in a project, are not listed as an animal handler must complete and submit a **Risk Acknowledgment Form** to the ACUC prior to beginning work on animal study. https://research.virginia.edu/sites/vpr/files/2019-07/RISKACKNOWLEDGMENTFORM08-03-09.pdf
	All **rooms** (outside of the vivarium) where ***LIVE* animals are taken** must be listed in the protocol (even if taken for euthanasia).
	Animal transfers between **protocols** for different or the same PI must be submitted and approved using the CCM online system. Sharing of live animals is not permitted without approval. https://researchcompliance.web.virginia.edu/ccm/index.cfm
	If a **PI** plans to be **absent for 3 or more months**, the PI must officially designate an individual (faculty) who will be responsible for the laboratory in their absence and submit notification to the ACUC Office.
	Pets/non-research animals are prohibited in labs designated as animal research areas. Personnel who have exposure to rodents outside of the workplace should be aware of the potential transfer of pathogens to/from research animals and follow designated procedures to minimize risks.
	Visual images of research animals are restricted to educational/instructional and research purposes only. Posting of animal images on a publicly accessible website requires ACUC approval. Posting of any information regarding animal use at the University on any social media website is strictly prohibited. No filming or photographing by the news media may take place without prior approval and guidance from University Public Relations and ACUC.

SAFETY	
	All animal handlers should know how to **report animal bites** or **work-related injuries** to their immediate supervisor and how to contact UVA WorkMed (243-0075) or Student Health (924-5362) for treatment.
	NO food or drink in proximity to areas where animals are used or housed.
	Gloves and protective clothing must be used at all times when handling animals.
	Rodent cages must have **filter tops** on them at all times for protection of the animals and to minimize allergen exposure to personnel. Alternatively, cages can be kept in a biosafety cabinet or chemical fume hood.
	All work involving ABSL-2, ABSL-3, human-derived material, radioactive material, and hazardous chemicals must have **prior approval by the ACUC** and the respective safety committee.
	Personnel working with hazardous materials must undergo appropriate **training as required by EHS**.
	Personnel working with hazardous materials within animals must complete the Animal Facility – **Animal Biosafety Level 2 Procedures** online module and associated **tour** with the vivarium supervisor prior to ABSL-2 access.
	Proper **PPE** (personal protective equipment) must be worn when working with hazardous animals or materials.
	All forms of **hazardous communication** must be employed for animal cages with biohazards, hazardous chemicals, or radioactivity – notify the vivarium supervisor prior to experiment; individually label each cage with hazard sticker; post appropriate door signs. Remove hazardous communication in the vivarium when hazard no longer exists.
	Follow appropriate **disposal guidelines** for hazardous *materials and carcasses* biohazard - http://ehs.virginia.edu/biosafety/bio.waste.html chemical - http://ehs.virginia.edu/ehs/ehs.chemicalsafety.cwc.html radioactive - http://ehs.virginia.edu/ehs/ehs.rs/rs.waste.html
	Biosafety cabinets and fume hoods should be **certified** annually or if relocated/repaired.
	Precision anesthetic gas vaporizers and/or gas analyzers must be validated at least **once every three years** or anytime a vaporizer is placed back into service after a year of no use. Maintenance record (sticker) should document validation.
	Anesthetic gases (e.g., isoflurane, methoxyflurane) must be properly **scavenged** or used in a fume hood.
	Activated charcoal canisters (F/AIR, VAPORGUARD) must be **weighed regularly** and within specs (dispose after 50 grams). Full canisters should be treated as hazardous chemical waste and **picked up** by EHS.
	Charcoal **canister vents must not be blocked** (F/AIR canisters must be hung in order to keep vents uncovered on the bottom of the canister). Soda-sorb should not have color changes.
	Gas cylinders must be adequately **secured**.
	Disinfectants must be labeled and maintained in accordance with manufacture instructions to maintain potency.
	Sharps boxes must be replaced when **3/4th full**. Contact EHS for pickup (2-4911).
	Eyewash station testing/flushing should be performed regularly and documented on eyewash station tag.

ANIMAL WELFARE	
	Animal handlers should know how to contact veterinary staff during regular business hours, after hours, weekends, and holidays (https://research.virginia.edu/center-comparative-medicine/organization-staff).
	Animal handlers **must know the clinical signs of pain** for the species to which they are working.
	Analgesia (or euthanasia) must be promptly provided to all animals demonstrating pain or suffering, unless specifically exempted as a Pain Category E experiment.
	Sick or injured animal(s) with weight loss of 20% or more must be noted on the **vivarium health report** or euthanized.
	Animal handlers **must know the humane endpoints (criteria for euthanasia)** approved in the protocol and euthanize animals appropriately.
	Clinical monitoring must be performed and documented for Pain Category E experiments and as required by the protocol. Frequency of observations and assessment criteria must be in accordance with protocol.
	Experimental endpoints, approved in the protocol, **must** be followed.
	Animal handlers must follow the **approved methods of euthanasia** described in the protocol.
	Death must be confirmed in all euthanized animals before disposal (i.e., by cervical dislocation, removal of vital organ, bilateral thoracotomy, exsanguination, decapitation, or perfusion).
	Cervical dislocation or decapitation **without anesthesia must be approved** in protocol except when performed on neonatal mice less than 5 days of age.
	Cervical dislocation is **not** permitted in rodents larger than 200gm, even to confirm death.
	Guillotines, when used on *live* animals (including anesthetized ones), are required to be sharpened annually ***OR*** after 250 animals. Annual sharpening must be documented with a "Guillotine Maintenance Record" *sticker*. When sharpening is based on animal use (after 250 animals), then a *usage log* must be kept designating species and animal use numbers.

	Guillotine weight limit for rats is 400gm. Decapitation using shears is not recommended for rodents <5 days of age.
	Carbon dioxide (CO_2) can only be used to euthanize small rodents (mice, rats, hamsters, gerbils, and guinea pigs), insectivores, and birds. Flow must be controlled by a **flow meter at 10-30% displacement**.
	For CO_2 euthanasia, the **maximum number of animals euthanized** in a mouse box at *one time* is limited to ≤10 adult mice or ≤15 pre-weanling mice per mouse cage. No more than 2 rats (200-450g) or one rat >450g per rat cage.

DRUGS, Treatments, Compounds, & Controlled Substances (CS)	
	Only the **specific drugs, methods, and materials approved in the protocol** may be used in animals, unless written documentation is provided from a veterinarian permitting and justifying their immediate use.
	Pharmaceutical grade drugs must be used if commercially available. Non-pharmaceutical grade drugs must be scientifically justified and approved in the protocol.
	Tribromoethanol (Avertin), as a non-pharmaceutical grade anesthetic and use must be scientifically justified and approved in protocol. Solution must be protected from light, refrigerated, and expires two weeks after dilution.
	Rodents should not come in **physical contact** with inhalational anesthetic solutions.
	CS in Schedules I – V require both DEA Registration and VBP CS Registration Certificate CS in Schedule VI only require only VBP. https://research.virginia.edu/compliance/compliance-programs/controlled-substances DEA Registration and VBP CS Registration Certificate **must be on file with CCM** in order to purchase CS from CCM
	CS must be kept in accordance with DEA/VBP regulations for storage and recordkeeping (i.e., inventory, usage, disposal).
	Expired unopened vials of CS **must** be sent to a **Reverse Distributer** for disposal. **Empty** vials should be disposed of properly (i.e., glass waste or sharps container).
	Compounded unusable vials of CS **must be disposed** of in the CCM Cactus Sink. Bulk vials containing less than 10% of the original volume may be disposed of through the CCM Cactus Sink.
	Vials **awaiting Reverse Distribution must be**: identified as "Expired," placed in a bag/box labeled "Do NOT Use," and stored with the other inventory until disposed of properly through the Reverse Distributer.
	Anesthetic, analgesic, and euthanasia drugs must be **used before expiration date** for **all procedures**.
	Compounding/diluting anesthetic or analgesic drugs must be performed in a manner that maintains sterility and potency. Vial must contain name of drugs in vial and expiration date (based on component due to expire earliest). Inventory and usage records must be maintained for compounded/diluted substances and have a unique identifying number.
	All **secure storage locations** of CS listed in the DEA and/or VBP Registration(s) must be included in the protocol.

VIVARIUM (Access & Facility Rules/Procedures)	
	All animal handlers requiring access to an animal facility **must complete the Animal Facility – Animal Facility Rules and Procedures (barrier) online training** module once and complete an **access tour** with the vivarium supervisor for *each vivaria* to which access is requested. Access will not be granted until training *and* tour have been completed.
	Barrier procedures must be adhered to by everyone entering a barrier facility/room, including wearing of protective clothing, disinfection of equipment, and proper handling of animals/cages.
	Species-specific physical and psychological **environmental enrichment** must be provided for all animals. Exemptions for providing environmental enrichment must be scientifically justified in the protocol.
	Housing social animals in pairs or groups is the standard method of housing. Single housing of *any animal* must be based on social incompatibility (includes end of study animals /singly housed breeders), veterinary-related concerns, or scientific justification for single housing due to an experimental paradigm.
	Singly housed animals must be provided additional environmental enrichment.
	Singly housed animals **placed in specific non-standard housing/caging** do not require social housing exemptions in the protocol or additional environmental enrichment. The scientific justification for the use of the non-standard caging must be in the protocol. The following non-standard caging types do NOT require an exemption for social housing and do not require additional enrichment: metabolic caging, cage on telemetry platform, EEG cranial headset/implant recording, CLAMS cage, plethysmography cage, operant conditioning chamber, exercise cages, or running wheel cages.
	Singly housed animals must be identified by placing a **yellow Avery dot/sticker** on the **cage card**. If/when animals are no longer singly housed; the yellow sticker should be removed or defaced. Exclusions: Yellow dots are *NOT required* if the animal is singly housed for a *current* veterinary treatment or *current* surgery cage card is present. Dots are not required for singly housed animals in specific non-standard housing/caging.
	Cage/tank cards must be present on *all cages* and contain the following information: PI, protocol number, source of animal, strain/stock, DOB or DOA (acquisition), CCM billing code, breeding information. Other useful information: sex, age, number in cage. CCM must be contacted for additional cage card labels when warranted.

	Newly received animals cannot be experimentally manipulated during the **acclimation period**. Rodents/fish/frogs – 2 days; large animals – 3 days.
	Non-standard husbandry/care must be approved in the protocol. Non-standard housing and care may include: an alternate light cycle, alternate temperature/humidity, alternate bedding or caging type (metabolic caging), alternate cage change schedule, cage changes performed by research personnel, or if there is a potential for more than one species being housed in a room with specialized equipment. Cages or entire room must be clearly marked.
	Non-standard *provision* of food and/or water must be approved in protocol. Special provisions include special diets or medicated/treated water. **Clearly label** any special food/water provision on cage with specialized quarter cards indicating type, start date, and end date.
	Animals with **restricted or scheduled access to food and/or water** must be approved in the protocol. **Daily written records** must be maintained *in the animal holding room* for EACH animal being restricted in order to document restriction and impact on animals for the duration of the study. **Clearly label** any restricted or scheduled access cages with type of restriction, start date, and end date.
	Pre-surgical **fasting** does not require protocol approval; however other types of fasting require approval (glucose tolerance testing). **Clearly label** all animal being fasted with start date and end date.
	Animal **feed**/diet (special or standard) must be *stored in a tight-fitting container and labeled* with the feed type and expiration and mill date. Feed taken to the laboratory must follow these same requirements.
	Animals in cages or on carts must be **concealed** at all times by a drape or bag during transport outside of the vivarium or laboratory. Place animals in clean caging *before* the animal leaves the vivarium especially if the animal returns to the vivarium following surgery and/or biohazard inoculation. Public corridors and elevators should be avoided during transport.
	CCM transportation services must be used to transport animals between different animal facilities. Delivery requests *must* be submitted and approved using the CCM online system. Transporting animals to different housing location is not permitted without approval. https://researchcompliance.web.virginia.edu/ccm/index.cfm
	Animals *must* always be sufficiently **recovered** from anesthesia prior to return to the vivarium or transport pickup. Animal must be able to freely move around in the cage.
	Animals that are **returned to the vivarium from the laboratory** must be returned to the correct room ("quarantine" or "return" room outside of the barrier facility), and have sufficient food and water available unless otherwise approved.
	Empty animal cages should be promptly **returned** to the vivarium for proper cleaning.
	Animal carcasses require proper and prompt **disposal in opaque** bags into **designated** Regulated Medical Waste containers within the vivarium. *Hazardous communication labels* must remain on carcasses and placed in corresponding waste containers.

BREEDING AND WEANING	
	Unauthorized breeding of animals is not permitted. All breeding must be approved in the protocol.
	Date of Birth (**DOB**) and projected Date of Weaning (**DOW**) must be listed on the cage card for all litters.
	Approved housing densities must be adhered to according to animal age and weight. **Overcrowding is not permitted.**
	Rodents: Preferably only **one** litter per cage. Two females, each with a litter (two litters total) - the litters must be *less than a week apart in age*, *AND* when the oldest litter reaches *12 days of age,* there are *no more than 12 pups in the cage* (combined). One litter must be removed with the corresponding female as needed. A single female having >12 pups is acceptable if the female is housed in a separate cage (with or without the male).
	Rodents: No more than **one litter** at a time **per female** per cage with a maximum of two females in the cage. The previous litter must be weaned prior to delivery of the next litter for a single female.
	Mouse and rat litters must be **weaned by Day 23** after birth or by Day 21 if a new litter is imminent.
	One-time exceptions to the weaning policy can be granted by the vivarium supervisor and must be documented on exception log. Ongoing **exceptions must be approved** in the protocol.
	CCM husbandry staff will perform certain functions *if the laboratory fails to do so* and a punitive technical charge will be applied. CCM will wean animals at Day 24; will separate females + litters if the two litters have greater than 7 days between DOBs; will remove pregnant female if three females are present in single cage; and will separate females with litters if at 12 days of age there are greater than 12 pups in the cage. OAW/ACUC will be notified of repeated violations.
	Research personnel **must report animal production information** in the CCM database monthly. Animals should be counted at weaning. Rodents used prior to weaning are reported at time of genotyping **or** experimental use. Amphibians are counted after metamorphosis.

	NON-SURGICAL TECHNICAL PROCEDURES *(all methods must be described in protocol)*
	Genotyping – Mice should not be older than *28 days* and *no more than 5mm* of tail should be biopsied. Mice older than 28 days *must* receive analgesia either topically or systemically. When >5mm is required or when a single mouse is biopsied more than once, local or general anesthesia is required. Disinfect the tail with 70% ethanol prior to biopsy and monitor for hemostasis after biopsy. Use clotting agent as appropriate on biopsied tail.
	Toe-clipping – *Must be scientifically justified* in protocol. Mouse neonates must be *no more than 10 days old*. For rodents, lizards, and amphibians, a *maximum of one digit per foot is permitted, and local anesthesia is required*.
	Footpad Injections –Freund's Complete Adjuvant (FCA) is only allowed if alternative adjuvants do not result in satisfactory results. *Only one hind footpad is permitted for use*. Animals should be *monitored for pain/distress daily for a minimum of one week*. Rodents *found in pain/distress* (swelling of injected foot, non-weight bearing on foot, limping, self-mutilation of foot, etc.) *must* be placed on *soft bedding*; *administered analgesic*; *document* analgesic administration on **Protocol Treatment Card**; and monitored daily until animal can bear weight on injected foot. If lesions develop, animal must be monitored daily until lesion has healed. Lesions must be treated with oral or topical antibiotic.
	Prolonged Restraint – Procedures where animals are placed into a restraint device for **longer than 5 minutes** must be described fully in protocol. This does *NOT* include tethered animals.
	Testing equipment, euthanasia chambers, anesthesia chambers, and guillotine/scissors should be *cleaned* of blood, fur, and excrements after every use.

	TUMOR STUDIES
	Tumor(s) must *never exceed 20mm in mice* and 40mm in rats in any dimension. Calibration curves depicting typical tumor volume increases over time for a specific model are recommended. Volumetric calculations from 2 or 3 dimensions (W, L, and H) provide more stringent data.
	In rodents, **baseline weights** must be *recorded* and **weight** *must be measured weekly*.
	Tumor size measurements and **animal condition assessments** must be performed and **documented**: • After *initial* inoculation/induction - monitor at least *1X/week*; • Once tumor *growth occurs* - monitor *2X/week*; • When tumor reaches *10mm* for mice or 20mm for rats – monitor *daily*.
	Visible or palpable tumors must be evaluated using calipers.
	Tumor ulcerations require additional monitoring and possibly treatment. • *Moist* tumor lesions must be treated (and documented on Protocol Treatment card) or euthanize animal • Tumor lesions (moist or dry) that are *<4mm must be monitored + documented 2x/week* • Tumor lesions (moist or dry) that are *>4mm must be monitored + documented daily*
	Rodents **must be euthanized** if the following occur, regardless of tumor size: *inability to access food/water, severe dehydration, anemic and lethargic, hunched, 20% weight loss, or moribund*.
	Death cannot be used as an endpoint.

	NON-SURVIVAL SURGERY/PROCEDURES
	Area should be **clean** for surgeries – no dirt, blood, fur, used syringes, etc.
	Surgical area should be **free** of unnecessary equipment and supplies.
	Surgeon must **wear** gloves and protective clothing during any animal procedure or surgery.
	Surgical instruments and equipment should be **cleaned** before use and between animals.
	External heat source should be applied to maintain the animal's body temperature during surgery if > than 1 hour.
	Drugs, suture, and surgical supplies should be used before their **expiration dates**, unless used for terminal experiments, where they should be clearly labeled "for acute use only."
	Anesthetic, analgesic, and euthanasia drugs **must** be used before expiration date for **all** procedures.
	Surgeon must **monitor** the depth of anesthesia regularly and adjust as needed to maintain appropriate depth of anesthesia.
	Anesthetized animals must **never be left unattended**.
	Method of **euthanasia** used must be in protocol.

RODENT SURVIVAL SURGERY	
	There must be a **dedicated area** for survival surgery in the laboratory or vivarium.
	The surgery area **must be clean** (no blood, fur, used syringes, etc.), **sanitizable**, and **free** of unnecessary equipment and supplies. The "Rodent Survival Surgery Reminders" poster **must** be placed in surgical area.
	Surgeon must be properly **trained** in aseptic technique, surgical skills, and particular procedure being performed.
	Surgeon **must always wear** *sterile surgical gloves* (in-date), mask, head cover, and protective clothing (over street clothes).
	Surgical instruments must be **initially *entirely* sterilized** (autoclave or cold sterilant).
	Sterilized instruments must be used **within six months** of sterilization date (date packs).
	Surgical instruments should be cleaned of organic material and placed in a hot bead sterilizer or soaked in 70% alcohol between rodent surgeries performed **consecutively** on the same day.
	Surgical instrument packs and *sterile surgical gloves* must be replaced with a newly sterilized pack or re-sterilize initial pack after **five** consecutive animal surgeries performed on the same day.
	Surgical site must be sufficiently **clipped**, plucked, or depilated (for sensitive skin only) to remove hair around surgical site. Animal hair should not be present within the wound closure.
	Surgical site must be **prepped** by scrubbing with *3 alternating rounds* of antiseptic scrub and 70% alcohol. Antiseptic and alcohol solutions must be used before expiration date. *Do not over-wet* the animal with scrubbing solutions.
	Draping the rodent surgical site is required.
	Drugs, fluids, suture, and surgical supplies must be **within their expiration dates**.
	Depth of anesthesia must be **monitored** regularly and adjusted as needed to maintain appropriate depth of anesthesia.
	An external **heat** source should be applied to maintain animal's body temperature during surgery and recovery.
	Ophthalmic ointment must be applied.
	Each tissue layer must be **closed** individually with absorbable suture material.
	The skin incision must be closed in an **interrupted** suture pattern. NO silk sutures are permitted in the skin.
	The **analgesic** dose listed in the protocol must be administered either pre-op, intra-operatively, or immediately post-op, and repeated as listed in the protocol (or as recommended by veterinarian).
	Animals must be observed or **monitored** regularly until recovered from anesthesia (conscious and at least sternally recumbent). Anesthetized animals must **never be left unattended**.
	Surgery cage cards must be completely filled out and placed on cages of all post-op animals.
	Animals must be **checked** at least once daily until the incision is completely healed for at least three days post-op for minor surgeries and four days post-op for major survival surgery (enters abdomen, thorax or cranium) or for those that are severely debilitating (orthopedic, metabolic derangement, injury, trauma).
	Post-operative health problems must be **reported** to the veterinary staff.
	Non-absorbable sutures or wound clips must be **removed** within 10-14 days following surgery (unless justified in protocol or unless animal is euthanized within 14 days of surgery).
	Written records must be maintained of anesthetic administration, surgery performed, post-op care, drugs administered, and any complications. Records must be kept for a minimum of one year after the disposition of the animal.

LARGE ANIMAL SURGERY/PROCEDURES/GENERAL ANESTHESIA (includes all USDA Regulated Non-Rodent Species) *All items listed for rodent survival surgery **above apply** to larger species, and additional items listed below. Surgery cage cards are not used and new surgical instrument packs should be prepared for each large animal surgery.*	
	Mandatory **pre-planning meeting** required – new species, new technique/surgery, or new protocol.
	Survival surgery must be performed in a **dedicated surgical suite** with separate anesthetic induction/prep, surgical room, and recovery spaces. Area must be clean and sanitized regularly. ACUC sub-committee must inspect proposed dedicated surgical suite prior to approval in protocol if the location is outside of the vivaria.
	For survival surgery, the surgeon **must wear** a head cover and face mask, and wash hands with antiseptic surgical scrub preparation prior to donning a *sterile gown* and *sterile surgical gloves*.
	A written **anesthetic record** must be generated every time the animal is anesthetized. Subsequent episodes (<1hr) for same animal on different date can be recorded on same record noting time (start and completion), date, and type of procedure. Record must include: PI, protocol #, species, animal ID, description of procedure, and name/phone # of contact person. When anesthetic records involve surgery, the surgeon's name must be included.

	After anesthetic induction, body temperature, heart rate, respiration rate, and depth of anesthesia must be taken and recorded at a minimum **every 15 minutes**. Other useful parameters – mucous membrane color, capillary refill time, character of respirations and heart sounds, hydration state, pulse oximetry, blood pressure, arterial oxygen saturation, ECG, and end-tidal CO_2. Changes in anesthesia or administration of additional medications must be recorded.
	Anesthetic records must document **all medications** administered (route, dose, time) including sedatives, anxiolytics, antibiotics, and analgesia. Administration of intra-operative fluids should be noted (type, rate, total volume given). Pre-emptive analgesia must be given and recorded. All acute post-op manipulations must be recorded.
	An external **heat** source should be applied to maintain animal's body temperature during procedures lasting longer than 15 minutes and during recovery.
	Continuous monitoring is required for the *entire period* that the animal is unconscious. Once swallowing reflex is regained and animal is extubated, the monitoring requirement is reduced from continuous to every 15 minutes until animal is self-righting or is conscious. Written assessments should be recorded every 15 minutes.
	Prevent collapse of dependent lung of unconscious animals in lateral recumbency by rotating animal at least once every 30 minutes to lie on other side.
	Following acute post-op period **until 72-96 hours** following invasive procedure, animals should be checked at least once daily and more frequently if clinically indicated. These observations are in addition to animal care staff observations. Assess attitude/behavior due to pain/distress, activity, food/water consumption, urine/feces production, incisional issues, swelling, and inflammation. Observations are documented on post-procedural monitoring records.
	Post-procedural monitoring records must be maintained and include time of observation, any abnormal findings, administration of drugs, and observer's name and initials. Abnormal findings should include a follow up plan of action and resolution. Treatment plans should include diagnosis, type, frequency, and duration of treatment, and schedule for re-evaluation. Veterinarian must be consulted.
	Animals are "discharged" from post-operative care program at the end of the 72-96 hour period and return to normal housing procedures.
	Euthanasia route, dose, date/time, and reason must be recorded on the anesthetic record (non-survival procedures), post-procedural monitoring records, or on the CCM Daily Observation Record.
	Anesthetic, surgical, and post-operative care records **must stay associated with the animal** (typically in the vivarium). After final disposition (death, euthanasia, shipment, transfer, etc.) of animal, health records must be **retained for at least 1 year** either by the researcher or veterinary staff. All components of the health record **must be readily available for inspection**.

TEMPORARY (SATELLITE) HOUSING FACILITIES	
	ACUC sub-committee must inspect proposed temporary/satellite space **prior to approval** in protocol.
	Any area in which rats and mice (purpose bread for research), fish, birds, or amphibians are maintained for **24 or more hours** outside of the vivarium (or inside of the vivarium and maintained by research staff) must be listed as a temporary housing location on an approved protocol.
	Any area in which USDA regulated species (hamsters, gerbils, guinea pigs, rabbits) are maintained for **12 or more hours** outside of the vivarium must be listed as a temporary housing location on an approved protocol.
	Animals must be observed and monitored **daily,** including weekends, holidays, and during agency closings.
	Appropriate **light** cycles must be provided.
	Daily observations **must be recorded** on a husbandry log. Log must include: daily temperature and humidity; daily health observations; feeding and watering; and cage changing. Animal observation/husbandry record *must be sent* to CCM weekly. Records must be maintained for one year.
	Food must be stored in a container with a tight-fitting lid (e.g. Tupperware) and labeled with the food type and milling date or expiration date.
	Sick animals or animals experiencing unanticipated pain/distress must be reported to the veterinary staff or euthanized in accordance with approved methods in protocol. Sick animals should be reported on daily observation log.
	Routine **cage cleaning** and changing must be performed and documented. • Rodents – change bottoms at least twice per week (single housed – once a week); water bottles change at least once per week; wire lids and filter tops change every two weeks. Clean cage components must be obtained from CCM. Do not dump cage bedding in lab. • Rodents receiving non-standard care (standard listed above) or any non-rodent species must have an **approved SOP** for husbandry practices.
	The room, or secondary enclosure, containing the animals and the surfaces harboring the cages must be wiped down as needed and **sanitized** with disinfectant solution at least every two weeks, and documented on logs. Sanitation should be monitored in some manner for efficacy (CHARMs testing).

	Environmental enrichment must be provided to animals while in temporary housing locations.
	A signed **Memo of Understanding** (MOU) must be posted in room if located within a CCM vivarium space.
	Report animal use to OAW semi-annually for reporting purposes.
	GFI electrical outlets must be installed and potential electrical hazards must be eliminated if using water in behavioral testing (no extension cords).

PI-MANAGED PERMANENT ANIMAL HOUSING FACILITIES *All items listed for temporary housing facilities above apply **and** additional items listed below*	
	Area is scientifically justified and approved in protocol. May be within confines of CCM-managed vivarium or outside of the CCM-managed vivarium; however, PI & laboratory provides all husbandry and maintenance of the space. Animals are never returned a CCM-managed vivaria.
	Husbandry SOPs, Disaster Plan, and Environmental Enrichment Plan are approved by the ACUC initially **and** every three years. Recently approved versions should be available in the space for reference for animal handlers and inspectors/visitors.
	All animals **must** be maintained in accordance with Guide, Animal Welfare Act, UVA PHS Assurance, ACUC policies and procedures, and PI SOPs.
	Daily **logs** of animal health observations, husbandry, and mortality must be maintained similar to temporary housing requirements described above.
	Proper **quarantine** procedures must be used for sick animals. Records of treatment should be available.
	Report animal use to OAW semi-annually for reporting purposes.

AQUATIC SPECIES *(additional requirements)*	
	Proper **water quality** must be maintained and recorded. Un-ionized ammonia must be calculated.
	Chlorine, chloramines, chemical and reactive bi-products must be removed or neutralized prior to use in aquatic system.
	Bio-filter must be of sufficient size to process bio-load.
	GFI electrical outlets must be installed and potential electrical hazards must be eliminated (no extension cords on wet floors or over open tanks of water).
	Nets must be cleaned, disinfected and managed to avoid contamination of system.
	Aseptic technique must be observed for all survival surgeries, including proper attire, equipment sterilization, and animal prep.
	Proper anesthesia, routine analgesic administration, animal recovery, post-op monitoring, and euthanasia procedures must be followed with animals undergoing survival surgery.
	No more than 6 oophorectomies are permitted on any single frog - with sufficient recovery time between surgeries. Individual animal identification and surgical records must be available for inspection.

Resources:

UVA ACUC Policies - https://researchcompliance.web.virginia.edu/acuc/pi/policy/iacuc_policies.html

PHS *Policy on Humane Care and Use of Laboratory Animals* - http://grants.nih.gov/grants/olaw/references/phspol.htm

Guide for the Care and Use of Laboratory Animals – 8th Ed. - http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf

USDA *Animal Welfare Act* - http://www.nal.usda.gov/awic/legislat/awa.htm

APHIS Animal Welfare Inspection Guide - https://www.aphis.usda.gov/animal_welfare/downloads/Animal-Care-Inspection-Guide.pdf

NIH OACU guidelines - http://oacu.od.nih.gov/ARAC/index.htm

AVMA *Guidelines on the Euthanasia of Animals*, 2020 - https://www.avma.org/KB/Policies/Documents/euthanasia.pdf