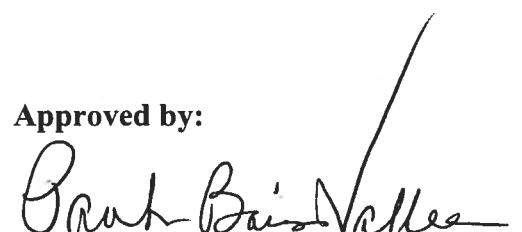


Lifespan System-wide Policy	Subject: Lifespan Policy for the Responsible Conduct of Animal Research and Use of Central Research Facilities	File Under: ORA CRF 001
Issuing Department: Lifespan Office of Research Administration		Latest Revision Date: April 9, 2018
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Revised November 19, 2014		 Peggy McGill, MA, CRA, Vice President, Research Administration
Revised January 1, 2017		
Revised April 9, 2018		

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- I. Purpose:** The Purpose of this Policy and Procedure Manual is to define and describe the policies and procedures regulating the use of animals in research and the appropriate utilization of the Central Research Facilities
 - II. Eligibility:** The entire research community of The Rhode Island Hospital (collectively known as Lifespan for the purposes of this manual)
 - III. Content:** The Manual is attached

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- 1. ORA Organizational chart**
- 2. Zoonosis of Concern in Animal Care Facilities**
- 3. Selection and Use of Anesthesia and Analgesia**
- 4. Guidelines for Rodent Survival Surgery**
- 5. Post-Op Animal Treatment Form**
- 6. Animal Health Program**
- 7. Cage Card Sample**
- 8. Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols**
- 9. Cadaver and/or Animal Parts Form**
- 10. Tumor Monitoring Form**
- 11. Notice of Intent to Use Avian Embryos Form**

I. Purpose and Scope of Manual

The purpose of this manual is to provide researchers with an overview of responsibilities in conducting animal research at Lifespan, as well as details in procuring, housing and other aspects of animal care. In addition, we have provided details in safe working practices in the Central Research Facilities (CRF).

All research at Lifespan that involves animal subjects must be reviewed and approved in accordance with federal law and Lifespan policy. The Animal Care and Use Program at Lifespan is consistent with the [Guide for the Care and Use of Laboratory Animals \(the Guide\)](#), the [Public Health Service Policy on Humane Care and Use of Laboratory Animals \(PHS Policy\)](#) and the [Animal Welfare Act Regulations \(AWRs\)](#).

Lifespan's Animal Welfare Committee (AWC) or Institutional Animal Care and Use Committee (IACUC) is charged with overseeing compliance with these federal regulations. The goal of these regulations is to ensure the safety, respect, and dignity of animal subjects involved in scientific research, and is a cooperative effort between the IACUC, Administration, Principal Investigators (PI), laboratory staff, and animal care staff. Details regarding the Animal Care and Use Program, IACUC function, operation, and review requirements are included in the [Lifespan Institutional Animal Care and Use Committee \(IACUC\) Policy and Procedure Manual](#), ORA RRC 002, IACUC, November 2014. See also [Appendix I- ORA Organizational Chart](#).

All forms and additional guidance and informational links may be found at
<http://www.lifespan.org/research/administration/animal-research.html>.

II. Description of the Lifespan Facilities

The Central Research Facilities (CRF) consists of 19,000 net sq. ft. in the following four functions: Central Animal Facilities (CAF); Washing/Sterilizing Facilities; Operating Rooms/Veterinary Services; and Research Operations. The CRF functions are located at Rhode Island Hospital (RIH) in the Middle, Aldrich and Nursing Arts Buildings; the Claverick Street Building; and the Coro West and Coro East Buildings.

Lifespan has an Animal Welfare Assurance on file with OLAW. The Animal Welfare Assurance number is A3922-01. The USDA license # is 15-R-0002, issued 7/31/1967.

The Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs – Lifespan's institutional accreditation by AAALAC dates to May of 1970. (The original accreditation was for The Miriam Hospital; Rhode Island Hospital first received accreditation in 1996 after the two hospitals were joined under the Lifespan parent organization in 1994). AAALAC International has continued full accreditation for Lifespan's Animal Care and Use Program under file number 205.

The facilities are monitored by a variety of security measures and entrance into the CRF is by permission only.

III. Training and Orientation Procedures

A. Requirement

The Animal Welfare Act and Public Health Service Policy requires that research facilities ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use be qualified to perform their duties. In addition the qualifications of the personnel must be reviewed frequently enough to assure continued compliance. This training and instruction must include guidance in the following:

- Humane methods of animal care and use,
- Methods to limit the use of animals or minimize animal distress,
- Proper use of anesthetics and analgesics,
- Methods to report deficiencies in animal care and treatment
- Utilization of information services, e.g., the National Library of Medicine and the National Agricultural Library.

B. Implementation

The hospital has implemented a formal educational program on animal care and use to assure compliance with these requirements. The IO delegates the responsibility for the implementation and the continued development of this program to the Director of the Central Research Facilities and the Attending Veterinarian. The educational program has been approved by the Institutional Animal Care and Use Committee and is reviewed semi-annually as part of its responsibility to review the Animal Care Program.

- *All personnel involved in animal research, in any capacity, must attend the CRF orientation/training. All newly hired research investigators, personnel, volunteers and students must contact the CRF office at 444-5788 to schedule training and orientation.*

At the time of initial contact, the CRF user will complete a request for Laboratory Animal Procedures and Privileges (LAPP) as well as the Health Surveillance Questionnaire (HSQ). The content and delivery of the training/orientation will be determined by the CRF management.

Additional training in anesthesia, aseptic surgery techniques and the use of the operating room for surgery requests must be requested by the research personnel by contacting the Operating Room Supervisor at 444-6366. Mandatory training for use of the autoclave units may be arranged by contacting Vet Services through the CRF Office at 444-5788.

An annual training refresher is required for anyone utilizing animals. The training is customized for rodent users or large animal users. Annual Training is available on-line at CITI (Collaborative Institutional Training Initiative) www.citiprogram.org. Training completion dates are recorded on the Animal Care and Use Protocol form (ACUP) and/or the annual progress report forms for continuing review and are verified by the IACUC Coordinator during the pre-review process.

C. Educational Program

The program is intended to assure the continued excellence in animal care and scientific investigation as well as to comply with all federal, state and local regulations concerning animal related research. Assistance and guidance are provided through various forums including: (1) an Introduction/Orientation to the Central Animal Facilities, (2) veterinary consultation with the Principal Investigators during the preparation of a new Animal Care and Use Protocol (ACUP); (3) individual or group instruction on specific animal use techniques; (4) continuing education; (5) training for new animal care technicians. Additional details concerning these forums follow.

- 1. Introduction/Orientation:** All personnel using animals at Rhode Island Hospital or submitting ACUPs are required to attend an Orientation meeting at the Central Research Facilities (CRF). At that time, a PowerPoint Presentation will be given which includes an overview of the federal regulatory and accreditation agencies.

Each person is instructed on the methods for reporting deficiencies in animal care and treatment and is provided a link to the website where the CRF Policy and Procedures Manual resides. The orientation packet includes the RIH policy on humane animal care and handling, general rules and procedures in the animal facilities, reference tables for typical laboratory animal species and membership rosters for the IACUC and the Institutional Biosafety Committee. A Lab Animal Privileges and Procedures Training Documentation Form of each person's past experience with animals is completed. This form must be kept accessible in the laboratory and updated as new training is completed. After the orientation presentation, a tour of the Animal Facility is given.

- 2. Preparation of a new application:** The Veterinarian provides consultation to the investigators during the planning and implementation of animal use proposals, which the Principal Investigator then indicates on the ACUP application form prior to submission to the Institutional Animal Care and Use Committee. This consultation is used to advise the investigator on the selection of experimental models, including consideration of alternatives to painful procedures; give directions and recommendations for the use of anesthetics, analgesics and euthanasia methods, and the prohibition of the use of paralytics without anesthesia. The Attending Veterinarian also makes an assessment concerning the qualifications and training of the investigator and staff to provide humane care for the animals and to perform the procedures so that pain and distress will be minimized.
- 3. Individual or Group Instruction:** Veterinary Services provides instruction on humane methods of animal maintenance, restraint, and experimental technique as needed or at the request of a person or laboratory. Areas of interest might be common technical procedures including various methods for giving injections, blood sampling or orogastric gavage. Veterinary Services provides instruction on aseptic surgical techniques or anesthesia. These personnel can be contacted through the CRF (444-5788).
- 4. Continuing Education:** Information is provided to the research community through internal memos. Issues addressed would include changes in the Animal Welfare Act, NIH

guidelines for the care and use of laboratory animals, publications of the National Agricultural Library or the National Medical Library, and information on animal issues obtained at national and regional seminars. The Central Research Facilities also has numerous books and other materials which can be consulted. Webinars are offered by CRF (AALAS webinars or similar) and by the IACUC (OLAW webinars or similar). Members of IACUC are provided with educational materials and reprints. As noted in B above, the IACUC requires annual training for every person involved with the care and/or use of animals.

5. Training for Animal Care Technicians: Each technician, including volunteers and interns, receives extensive one-on-one instruction on proper care and handling of each species housed at the hospital prior to receiving work assignments. The majority of the Animal Care Technician training is provided by Veterinary Services and CRF Managers. Volunteers and interns receive their instruction while under the direct supervision of their assigned Animal Care Technician. The Attending Veterinarian and/or investigators present specific animal requirements to the staff and discuss zoonosis, radiation or toxic hazards that may be involved in animal research. Presentations on animal models or AALAS webinars are also offered. All technicians are strongly encouraged to attend continuing education and seek certification by the American Association of Laboratory Animal Science.

6. Use of Animals in Training Courses: Instructors (or appropriate designees) of any course involving animals must attend the Central Research Facilities (CRF) Orientation. Contact Central Research Facilities (444-5788) to make arrangements for orientation.

Students of any courses involving animals may attend a shortened orientation as long as their contact with animals is limited to procedures under the direct supervision of the instructor (or appropriate designee). Instructors (or appropriate designees) should inform all participants of the existence of the IACUC, that this course has been approved by the Committee and that anyone is welcome to discuss the hospital's animal care and use policies with the IACUC Chair, Director of CRF or Attending Veterinarian if they have any questions.

Pre-op preparation of large animals for procedures and post-op care, if any, is the responsibility of the Research OR/Vet Services. A tutorial on rodent surgical techniques is available on-line through the AALAS Learning Library or can be discussed with the veterinarian. Training by field experts may be considered. Contact the CRF (444-5788) for access to the Library.

7. Health Surveillance and Training Requirements for RIH Research/Training Course Staff

The following table provides an overview of the training required for faculty and staff involved in research or training at Lifespan (or Women & Infants) that involves the use of animals or animal tissue.

	Principal Investigators		Research Staff			CRF Staff			IACUC Members
	Hands-On	Admin(1)	Principal Researcher	Research Assistants	Technicians	Vet Supervisors	Vet Coordinator	Animal Care Technicians	
CRF Orientation for new employees	Required	Required	Required	Required	Required	Required	Required	Required	
Annual Health Surveillance	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually	Required Annually
CITI Training Modules									
Essentials for IACUC Members									Required
Working with the IACUC	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Required Every 3 years	Encouraged	
Annual rodent training	Users (2) Annually		Users (2) Annually	Users (2) Annually	Users (2) Annually	Required Annually	Required Annually	Required Annually	
Annual large animal training	Users (2) Annually		Users (2) Annually	Users (2) Annually	Users (2) Annually	Required Annually	Required Annually	Required Annually	
Working with Mice in Research									
Working with Rats in Research									
Working with Rabbits in Research									
Working with Swine in Research									
Post-Procedure Care of Mice & Rats									
AAALAS Learning Library (4)									
Rodent Surgery	Users (2) required at initial review		Users (2) required at initial review	Users (2) required at initial review	Users (2) required at initial review	Required	Required	Encouraged	
Hands-On Procedural Training									
All procedures performed independently (documented on training form)	Required (3) at initial review		Required (3) at initial review	Required (3) at initial review	Required (3) at initial review	Required (3) at initial review			

Notes:

- 1) Administrative PIs are those who direct research or training programs, but who are not personally involved in working with animals.
- 2) Users are defined as anyone who is involved in direct hands-on use of animals, or supervising/training of others who are.
- 3) Technical proficiency must be documented on the Lifespan Animal Privileges and Procedures Training Form via signature by an expert assessor before procedures may be performed independently on live animals. Expert assessors include anyone with documented proficiency in the procedure, such as a more senior lab member (e.g. PI, senior researcher, lab manager and technician), a CRF staff member, or one of the attending veterinarians.
- 4) The AALAS Library is a subscription service. Contact the IACUC Office at 444-2093 to gain access to the library.

The Central Research Facilities Office (444-5788) can be contacted for information or assistance concerning the care and use of animals or for specific technical needs.

IV. Reporting Animal Care and Use Concerns

Individuals having concerns involving animal care and use within Lifespan facilities are responsible for reporting these concerns either through their supervisor or independently to the IACUC and can be made through various persons, e.g., any member of the IACUC, IACUC Manager/Coordinator, Director of CRF, CRF managers, veterinarians, or the Institutional Official (Vice President, Research Administration, verbally or in writing. IACUC contact information is posted on the IACUC webpage as well as provided to all researchers during their initial orientation with Central Research Facilities (CRF).

Veterinary and CRF management staff telephone numbers are posted within each animal facility. Alternatively reports may be submitted anonymously to Corporate Compliance via the Employee Response Line at 888-678-5111.

Although written concerns are more convenient to handle, complainants may not be willing to submit them in this manner. In such cases, the individuals who receive concerns should document them fully to ensure that the issues are clear and to prevent misunderstandings.

Lifespan will take appropriate steps to protect the confidentiality of those who report concerns as well as anyone against whom allegations are directed, while allegations are under investigation.

Lifespan policy prohibits unlawful retaliation against employees as a consequence of good faith actions in the reporting or the participation in an investigation pertaining to allegations of wrongdoing.

See [Lifespan Institutional Animal Care and Use Committee \(IACUC\) Policy and Procedure Manual](#), ORA RRC 002, IACUC, November 2017, Section 8.

V. Security, Safety and Biosafety within the CRF

A. Admittance to the CRF Animal Facilities

The Central Animal Facilities (CAF) are restricted areas and secured at all times. Only personnel authorized by the CRF Director are permitted into the animal facilities. No one will be given access to the CAF until mandatory training is completed and documented. The Principal Investigator must send the authorization for facility access to the CRF Manager. All keys and access materials must be returned to the CRF office upon termination from Lifespan.

Animals may be transported to and from the CAF with an IACUC protocol approval, but under no circumstances are animals to be housed outside the CAF overnight.

B. Infection Control

All persons using the facilities are required to follow the RI Hospital Infection Control Policies and to use Standard Precautions. <http://intra.lifespan.org/policies/rih/Epidemiology/>
All orientation records and updates must be documented.

All employees having contact with human blood and body fluids are encouraged to receive the Hepatitis B vaccine.

All employees working with animals must have a full primary series of tetanus and a booster (Td/Tdap) every 10 years. Rabies vaccination is available but is not required.

C. Autoclaves

The sterilization process monitoring includes the function of the sterilizer, type and method of packaging and the loading of the sterilizer. Sterilizers are monitored with a biological spore test weekly and records of the monitoring are maintained. All persons responsible for use of sterilizers must be oriented to the proper use of sterilizers and that orientation must be documented. The CRF is responsible to see that the sterilizers are monitored, and that education is documented.

1. A log must be kept by each autoclave with the names of every user as well as their instructors.
2. Every load must have a steam indicator and a steam load record log.
3. Once a week, a spore test must be run with a normal load. The spore test pack is sent to the Veterinary Services office. The spore test pack is placed in an incubator for the appropriate process time.
4. The spore test results are logged by Veterinary Services and sent quarterly to the Infection Control Department along with preventative maintenance service reports. These reports are kept on file in the Veterinary Services office.
5. The CRF has established a maintenance service agreement. Preventive maintenance is performed regularly on all sterilizers. All preventive maintenance documentation is on file in the CRF. A log is kept for the service visits.

D. Animal Biosafety Criteria:

The hospital safety officers are charged with enforcing biosafety guidelines. In general, investigators are required to follow the recommendations presented in Section IV of the *Biosafety in Microbiological and Biomedical Laboratories Manual*, published by the Centers for Disease Control and The National Institutes of Health.

<http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf>. These recommendations describe four combinations of practices, safety equipment, and facilities for experiments on animals infected with agents, which are known to or believed to produce infections in humans. These four combinations, designated Animal Biosafety levels 1-4, describe animal facilities and practices applicable to work on animals infected with agent assigned to corresponding biosafety levels 1-4. The high confinement requirements for Animal Biosafety levels 3 and 4 cannot be met at any of the RIH facilities.

E. Personnel Occupational Health Program (POHP)

Personnel hired to work in the Central Research Facilities (CRF), or any biomedical research area, are given pre-employment physical examinations by Employee & Occupational Health Services (EOHS).

1. Pre-Employment Phase

Each job applicant for a CRF or biomedical research laboratory position will receive the standard pre-employment medical examination at the Employee & Occupational Health Services (EOHS). In addition, the following examinations may also occur: history for allergies especially to animals and animal by-products, a history of orthopedic problems, e.g. bad backs, knees and problems preventing lifting, carrying, reaching and stretching in job context, and medical evaluation for ability to wear respirator masks.

2. New Employee Phase

Before assignment to animal care duties, all new biomedical lab personnel will be immunized against tetanus (or provide written evidence of recent immunization or booster), scheduled for a hospital orientation and receive departmental training. The CRF Operating Room and the Cardiovascular Research Department utilize fluoroscopy for procedures. All personnel who operate the fluoroscopy units are required to contact the Hospital Radiation Safety Office for specific training and hands-on instructions.

3. Daily Operations Phase

- a. Personnel showing signs of non-work related illness during the work day may be referred to EOHS for treatment. Clearance from the EOHS is required before the technician can return to work.
- b. Job injuries or illness recognized or otherwise occurring during the work day, including all animal bites and scratches, will be reported immediately to the laboratory supervisor and referred promptly to the EOHS. A formal detailed record of diagnoses and treatment activity will be maintained by the EOHS of each incident. Clearance from the EOHS is required before the technician can return to work. A copy of each incident report will be sent to the Lab Supervisor.

4. Volunteers and Students

Volunteers / students working in a research laboratory with protocols using animals are also covered by the POHP. Volunteers/students receive an interview at the time orientation to assess history for allergies, especially to animals and animal by-product, history of orthopedic problems, i.e. bad back, knees and problems relating to lifting, carrying, reaching and stretching, and evaluation for ability to wear masks.

One or more of the following services will be provided or offered according to the area and the animals the volunteer / student will be working with: health screening, hepatitis B vaccine, TB surveillance, tetanus toxoid.

5. Contractors and Visitors

Non-RIH/Lifespan contractors and visitors entering the CAF must follow all PPE requirements. If a respirator is needed, that person must be cleared by their employer's occupational health program.

F. Standard Precautions

Standard Precautions includes the following elements and must be followed by **ALL PERSONNEL AT ALL TIMES**. These precautions apply to contaminated medical equipment. Body substances included in standard precautions are: blood (human and animal), urine, stool, oral secretions, wound and tissue. The precautions take into consideration the degree and risk of exposure. Appropriate judgment must be used in determining the protective measures needed for maximum protection.

1. Wear gloves whenever hands will be in contact with blood or body substance (blood, urine, stool, oral secretions, wound or other drainage, or tissue). This includes all contact with animals or soiled animal equipment. Discard gloves and perform hand hygiene.
2. In the event of an accidental skin exposure, hands or other exposed areas must be washed with soap and water as soon as possible.
3. Care must be taken to avoid needle stick injuries. Used needles must not be recapped or bent, but must be placed in the puncture resistant containers designed for such disposal.
4. Report significant exposure (needle sticks, mucous membrane splash) to EOHS for evaluation and follow-up.

G. Respiratory Protection

The primary objective is to prevent potential occupational exposures caused by the inhalation of contaminated air. Central Research Facilities will attempt to accomplish this by accepted engineering control measures and practices (e.g., biosafety cabinets, changing and dumping stations). When effective engineering controls are not feasible or practical, appropriate respirators shall be used.

Respirators which are suitable for the intended purpose shall be provided to all employees. N95 respirators will be provided by Central Research Facilities when such equipment is necessary to protect the health of the employee. Full or half face respirators, or PAPR (powered air purifying respirator) will be provided by the employee's department. Central Research Facilities shall adhere to the Hospital's [Respiratory Protection Program](#) (Environmental Safety Department, policy SM-15) which includes the requirements as outlined within OSHA 29 CFR 1910.134, *et al.*

The procedures for the use and maintenance of respirators for employees while conducting their normal animal care work duties and instructions on selecting the appropriate respirator for each specific function or area/room are described below. There may be additional requirements depending on the hazard or potential exposure. In such cases, Central Research Facilities management in conjunction with the Safety Department will determine the appropriate respiratory protection in accordance with the OSHA Standards.

Animal care technicians, investigators, laboratory personnel, and CRF management staff are to don air purifying respirators depending upon work/room functions. When donning the chosen air purifying respirator, a user seal check (i.e., fit check) must be conducted prior to entering the work area.

1. Conventional animal rooms

A number of engineering controls will be implemented to limit exposure to contaminated air. These will include the increased use of ventilated cages, microisolator covers for cages, use of fan driven, HEPA filtered environmentally isolated caging units, use of portable changing stations for changing cages, and use of filtered dumping stations/hoods for dumping cages. N95 particulate respirators must be donned while dumping cages if dumping stations/hoods are not available for use. Once the dirty cages are in the washer, animal care personnel may remove their respirator.

2. ABSL2 rooms

In ABSL2 rooms, personnel are to don N95 respirators for any procedure being done in these rooms including checking cages, cage changing, opening cages, or handling the cages or animals for any reason. Each protocol requiring animals to be housed in ABSL2 rooms will be evaluated for respirator usage by the Biohazard and Laboratory Safety Committee. They may deem that full face respirators be used for handling animals that are part of certain protocols.

3. Yearly Fit Test

All personnel that wear respirators (N95 particulate, full face or other respirators) are to be fit tested yearly by the RI Hospital Safety Office. If personnel have problems wearing indicated respirators, there may be alternative respirator types/styles that may be more suitable/comfortable. In such instances, personnel should report to their supervisor who will coordinate with the Safety Office for appropriate recommendations.

H. Eye Protection

Eye protection (safety glasses, chemical-resistant goggles, or face shield) must be worn in the animal facility when a splash risk exists while handling chemicals, including detergents, disinfectants and/or hazardous materials. Use the appropriate eye protection for the kind of hazard in the work area. Ordinary prescription glasses are not considered effective eye protection since they lack necessary shielding. Safety glasses with side shields offer minimal protection; splash goggles and face shields offer greater protection for procedures involving liquids. Chemical-resistant goggles can be worn over the glasses. Safety glasses or chemical-resistant goggles shall be worn over contact lenses when handling chemicals. Safety glasses protect from impact. Goggles protect against impact, dust, and splashes. Face shields are generally worn over safety glasses or goggles to protect the face from dusts,

sprays or splashes. Only equipment certified by the American National Standards Institute qualifies as protective eyewear. The safety office encourages laboratory personnel to wear eye protection at all times when in a laboratory.

I. Precautions for Invasive Procedures:

The standard precautions listed above in Section F, combined with those listed below, should be the minimum precautions for ALL invasive procedures (procedures that involve entry into tissues during which bleeding occurs).

All workers who participate in invasive procedures must routinely use appropriate barrier precautions to prevent skin and mucous membrane contact with blood and other body fluids. Gloves and surgical masks must be worn for ALL invasive procedures.

Protective eye wear or face shields should be worn for procedures that commonly result in the generation of droplets, splashing of blood or other body fluids, or the generation of bone chips.

Gowns or aprons made of materials that provide an effective barrier should be worn during invasive procedures that are likely to result in the splashing of blood or other body fluids.

J. Precautions for Laboratories:

The Standard Precautions listed above in Section F, combined with those listed below, should be the minimum precautions for workers in laboratories.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
2. All persons processing blood and body fluid specimens (e.g., removing tops from vacuum tubes) should wear gloves. Masks and protective eye wear should be worn if mucous membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.
3. For routine procedures, such as histologic and pathologic studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets (Class I or II) should be used whenever procedures are conducted that have a high potential for generating droplets from open containers. These include activities such as blending, sonicating, and vigorous mixing.
4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.
5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under Standard Precautions should be followed.
6. Laboratory work surfaces must be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.

7. Contaminated materials used in laboratory tests must be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.
8. All persons should perform hand hygiene after completing laboratory activities and should remove protective clothing before leaving the laboratory.

Implementation of Standard Precautions for all specimens eliminates the need for warning labels on specimens since all specimens should be considered infectious.

K. Precautions for Personnel Working with Animals

Personnel working with animal subjects must maintain high standards of personal hygiene. Though rare, transmission of disease between animal and man has been clearly documented.

1. All personnel should wear sterile or disposable gowns over scrubs or over street clothing, gloves, or other appropriate apparel when working with animals. Laboratory apparel should be changed frequently to maintain cleanliness and minimize the potential for cross contamination between animals and between rooms. Personal Protective Equipment (PPE) is described and listed on all animal room doors.
2. All personnel should sanitize their hands thoroughly before entering and upon leaving an animal room to insure personal protection and to minimize any potential for cross contamination between animals and rooms.
3. Dispose of broken glass, needles, and other sharp hazards in proper containers.
4. Eating, drinking, and smoking are not permitted in the animal facility. Food and drink may only be consumed in the CRF offices and staff room.
5. Pets are not allowed into any of the animal care facilities under any circumstances.

L. Zoonotic Diseases

A zoonotic disease, or zoonosis, is an infectious disease which can be transmitted between humans and animals. Of the hundreds of zoonotic diseases known, only a handful are of concern in the research animal facility. Modern animal production techniques and animal facility operating procedures are designed to minimize the threat of zoonotic diseases, both to personnel and valuable animal colonies. When human infection does occur, it often is the result of failure to follow accepted procedures.

Prevention and control of disease in a research facility includes vendor selection, animal receiving, quarantine, facility design, animal housing, personnel traffic patterns, sanitation practices, vermin control, veterinary care and necropsy.

A list of common zoonoses of laboratory animals can be found in *Appendix 2 Zoonosis of Concern in Animal Care Facilities*.

Consult with a physician knowledgeable about animal-related diseases if you have any medical condition that may make you more susceptible to certain animal-related diseases. Such medical conditions include but are not limited to splenectomy, alcoholism, immune system problems (e.g. AIDS, chemotherapy, systemic steroids such as cortisone, cancer), tuberculosis, pregnancy, or a history of heart disease or heart surgery (even though you may not have any heart symptoms now). If your personal physician is unfamiliar with animal

diseases, have him or her contact a Lifespan Employee Health physician or the Lifespan Attending Veterinarian for additional information.

Women who are pregnant can work in animal facilities, but certain tasks may present a hazard to the unborn fetus. Women who become pregnant should notify their instructor/supervisor. The employee should consult with a Lifespan Employee & Occupational Health Services physician to review their duties while pregnant.

Disease Transmission and Prevention:

1. Presence of the Zoonotic Agent in the Animal

The first consideration in control of zoonotic disease is the presence of a potential disease-producing agent in the animals. Zoonoses are most effectively avoided by purchasing animals which do not harbor these agents. Most approved vendors supply information on the disease status of animals shipped from their production facilities.

2. Escape of the Zoonotic Agent from the Animal

Natural routes by which zoonotic agents are shed from animals include saliva, feces, urine, exudative skin lesions, and vectors such as biting insects. Surgery, biopsy, necropsy and removal of any animal tissue (including blood) can serve as a means of transmission.

3. Transmission of the Zoonotic Agent to a Human

Direct contact with animals or animal products is the primary method of disease transmission. Recommendations for avoiding this route of disease transmission include wearing gloves and washing hands. Puncture wounds inflicted with needles used on animals are also common sources of infection. Aerosol transmission of disease-producing organisms can be minimized by working within a Biosafety cabinet and/or wearing a face mask or respirator when working with animals or animal products.

4. Zoonotic Agent Enters Human Host

Zoonotic agents can utilize four routes of entry into the human host: ingestion, inhalation, parenteral inoculation, and contact with mucous membranes (e.g. eyes or mouth). Gloves, masks, and in some cases splash-proof eye protection are used to prevent entry of zoonotic agents into humans. Hands are washed before and after handling animals or animal products. No eating, drinking, or smoking is allowed in the animal or treatment rooms. Needles must be disposed of in a puncture-proof container.

5. Human Host Contracts Disease

The susceptibility of the human host for disease is dependent on a number of factors. One of the most important of these factors is the status of the host's immune system. Manipulation of the immune system through vaccination is used in some instances where potential for zoonotic disease is great. Vaccines developed for some of the zoonotic diseases are available to personnel with high risk of exposure. Tetanus (Tdap) is the only inoculation currently required for CRF personnel.

M. Biosafety Levels for Animal Diseases (Zoonotic Agents)

Criteria and practices for zoonotic agents are based on recommendations found in Section IV of the CDC's manual, "Biosafety in Microbiological and Biomedical Laboratories" <https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf>.

Animals suspected or known to carry a zoonotic agent are assigned to a particular biosafety level. The standards of practice found in the CDC manual will be instituted by the CRF indefinitely or until the animal is determined to be free of the particular agent. The CDC manual describes Animal Biosafety Levels 1 – 4 but only Animal Biosafety Levels 1 and 2 are allowed in the CRF program and at the CAF sites.

- **Animal Biosafety Level 1 - no risk**

Animal diseases listed in Biosafety Level 1 are considered species-specific and as such do not fit the definition of zoonoses. These agents are not associated with disease in healthy adult humans. However, animals with diseases in this category may be banned from the CRF to prevent spread of infection to animals under study.

Examples: mouse hepatitis, mouse pox, rat parvovirus, rabbit pox

- **Animal Biosafety Level 2 - moderate risk**

Animal diseases listed in Biosafety Level 2 include most infectious zoonotic agents. The primary hazards of these diseases are associated with parenteral inoculation or mucous membrane exposure. Aerosols are not a common means of exposure to agents in this class. Animals inoculated with BLS2 agents are handled as such (i.e. in the Biohazard suite, within a biosafety cabinet) at an ABSL2 level. Examples are salmonellosis, and enterococcus. Immunodeficient animals carrying human source tissues and tumors are also considered ABSL-2.

N. Safety Procedures for the Use of Non-Formalinized (unfixed) Animal Tissue

Humans can contract potentially serious zoonotic diseases after being exposed to non-formalinized animal tissue just as they can after exposure to live animals. Non-formalinized animal tissue originating off campus can also be a source of infection for the laboratory animals at Rhode Island Hospital. Precautions must be taken to protect hospital employees and patients from possible exposure to the more pathogenic zoonotic disease organisms and to protect the integrity of our research animal populations.

1. Containers for Transportation

Non-formalinized animal tissue being transported to the Hospital or from one area to another area within the Hospital must be transported in a sealable container that can be autoclaved.

2. Hood Requirement

A Class I or II hood (a biological Safety cabinet with HEPA-filtered recirculated mass airflow within the work space plus HEPA-filtered exhaust air) is required while utilizing non-formalinized animal tissue from ruminant livestock species (sheep, goats, cattle) and from non-human primates.

3. Protective Equipment

Protective equipment such as disposable masks, gowns, safety glasses and gloves that are appropriate for handling potentially infectious material must be worn when working with non-formalinized tissue from ruminant livestock species and non-human primates (although these species are not housed in the CRF). Lab coats and gloves are appropriate when working with other animal tissue.

4. Decontamination/Disinfection

After each use, first wash surfaces with water to remove chemical. Then disinfect with fresh or stable 10% bleach solution or suitable disinfecting agent (such as Rescue H2O2).

5. Waste Disposal

Autoclave the waste before leaving it for disposal.

6. Use in Central Animal Facilities

- a. Non-formalinized animal tissue must not be brought into any of the Lifespan animal facilities without permission by the Veterinary Services staff. The origin of tissues must be identified.
- b. If such tissue is to be brought to the Research Operating Room in the Aldrich Building, it must be in a sealed, covered container while in transit. The route would be up the Aldrich elevator and then through to the Research Operating Room. The Central Research Facilities Office (444-5788) must be notified in advance when non-formalinized animal tissue will be brought to the Research Operating Room.

7. Responsibilities and Compliance

In the interest of the safety of employees, patients and the animal population, research personnel will be responsible for compliance in their area with the above procedures. Reports of noncompliance will be brought to the attention of their supervisor and the Director, CRF and the Vice President, Research Administration.

O. Use of Biohazardous or Chemically Hazardous Substances in Animal Research

Lifespan's Policy is to inform personnel of potential health hazards in the work place (Right to Know Act, <http://intra.lifespan.org/rih/environmentalsafety/documents/RighttoKnow.pdf>). Before using any potentially hazardous substance or procedure, a detailed set of Standard Operating Procedures (SOP) for that substance or procedure needs to be written and provided to staff members. Prior approval by the Institutional Biosafety Committee (IBC) is required for:

- Chemical agents that have been assigned a safety rating of 4 or greater in any category on the SDS sheet
- Any compound listed as a carcinogen, mutagen or teratogen in the Chemical Hygiene Plan
- Any toxin including such proteins as ricin, cholera toxin and bacterial toxins
- Any organism included in the list of Risk Group 2 (RG2) or higher organisms in appendix B of the [*NIH Guidelines For Research Involving Recombinant or Synthetic Nucleic Acid Molecules*](#) for Research Involving Recombinant or Synthetic Nucleic Acid Molecules or organisms that require Biosafety Level Containment Level 2 or greater as defined by the Centers for Disease Control (CDC) manual [*Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)*](#).
- Any organism that will be administered to live animals. [note, separate IACUC submission for any work using animals is also required]

To insure that all involved personnel are fully informed, investigators, technicians, students/volunteers, and CRF personnel will attend a mandatory training session and will comply with the SOP. This training must be documented and records maintained by the CRF office.

Note: If materials used in animal research are radioactive as well as hazardous, additional safety measures must be taken (see section on Use of Radioactive Materials in Animal Research).

Implementation of appropriate safety precautions is required by the CRF before investigators initiate studies employing hazardous substances in animal research. Investigators should be thoroughly aware of the disposition of the hazardous substance and/or its metabolites in designing the appropriate safety protocols. These precautions should maximize the safety of personnel exposed to substances either known to be or suspected of being hazardous.

Consultation is available from the Institutional Biosafety Committee (IBC), veterinary staff, the CRF staff, and the Hospital's Safety and Radiation offices.

Protocols are reviewed with respect to the use of hazardous materials as part of the protocol review process. CRF policy is based on the recommendations of the NIH, NCI, OSHA, and other federal and state regulations. The following guidelines should be incorporated into protocols involving hazardous substances.

Guidelines for Use of Hazardous Substances:

1. Use of hazardous substances in the CRF requires prior approval from the IBC. The animals dosed with chemical hazards may be housed in standard housing rooms in contained cages, but must be clearly identified with a hazard label. Animals dosed with biological hazards must be housed in a room designated as BSL-2. Room doors and cages must be clearly marked with the type of hazard involved, name(s) and telephone numbers of responsible investigators, and appropriate precautions to be followed.
2. Personnel working with hazardous substances, including CRF personnel, must be identified and thoroughly trained about the relevant safety precautions, potential hazards, and procedures for decontamination.
3. Protective apparel must be worn when working with hazardous substances and may include a disposable gown, N95 or other suitable respirator, eye goggles, respirator, double disposable gloves, head cover, double shoe covers, among others. The characteristics of the particular substance should be considered in selecting appropriate protective apparel.
4. Biological safety cabinets must be used when activities have a high potential for creating aerosols: intranasal inoculations, necropsy of infected animals, dumping of contaminated bedding, and manipulation of large volumes of materials. The class of safety cabinet used must reflect the risk level of the hazardous agent and/or operations.
5. Animals being used in hazard protocols must be housed in caging that confines the feed, feces, urine, and bedding in the enclosure. Static cages with filter tops or disposable cages are typically used during the hazardous period.
6. Investigators will be responsible for changing cages housing animals in hazard protocols, unless other arrangements are made with the CRF office. Certain protocols may require a laminar flow, HEPA filtered, bedding disposal cabinet.
7. All materials contaminated or in contact with hazardous substances must be decontaminated and disposed of properly. Procedures may include autoclaving,

incineration, and precautions for chemical and physical cleaning. All materials to be disposed of should be double bagged in red hazard labeled bags.

For detailed information, see Appendix 8 Procedures for the Care and Handling of Rodents on Biosafety Level 2 (ABSL-2) and Other Hazardous Containment Protocols.

P. Use of Human Tissues in Animal Facilities or Laboratories

In the pursuit of medical training and research it may be necessary to utilize donated human cadaver parts. In all cases, the academic community will treat these (parts) with respect and diligence in gratitude for their donation and strive to achieve the highest level possible of medical science and research.

Any research activity at Rhode Island Hospital utilizing human body parts must be approved by the IBC before starting or, if the body parts are to be received from off campus, before transportation to the Hospital. If any human tissues are to be brought into a CRF area, advance written permission must be obtained by the CRF Director and the area Supervisor/Manager.

To receive approval, the researcher must file a research application with the IBC. The application must include a description of the activity, certification of origin, how to be transported, where to be stored, the facility or location where the proposed research/educational activity will take place and means of disposition/ disposal. The Committee may endorse or may make implementation contingent upon compliance with some recommendations.

The following SOP (Standard Operating Procedure) will be followed for embalmed and unembalmed body parts:

1. Obtain specific shipping instructions from the source prior to the shipping date.
2. Transportation shall be in a sealed autoclavable container.
3. Standard (Universal) Precautions are to be followed at all times.
4. Disposition/Disposal will be per instructions from the source which may include returning to the source of origin. Lacking instructions, the State of Rhode Island Regulated Medical Waste Rules and Regulations will be followed.
5. Supplier of body parts will describe any and all infectious disease screenings that are performed on their products.

Q. Inactivation of Recombinant DNA Materials

As specified in the NIH Guidelines for Research Involving Recombinant DNA Molecules, liquid and solid waste generated in recombinant DNA work must be decontaminated before disposal. Decontamination will be carried out by bleach treatment or steam autoclaving as appropriate. For example, bench tops and spills are best treated with bleach while culture plates, used pipettes and tubes will be autoclaved. Because of variables affecting the effectiveness of autoclave steam inactivation, the following protocol has been adopted by the RIH Recombinant DNA Committee (RIH-RDC).

All prior RIH guidelines pertaining to use of autoclaves must be followed, including logging of loads, use of indicator strips, schedule of testing, training for specific autoclaves, etc. The autoclave used for sterilization of animal facility supplies by CRF in the Nursing Arts Building is not available for waste inactivation.

Recombinant DNA waste materials must be clearly labeled. Transport to the autoclave must be in leak proof outer containers and resting in a tray or bucket inside the autoclave. Avoid transport through patient areas. Arrange materials so that steam circulates freely around them. No more than 15 pounds of material (less than 200 culture plates) may be autoclaved in one load.

For bagged dry materials, (e.g. empty pipettes), add 100-200 ml of water to the bag to generate steam and leave the bag top open inside the autoclave.

Set time for 45 minutes after reaching the temperature of 250° F. This is more than necessary to kill bacteria but is the time needed to insure complete activation depending on load variations. Check that the settings are correct. Use care to avoid burns from hot liquids when removing items from the autoclave.

R. Use of Radioactive Materials in Animal Research

Investigators planning to use radioactive materials in animal subjects must submit an application to the Radiation Safety Office for authorization by the Radiation Safety Committee prior to submission of the application to the IACUC for approval. Investigators are responsible for the safety of all personnel associated with any project. Consultation is available from the Radiation Safety Officer and/or the CRF and veterinary personnel.

The CRF Operating Room and the Cardiovascular Research Department utilize fluoroscopy for procedures. All personnel who operate the fluoroscopy units are required to contact the Hospital Radiation Safety Office for specific training and hands-on instructions. Only staff required during the surgical procedure or persons in training shall be present in the room during the operation of the fluoroscopy unit. All required staff shall be protected with protective lead, including thyroid shields, or protective lead barriers. Each individual is required to wear a radiation dosimeter. A fluoroscopy time log must be maintained.

1. Responsibilities of the Investigator (radioisotopes administered to animals)

- a. Must obtain approval from the CRF Director to ensure that appropriate facilities are available for the housing of animals and/or experiment.
- b. Must obtain approval of experimental protocol(s) by the appropriate RIH Committees, (e.g. Radiation Safety Committee and the Animal Welfare Committee).
- c. Must advise all appropriate CRF and laboratory personnel of the nature and potential health risk of the radiation hazard to be used in the experiments.
- d. Must keep detailed chronologic record of all experiments which includes numbers of animals used, use of radioisotopes (material, amount and route of administration) and the date and method of sacrifice of animals at the end of an experiment.
- e. The daily animal care during the use of radioisotopes will be the responsibility of the investigator and/or designated laboratory personnel and will include the following duties:

- Placement of appropriate signs indicating the nature of the hazard on door(s) to the room(s) where animals are housed during experimentation, as well as in the cage(s) where animals are kept.
 - Labeling of the waste cans in the experiment room with signs which indicate the nature of the hazard and the appropriate procedures established for the particular hazardous agent being used.
 - Recommend and provide the appropriate protective clothing, gloves, and/or masks by personnel working with the animals and/or biohazard agent.
 - Providing daily food and water to the animals.
 - Providing change or changes of bedding and cage washing at intervals established by the CRF.
 - Recording on cage cards the date of death or euthanasia of animals along with the initials of the person making the record.
 - Disposal of waste material and animal carcasses according to procedure guidelines established by the appropriate review committee.
- f. Any deviations from the above duties must receive authorized approval from the appropriate review committee, radiation safety office and/or the CRF Management.

2. Responsibilities of the CRF

- a. Will provide equipment (if available) for animal housing.
- b. Will supply feed and bedding for maintaining animals during the experimentation period. At the end of the experiment, excess feed and bedding must be disposed of and not returned to the CAF stores.
- c. Will provide cage cards for project and animal identification.
- d. Daily census and check of animal's well-being will be made by the CRF supervisor or designated person. Cage cards from cages in which animals died or were euthanized will be collected and placed in the CRF Supervisor's inbox and will be removed from census/per diem charges.

3. Radiation Safety Office Responsibilities

- a. Provide information on the appropriate protective clothing, personnel monitoring, procedures and safe working times, etc., if required.
- b. Regular monitoring of room for contamination outside of containment areas (i.e., waste containers and cages).

S. Chemical Safety

1. Disinfectants

The concentrated forms of some of the disinfectants used in the CRF are caustic to the skin and are only handled by CRF personnel. Only diluted working concentrations of

disinfectants are provided to non-CRF personnel. Gloves are worn when handling and diluting bottles of concentrated disinfectants. If skin comes in direct contact with these chemicals, the area is immediately flushed with water for two minutes, the CRF office is informed of the incident, an incident report filed, and a visit made to EOHS by the affected personnel.

2. Detergents and cleaning solutions

Detergents and other solutions used in cage washing are supplied in concentrated forms that may cause skin irritation. CRF personnel must wear gloves when dealing with these chemicals; rinsing with copious amounts of water is indicated in case of direct skin contact. The incident must be reported to the CRF office and the EOHS office and an incident report filed.

3. Pesticides and Pest Control

CRF, in cooperation with the Environmental Services Department (ESD) at the main campus, and the Property Management Department at Coro and Claverick, has a pest control program. A commercial pest control company is under contract and makes an independent assessment every two to four weeks. CRF staff visually monitors for pests daily and communicate with the pest company via log books which are kept in the ESD. Insect sticky boards are in use and live catch rodent traps are checked daily by the animal care staff.

Whenever possible, pest control is by means of sanitation and/or mechanical devices. If chemical pest control is required, all investigators affected will be consulted about any proposed treatments. Trained personnel from a pest control agency, which meets the requirements for animal and human safety, will apply pesticides. Such treatments will be performed only with authorization from the CRF Management, the attending veterinarian, and the investigators involved.

4. Anesthetics

Isoflurane is a nonflammable volatile liquid used for animal anesthesia. This agent is typically used in a precision vaporizer, where waste gases are absorbed in an activated charcoal filter or scavenged to the outside of the building via a vacuum line. For some small animal procedures, isoflurane may be used in a closed jar in a fume hood which will exhaust the waste gas.

Because of the explosive potential as well as the flammable properties of ethers and because of non-ideal anesthetic properties, their storage and use within the animal facility is PROHIBITED. Departments which feel they need the ether for a particular use in the laboratory must submit a request in writing for review by the Institutional Biosafety Committee. Such requests will also be forwarded to the RI Hospital's Safety Manager and Chairman of the Environment of Care Committee (EOC).

Parenteral (injected) anesthetics used in the CRF generally do not pose safety hazards when properly used. Adequate animal restraint is required for animal injections. Accidental inoculation of a human with any animal anesthetic must be reported immediately to the CRF office and EOHS. Medical treatment is required because many parenteral anesthetics are highly alkaline. Left untreated, tissue necrosis can occur. ***All used needles and syringes must be disposed of in sharps containers.***

T. Physical Safety

All of the accidental injuries listed below must be reported immediately to the CRF office and the Employee and Occupational Health Services office (EOHS). The initial report is filed by the CRF Supervisor while EOHS documents the accident and ensures that further treatment for problems related to the accident will be provided. Reporting to the CRF Supervisor's office ensures that any hazardous situation can be corrected immediately.

1. Wounds Inflicted by Animals-Bites

Animal bites can cause severe mechanical damage and, in some instances, pose a serious threat due to disease transmission.

Prevention of animal bites is based on knowing and practicing good animal handling techniques. Familiarity with the animals and their behaviors is helpful, but unpredictable events will occur regardless of past experience. Animals exhibiting aggressive behavior should be reported at once to CRF personnel. Do not attempt to handle these animals without assistance.

If bitten by an animal, the site of injury should be immediately washed with soap and water, except in cases where the wound is severe and accompanied by extensive bleeding. Sites of bleeding should be wrapped in clean cloth and pressure should be applied to control bleeding. Go to EHOS for examination, treatment and documentation of the incident. If necessary, allow someone to assist you in obtaining medical treatment at EOHS. Also describe the incident to the CRF Supervisor and indicate the condition, location, and status of the animal in question.

2. Wounds Inflicted by Animals-Scratches

Animals most likely to inflict scratches are rabbits. There is no known zoonotic disease associated with rabbit scratches, but the mechanical damage caused by the hind claws of a rabbit can be extensive.

Proper techniques for handling animals will prevent the infliction of most scratches. It is important to realize that scratching is a rabbit's primary defense mechanism when cornered or frightened. The techniques for handling rabbits are devised to prevent scratching while providing adequate support for the rabbit's back. Animals exhibiting aggressive behavior should be reported to CRF personnel. Do not attempt to handle aggressive animals without assistance.

Actions to take if you are scratched by an animal are the same as those to be taken following an animal bite (see previous section). Be sure to notify the CRF Supervisor and obtain medical attention.

U. Other Accidental Injuries

The CAF poses many of the same hazards as any general laboratory. Accidental injuries due to safety problems such as those described below should be treated immediately in accordance with general first aid principles. Report the accident to a CRF supervisor and obtain medical attention at EOHS. The initial report filed with EOHS documents the accident and ensures that further treatment for problems related to the accident will be provided.

1. Burns

Steam released from equipment used in cleaning and disinfecting procedures is a major hazard. Only personnel who have been trained in the use of these items should handle autoclaves, cage washing machines, and portable steam cleaning units. Periodic inspection and maintenance are required to ensure that equipment is in proper working condition.

2. Falls

The major cause of falls in the CAF is water on the floor. Wet floors are common in all areas due to necessary mopping and sanitation procedures. This hazard is prevalent in cage washing areas and animal rooms, which are cleaned with large quantities of water. CRF and research staff should be mindful when working in or walking through these areas to decrease the chances of a fall due to wet floors.

3. Skin Lacerations or Punctures

Many of the materials in the CAF have the potential for causing laceration or puncture of the skin. Skin trauma can lead to a variety of local and systemic infections. Tetanus prophylaxis is mandated for all CRF and research personnel.

Animal cages are inspected for safety hazards prior to cleaning. Broken and bent wires on animal cages are repaired to ensure animal and human safety. Cracked plastic animal housing is discarded. Hypodermic equipment must be disposed of in proper containers. These devices are found in the CAF procedure rooms.

4. Miscellaneous

a. Animal-Associated Allergens

Many species of animals are known to cause allergies in humans. Reduction of exposure to animal allergens is recommended for all personnel working with animals. While a surgical mask will reduce exposure to hair and dander, only an N95 respirator (or equivalent) can adequately reduce animal allergen exposure. To reduce the risk of acquiring allergies, it is strongly recommended that an N95 respirator be worn during the handling of animals and their bedding to reduce allergen exposure (after appropriate fit-testing). Safety glasses and protective clothing should routinely be used to prevent exposure to allergens and to prevent the transport of allergens outside of the animal room and facility. Rodent urine can produce severe allergic reactions and skin contact must be avoided; in the event of contact, wash off immediately with soap and water. All allergic reactions MUST be reported to the CRF supervisor and EOHS. Signs are posted at the entrances of the animal facilities to warn of the possible exposure to allergens.

b. Glass and/or Sharps

Glass and/or sharps are to be disposed of in appropriate containers.

V. Reporting Safety Concerns

Individuals having concerns involving safety within Lifespan facilities are responsible for contacting the CRF Management, the Safety Office, and/or the Vice President, Research Administration, verbally or in writing. Contact information is provided to all

researchers who work with animals during their initial orientation with the Central Research Facilities (CRF) Management. Telephone numbers for CRF management staff are posted within each animal facility. Contact information is also posted on the IACUC webpage. Complaints may be submitted anonymously to Corporate Compliance via the Employee Response Line at 888-678-5111.

Although written concerns are more convenient to handle, complainants may not be willing to submit them in this manner. In such cases, the individuals who receive concerns should document them fully to ensure that the issues are clear and to prevent misunderstandings.

Lifespan will take appropriate steps to protect the confidentiality of those who report concerns as well as anyone against whom allegations are directed, while allegations are under investigation.

Lifespan policy prohibits unlawful retaliation against employees as a consequence of good faith actions in the reporting or the participation in an investigation pertaining to allegations of wrongdoing.

VI. Veterinary Care

A. Role of Veterinary Care

Veterinary care at Lifespan is provided by laboratory animal veterinarians, including American College of Laboratory Animal Medicine Diplomates, through an agreement with Brown University. Lifespan has given assurance that the veterinarians have access to RIH management and have appropriate authority to ensure the provision of adequate veterinary care in the animal facilities.

The veterinarians are responsible for supervising a program of veterinary care which has been approved by the Institutional Animal Care and Use Committee and is in compliance with the Animal Welfare Act Regulations and the Public Health Services Policy on Humane Care and Use of Laboratory Animals. The program includes: (1) details on the facility, personnel, equipment, and services available for appropriate animal care; (2) acceptable methods to prevent, control, diagnose, treat health problems and injuries, and the availability of emergency weekend and holiday care; (3) guidance in the care and use of animals regarding handling, immobilization, anesthesia, tranquilization and euthanasia; (4) assurance that appropriate surgical areas for survival surgery are maintained and utilized, and that sterile technique is used; (5) assurance of adequate pre-procedural and post-procedural care including the appropriate use of anesthetics and analgesics; and (6) assurance that appropriate methods of euthanasia are utilized.

The veterinarians are at RIH on a mutually agreed upon schedule and maintain frequent contact with the management of the Central Research Facilities. The veterinarians maintain an on-call schedule and are available in the case of emergencies, after hours, and on weekends/holidays. Their telephone numbers are posted in the animal facility.

The veterinarians also have frequent contact with CRF supervisors, the veterinary technicians, the Research O.R. Supervisor and the CRF technicians to discuss problems. The veterinary and animal care technicians are responsible for daily monitoring of the animals and recording changes in animal health, behavior, and wellbeing. Any health concerns or

abnormal findings are reported to the veterinarian in an accurate and timely manner, via phone call, email, or in person.

The Director of the Central Research Facilities and the Chairperson of the IACUC are contacted about significant deficiencies or to propose changes in the animal care program or facilities. The veterinarians serve on the IACUC, the Animal Welfare Executive Committee, the Institutional Biosafety Committee.

B. Veterinary Consultative Services

1. The veterinarians are available in person or via cell phone for consultation on a wide range of subjects including:
 - Selection of appropriate animal species for *in vivo* studies.
 - Information on animal models of human diseases.
 - Anatomical and physiologic characteristics of individual animal species.
 - Techniques of anesthesia, analgesia, chemical restraint, and euthanasia
 - Design of appropriate post-operative care programs.
 - Technique of collection and storage for blood, body fluids, and tissues.
 - Effects of intercurrent animal disease on experimental results.
 - Utilization of specialized surgical techniques.
 - Experimental design.
2. Investigators are required to consult the Attending Veterinarian during the planning phase and prior to submission of the Animal Care and Use Protocol (ACUP). The consultation date is indicated on the protocol forms. This consultation is used to advise or evaluate:
 - the selection of experimental models
 - consideration of alternatives to painful procedures
 - directions and recommendations for the use of anesthetics and analgesics
 - acceptable euthanasia methods, and the prohibition of the use of paralytics without anesthesia
 - the qualifications and training of the investigator and staff to provide humane care for the animals, and to perform the procedures so that pain and distress will be minimized
 - current laws and regulations concerning animal care and use
3. The veterinarians can provide health certificates for animal shipments from the facility.
4. The veterinarians, using external diagnostic facilities, when needed, evaluate clinical problems in all housed species and the veterinary technician or the CRF staff administers treatments under her guidance.

C. Reporting of Sick or Injured Animals (Clinical Medicine)

All personnel utilizing animal subjects are expected to contact Veterinary Services or the supervisor's office if they believe an animal is sick, in discomfort, or otherwise requires aid. A veterinarian will respond and take appropriate action in consultation with the investigator. It is essential that clinical calls be initiated at the earliest sign of an abnormality. The veterinarian will keep investigators informed of the diagnosis, condition, etc., and the appropriate course(s) of action.

1. Objectives

- a. RIH is committed to providing veterinary care for all research animals in our facilities which is consistent with the objectives of the Institutional Animal Care and Use Committee approved protocol or is directed by the attending veterinarian to ensure the welfare of the animal.
- b. The daily care of each research animal requires accurate and knowledgeable observations to detect common rodent diseases, the appropriate disposition for large animals or surgical complications for either large or small laboratory animals. This is a shared responsibility of the personnel from the research laboratory, veterinary services, and the animal care technicians.

2. Procedures

- a. **Weekdays** - Business hours (7:00 AM – 3:30 PM)

Problems requiring prompt assistance, immediately contact Veterinary Services.
(These numbers are posted on each floor of each research facility.)

Veterinary Services Supervisor	Middle House and Claverick	401-444-5607*
Veterinary Services Supervisor	Coro East	401-255-4183**
Veterinary Services Coordinator	Coro West	401-601-7914**

*If unavailable, please contact the CRF Main Office (401) 444-5788

**If unavailable, please contact the Attending Veterinarian

Veterinarians	Work hours	Weekends
Dr. Tiffany Borjeson	(401) 444-6842	(401) 369-1845
Dr. Jessica Johnston	(401) 444-6842	(818) 568-8512

Veterinary Services will contact the Veterinarian directly when on premises or by phone. They will assure that the animal is receiving appropriate attention and that appropriate documentation is maintained. This will include instructions from the Veterinarian regarding immediate care instruction, diagnostic work up and treatment plan. For non-life threatening situations, every effort will be made to obtain approval from the PI or other laboratory personnel prior to initiation of treatment.

Veterinary Services will contact the CRF Supervisor/Manager regarding significant issues that might require further assistance or notification of CRF staff.

For problems requiring follow up assistance by Veterinary Services, the findings must be documented as completely and accurately as possible:

- For rodents, use one of the “Health Check” cards which are available in each of the rodent rooms and then post it on the animal’s cage.
- For non-rodents, provide the required information for identification of the animal and the clinical problem on the animal’s individual record.

b. **Weekend Daytime Hours** (7:00 AM – 3:30 PM)

For animal health problems requiring prompt assistance, immediately contact CRF Animal Care Technician on duty.

Main Campus	(401) 444-8146
Coro East	(401) 793-9818
Coro West	(401) 793-8761
Claverick	(401) 444-6978

If no answer, call the CRF Supervisor at (401) 255-4183 or (401) 477-9469.

The CRF Technician will contact the Veterinary Services staff member on-call as deemed necessary in consultation with the laboratory. If there is no answer with the CAF technician or the Veterinary Services staff member, the lab is to call the on-call veterinarian for urgent issues.

c. **Off-Hours** (Weekdays and Weekends, before/after work hours)

For problems requiring assistance before/after working hours, the PI/Lab staff member will contact the Veterinary Services Supervisor responsible for the building.

Main Campus and Claverick	(401) 477-9469
Coro East and West	(401) 255-4183

Veterinary Services will then contact the Veterinarian on-call, as deemed necessary. In the event that the contact person cannot be reached, the PI/Lab staff will call the on-call veterinarian. **See the On-Call List in each facility for the scheduled veterinarian's phone numbers.**

D. Utilization of Anesthetics and Analgesics

(See Appendix 3 Selection and Use of Anesthetics and Analgesics.)

Balanced anesthesia/analgesia will be employed to minimize surgical pain. A veterinarian must be contacted for assistance in designing appropriate anesthetic and analgesic regimens, which will be examined as part of the protocol review process conducted by the IACUC. Some agents have been shown to have undesirable physiologic effects which preclude their use in particular research situations. Investigators are urged to familiarize themselves with the agents used in their studies.

The following criteria should be considered in selecting agents for research studies.

- Species of animal(s)
- Is procedure acute or survival?
- Duration of anesthesia required
- Ease of administration
- Anesthetic effects
- Safety concerns
- Reversibility
- Recovery characteristics of the agents

General Principles:

- Some large species (i.e. Swine) should be fasted the day before anesthesia (8-12 hours).
- Animals should be intubated to provide airway control during procedures.
- IV catheters should be placed to provide easy access to a vein during an emergency.
- The animal's vital signs (HR, RR, temperature, ETCO₂, EKG, SPO₂) must be monitored at least every 15 minutes during anesthesia.
- Emergency drugs should be available
- Animals must be monitored after anesthesia until they are fully awake according to their protocol guidelines.
- Animals must be able to maintain sternal recumbency and must maintain thermoregulation before being returned to their cage.

1. Pre-Anesthetics

Tranquilizers or sedatives are commonly used as pre-anesthetics for general anesthesia. Animals premedicated with sedatives and tranquilizers are more manageable and require lower dosages of general analgesia.

2. General Anesthesia

- a. Inhalational anesthetics should be administered using a precision vaporizer. Anesthesia machines regulate the flow of oxygen and the concentration of the anesthetic gas.

Isoflurane delivered by mask or endotracheal tube via a precision vaporizer is recommended for all species. Vaporizers are available for use in the Claverick, Coro West, Coro East, and Middle House procedure rooms. Contact CRF for information regarding vaporizer availability and training. For very brief procedures in rodents, (e.g., tail biopsies for genotyping), it may be acceptable to use isoflurane or other inhalant anesthetics, without a precision vaporizer, in a “bell-jar” while precluding direct contact of animal skin with inhalant anesthetic, and must be an approved method within the IACUC protocol. In all cases the anesthetic vapors must be adequately vented in a fume hood to prevent inadvertent exposure of personnel.

Appropriate scavenging systems are required for personnel safety when using inhalational anesthetics. Additional information is found in the **SAFETY RULES** section of this manual.

- b. Injectable anesthetics may be appropriate for some procedures. There is however, a great deal of variation in depth and duration of anesthesia between individual animals. All injectable anesthetics should be on an approved protocol that has been reviewed by the veterinarian.

3. Local Anesthesia

The use of local anesthetics as an adjunct to other anesthetic protocols is encouraged. A local anesthetic is not required if the pain of giving the injection is as great and of the same duration as that produced by the procedure itself.

4. Analgesics

Analgesics are used in animal studies where pain may result from experimental manipulations. They should be used in animals for any procedures which would require analgesia in humans, whenever possible. *Appendix 3 Selection and Use of Anesthetics and Analgesics* provides dosages for the agents commonly used in animals.

E. Use of Controlled Substances in Animal Research

The Controlled Substances Act (CSA) was enacted into law by the [Congress of the United States](#) as Title II of the [Comprehensive Drug Abuse Prevention and Control Act of 1970](#).

The CSA is the [federal U.S. drug policy](#) under which the manufacture, importation, possession, use and distribution of certain substances is regulated. Controlled substances to be used for approved protocols must be obtained through the Rhode Island Hospital Pharmacy. Investigators using controlled substances must obtain a DEA Registration and a license to use controlled substances through the RI Department of Health, or must be an Authorized User under a DEA Registrant. Contact the IACUC Manager or the IACUC Coordinator for further information and assistance.

Investigators are responsible for the ordering, record keeping and security of any controlled substances required for their protocol. Inventory and use records must be kept for each controlled substance and must be retained for two years.

Controlled substances must be kept under a double-locked storage system. In other words, you must open two locks in order to access the drugs. (e.g. double lock narcotic cabinet, a locked drawer in a locked room). The keys to each lock must be stored separately, and only Authorized Users can have access to the keys.

F. Pharmaceutical Grade Drugs

Consistent with USDA and PHS policy, investigators using Lifespan facilities are expected to use pharmaceutical-grade drugs or chemical compounds in all live animal research, whenever they are available (even for acute procedures).

Pharmaceutical grade substances are defined as those meeting pharmaceutical standards, being >99% pure, with no binders, filters, dyes or unknown substances. Lists of pharmaceutical-grade chemical compounds can be found in the human or veterinary physician's desk references (PDRs)

The use of non-pharmaceutical-grade drugs or chemical compounds is only permitted after specific IACUC review and approval. Approval for the use of non-pharmaceutical-grade drugs or chemical compounds will only be granted where:

- Acceptable pharmaceutical-grade substances are not available and/or,
- The use of the non-pharmaceutical-grade substance is scientifically necessary.

Note: Cost savings alone is not an adequate argument for the use of non-pharmaceutical-grade compounds in animals.

In reviewing requests for the use of non-pharmaceutical-grade substances, the investigator must describe preparation and at minimum, the procedures used to ensure sterility.

All non-pharmaceutical-grade substances must be sterile and maintained in sterile containers labeled with the name and concentration of the compound, as well as its expiration date.

Heat-stable compounds may be sterilized by autoclaving, and those that are not heat stable can be sterilized by microfiltration. The investigator is responsible for determining the stability “shelf life” for the compound after being dissolved in solvent. If the stability “shelf life” is not obtainable, a fresh batch/aliquot of the solution must be mixed each day it is used. Whenever possible, items should be compounded for the project the day of use and discarded immediately after use. See Section VI. J. Expired Drugs and Medical Materials Policy

For USDA and PHS/OLAW policies, go to:

https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf and https://grants.nih.gov/grants/olaw/120301_NPG_slides.pdf

G. Standard Operational Procedures for Survival Surgery

1. Large Animal Survival Surgery

Aseptic surgical technique is used for all surgeries where the recovery of the animal is anticipated. In addition, all surgeries are to be performed in the areas approved by the IACUC as indicated in the ACUP.

Surgical procedures will be classified as either Minor or Major as evaluated by a veterinarian during the protocol preparation and approved by the IACUC. Typically, survival surgery will be classified as Major, where there is penetration of or exposes a body cavity, produces substantial impairment of physical or physiologic function, or involves extensive tissue dissection. Major surgical procedures will be conducted only in an operating room approved by the IACUC.

a. CRF Operating Room Scheduling

The operating rooms are scheduled on a first come first served basis. The Operating Room schedule is available for viewing through the Lifespan intranet. Please note: Only investigators with approved large animal protocols will be granted access to the schedule.

It is recommended that you schedule your procedure(s) in advance to ensure availability of the room and any specialized equipment needed (ex. Fluoroscopy unit). Please contact the Operating Room Supervisor (444-6366) to ensure that any special needs can be accommodated and for instructions to access the OR schedule.

b. CRF Operating Room Charges

There are fees for the use of the operating room, technical assistance, and supplies. (See <http://www.lifespan.org/research/administration/lifespan-core-research-services.html>)

2. Rodent Surgery Overview

These Guidelines were developed to be consistent with those described in the [Guide for the Care and Use of Laboratory Animals](#) and any applicable requirements of the Animal Welfare Act regulations and Public Health Service [Policy for the Humane Care and Use of Laboratory Animals](#).

- Adequately train all personnel to ensure that good surgical technique is followed.
- Conduct detailed pre-surgical planning to provide an opportunity for input from the surgeon, veterinarian, veterinary technicians, and the laboratory staff.

- Provide appropriate pre-operative and post-operative care of animals in accordance with established veterinary medical and nursing practices.
- Conduct all survival surgical procedures in a designated surgery area in the laboratory, which is uncluttered and not being used at the same time for other laboratory procedures. Alternatively, Central Animal Facilities procedure rooms or surgical areas may be scheduled.
- Use aseptic procedures for all survival surgery, regardless of the interval of survival: if the animal recovers from anesthesia it is a survival surgery. This includes at a minimum wearing a hair bonnet, surgical mask and sterile gloves, using sterile instruments and practicing aseptic technique.
- Conduct a continuing and thorough assessment of the surgical outcomes to ensure that the appropriate procedures are followed and potential complications are detected and addressed. In the event of unanticipated morbidity or mortality, consultation with the Attending Veterinarian or designee is expected and appropriate corrective actions including amending the Animal Care and Use Protocol (ACUP) should be taken.

The Principle Investigator and all personnel responsible for or performing rodent survival surgery must be trained in the following essential elements of good surgical technique. This training can be obtained through recommended on-line training in conjunction with hands on training by qualified personnel in the laboratory or by CRF Veterinary Services staff.

- Asepsis
- Gentle tissue handling including minimal dissection to avoid excessive tissue trauma
- Appropriate maintenance and handling of surgical instruments
- Effective hemostasis
- Correct use of suture materials and patterns

In developing the protocol, the PI needs to:

- Develop the details for the survival surgical procedures conducted in rodents in consultation with the Attending Veterinarian or his/her designee.
- Provide a detailed description for each of the following:
 - Perioperative care and support including pre-operative medications, hypothermic prevention, ophthalmic protection (ointment)
 - Aseptic techniques including skin disinfection
 - Anesthetics and tranquilizers
 - Perioperative analgesics and anti-inflammatory agents
 - Nursing care and/or other treatments
- Provide a brief description of the area where the surgery will be conducted.
- Provide a description of the qualifications and training of personnel who perform perioperative care and survival surgical procedures in rodents.

See **Appendix 4 Guidelines for Survival Rodent Surgery** for details relating to disinfectants, sterilization methods, and recommended anesthesia/analgesia.

3. Post-Operative Care

It is the responsibility of the investigator, in consultation with the veterinarian and CRF personnel to provide appropriate post-operative care.

The veterinarian is available for consultation in designing protocol specific post-operative care programs. The following essential components should be routinely incorporated into post-operative management of rabbits and larger mammals

- The animal should be kept warm by the use of heating pads, chambers or lamps, and body temperature should be taken and recorded until it is normal (for most species this means a rectal temperature of 99° F or higher).
- Animals should be rotated from side to side every 15 minutes until they are able to maintain sternal recumbency, and should not be left unattended until they have recovered consciousness and have complete control of their airway.
- Hydration should be assessed on a daily basis for at least three days after surgery. Any needed parenteral replacement fluids should be administered at a dosage of 40-60 ml/kg of body weight/day for animals which are not drinking post-operatively. Fluids should be given parenterally in animals which have had gastrointestinal procedures or which have depressed swallowing reflexes.
- Adequate nutrition is necessary in the healing animal. Caloric replacement should be instituted for animals that have not resumed eating by the second post-operative day. Caloric replacement may require supplemental feedings using specialized dietary formulations and feeding methods, or may necessitate intravenous hyperalimentation.
- Daily observations of the animals for alertness, activity, eating, drinking, and stool will be made for a minimum of three days post-operative or as otherwise stated in the protocol.
- The incision must be examined daily for evidence of wound dehiscence or infection. Sutures or wound clips should be removed 10-14 days post-operatively.

CRF Veterinary Services staff will work with the laboratory personnel, Central Animal Facilities (CAF) personnel and the veterinarian to help ensure that animals receive high-quality post-operative care.

The CRF post-op form (*Appendix 5 Post Op Treatment Form*) must be used to record the progress of the animal post-operatively independent of any information that may be recorded in the investigator's laboratory notebook. Alternately, a PI may provide a different form if it captures the necessary information. All treatments should be entered as they are administered. The post-operative care form and/or information are kept in the room or hallway of the animal for all lab members, veterinary technicians, and veterinarians to view.

Although rodents do not generally require such intensive care, investigators should monitor their recovery from anesthesia, evaluate incisions and ensure that they continue to eat and drink post-surgically. Fluid and nutritional supplementation should be instituted if necessary. For rodents, post-operative records should be kept according to

their protocol guidelines. These forms are kept with the animal in the room for the remainder of the study.

Emergency contact information for the persons responsible for post-operative care must be provided to the CRF office. This allows CRF staff to consult with research personnel in order to provide appropriate support or veterinary care to post-operative animals when problems arise, especially after hours, or during weekends and holidays.

H. Differentiating Between Major and Minor Survival Surgery-Veterinary Perspective

1. Overall Concepts

The Eighth Edition of the NRC *Guide for the Care and Use of Laboratory Animals* offers much guidance on the major/minor surgical categorization issue (regarding research procedures, as opposed to veterinary clinical procedures) in the first two paragraphs under the heading Surgical Procedures on page 117. It states:

Surgical procedures are categorized as major or minor and, in the laboratory setting, can be further divided into survival and non-survival. As a general guideline, major survival surgery (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (Brown et al. 1993). Minor survival surgery does not expose a body cavity and causes little or no physical impairment; this category includes wound suturing, peripheral vessel cannulation, percutaneous biopsy, routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice. Animals recovering from these minor procedures typically do not show significant signs of postoperative pain, have minimal complications, and return to normal function in a relatively short time. When attempting to categorize a particular surgical procedure, the following should be considered: the potential for pain and other postoperative complications; the nature of the procedure as well as the size and location of the incision(s); the duration of the procedure; and the species, health status and age of the animal.

Laparoscopic procedures and some procedures associated with neuroscience research (e.g., craniotomy, neurectomy) may be classified as major or minor surgery depending on their impact on the animal (Devitt et al. 2005; Hancock et al. 2005; NRC 2003; Perret-Gentil et al. 1999, 2000). For example, laparoscopic techniques with minimal associated trauma and sequelae (e.g., avian sexing and oocyte collection) could be considered minor, whereas others (e.g., hepatic lobectomy and cholecystectomy) should be considered major. Although minor laparoscopic procedures are often performed on an “outpatient” basis, appropriate aseptic technique, instruments, anesthesia, and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor should be evaluated on a case-by-case basis by the veterinarian and IACUC.

Note: The USDA has emphasized that any survival surgical procedure that goes beyond being considered as minor, must be categorized as major.

2. Practical Minor/Major Survival Surgery Differentiation at Lifespan

The veterinary recommendations to the Lifespan IACUC in differentiating minor and major survival surgery in the relevant species are as follows:

Rodents Minor survival surgical procedures in rodents should be limited to: tail biopsy and digit amputation commonly used for genotyping and identification; minimally invasive vascular cutdowns, subsequent artery/vein catheterizations and associated intravascular manipulations, and related incision closures; or to subcutaneous minimally traumatic tissue dissection and implantation of devices up to the size of osmotic pumps, and related incision closures. [Similar survival preparations that involve multiple manipulations and incisions, or significant tissue dissection, may (on a case-by-case basis) be considered by the veterinarian and the IACUC to be major surgery.] All open procedures invading a body cavity (i.e., thorax or abdomen), all procedures involving penetration of the cranium, and all procedures with more extensive/aggressive subcutaneous tissue dissection or which purposely injure or sever ligaments, tendons or muscle tissue, should be considered major survival surgical procedures.

Rabbits and Swine Minor survival surgical procedures in rabbits and swine are generally limited to skin biopsies, or to minimally invasive vascular cutdowns, subsequent artery/vein catheterizations and associated intravascular manipulations, and related incision closures. [Similar survival preparations that involve multiple incisions and/or manipulations may be considered by the veterinarian and the IACUC to be major surgery.] Depending upon the age, size and/or resiliency of the particular animals used in a study, the veterinarian may (on a case-by-case basis) consider some subcutaneous procedures with minimal tissue dissection and implantation of a compact/low mass (in relation to the size and body weight of the animal) foreign body as minor survival surgery. All open procedures invading a body cavity (i.e., thorax or abdomen), all procedures involving penetration of the cranium, and all procedures with more extensive/aggressive subcutaneous tissue dissection or which purposely injure or sever ligaments, tendons or muscle tissue, should be considered major survival surgical procedures.

3. Decision Making

Classifying survival surgical procedures as major or minor is a joint process involving the veterinarian and the IACUC, taken on a case-by-case basis (see page 30 of the Eighth Edition of the NRC *Guide*). Discussion and a sharing of viewpoints about a given preparation will, of course, take place during the ACUP review and approval process. The guideline here must be that in the event of a disagreement between the veterinarian and the IACUC, the most conservative categorization of what is to be done shall take precedence.

4. Suitable Sites for Non-Rodent Mammalian Surgical Procedures

All survival surgery in rodents and in non-rodent mammals must be done aseptically. While rodent survival surgeries can be done in a designated space (generally a procedure room or a constant portion of a laboratory which is dedicated to surgery and related activities when used for this purpose), major survival surgery in non-rodent mammals certainly requires dedicated facilities. Regarding functional areas in survival surgical facilities for non-rodent mammals, the Eighth Edition of the NRC *Guide* states on page 144: *For most surgical programs, functional components of aseptic surgery include*

surgical support, animal preparation, surgeon's scrub, operating room, and postoperative recovery. The areas that support those functions should be designed to minimize traffic flow and separate the related non-surgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers (AORN 1993) but may also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities. The IACUC can consider requests by Principal Investigators to perform minor survival surgery in non-rodent mammals in appropriate designated space in procedure rooms or laboratory areas, with scientific justification, on a case-by-case basis.

5. Multiple Survival Surgical Procedures

The Eighth Edition of the NRC *Guide* states (in part) on page 30: *Regardless of classification, multiple surgery procedures on a single animal should be evaluated to determine their impact on the animal's well-being. Multiple major surgical procedures on a single animal are acceptable only if they are (1) included in and essential components of a single research project or protocol, (2) scientifically justified by the investigator, or (3) necessary for clinical reasons. As with major and minor surgical procedures, evaluation of requests for multiple survival surgical procedures is done jointly by the veterinarian and the IACUC on a case-by-case basis.*

I. Conditions for Multiple Major Survival Surgeries

If multiple major survival surgeries are being planned they should be related to a particular experimental endpoint and meet the following criteria to comply with PHS Policy and the Animal Welfare Act Regulations.

1. Any investigator requesting multiple survival surgeries must plan the project with the Attending Veterinarian or designee before submitting the protocol for IACUC consideration. This gives the Attending Veterinarian an opportunity to provide early guidance on how best to minimize pain, distress and/or discomfort to the animals.
2. The protocol submitted to the IACUC must include a description of the surgical procedures, the time frame for their performance and scientific rationale for doing multiple surgeries. Cost is not an accepted consideration in the IACUC protocol evaluation process.
3. In order to be considered for IACUC approval, the surgical procedures must be directed at securing a single valid objective.
4. If possible, the multiple procedures should be designed to cause less animal disability and/or morbidity than would a single complex procedure.
5. The proposed interval between procedures should be long enough to ensure an adequate recovery of the animal.
6. Patient monitoring capabilities for any multiple survival surgeries must be available and adequate.

Conservation of a scarce animal resource may justify the conduct of multiple major survival surgeries on a single animal, and will be reviewed critically by the IACUC. As part of the

approval process, the Institutional Official must submit a request to the USDA/APHIS and receive approval.

J. Expired Drugs and Medical Materials Policy

Purpose: The Rhode Island Hospital IACUC has adopted the following policy to provide instruction on the appropriate usage of drugs and materials in animal research studies performed in Rhode Island Hospital animal facilities or in field studies.

Background: *The Guide to the Care and Use of Laboratory Animals, 8th Edition* states that pharmaceutical grade chemicals should be used, when available, for all animal-related procedures. The use of lower grade substances/compounds with undefined or higher levels of impurities or poorly formulated non-commercial preparations can introduce unwanted experimental variables or toxic effects. A pharmaceutical grade compound should be used when available. The use of pharmaceutical grade chemicals helps ensure that the substances administered meet established documentable standards of purity and composition, which may also prevent adverse effects on animals or research outcomes. Administration of non-pharmaceutical grade compounds to animals must be scientifically justified and approved by the IACUC.

According to the USDA Animal Welfare Regulations Animal Care Policy #3 on Veterinary Care, “the use of expired medical materials (e.g. drugs, fluids, sutures, anesthetics, sedatives, or analgesics) during any survival surgical procedure on a regulated species is not considered acceptable veterinary practice and therefore not consistent with adequate veterinary care as required by the regulations promulgated under the Animal Welfare Act.” The finding of expired drugs and/or materials during a USDA inspection may result in a citation for inadequate veterinary care.

Scope: This policy applies to all drugs, medical supplies and/or devices used for animal studies at Rhode Island Hospital.

1. Definitions:

- **Pharmaceutical-Grade Compound:** A drug, biologic or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the United States Pharmacopeia (USP)-National Formulary (NF), British Pharmacopeia (BP), European Pharmacopeia (EP), etc.).
- **Controlled Substance:** Any material containing any quantity of a substance with a stimulant, depressant or hallucinogenic effect on the higher functions of the central nervous system, and having the tendency to promote abuse, physiological or psychological dependence.
- **Drug:** A substance used as a medication, including controlled substances.
- **Medical Supplies or Devices:** Materials, other than drugs, for use in animals that have an expiration date (saline, sutures, ointments, gauze packs, capillary tubes, blood collection tubes, syringes, needles, surgical gloves, etc.).

2. **Policy:** The use of expired drugs, medical supplies and/or devices is not acceptable veterinary practice and does not constitute adequate veterinary care. The use of expired

drugs, medical supplies and/or devices may result in harm to the animal and may compromise research data.

Each researcher is responsible and accountable for ensuring that expired materials are not used or present in his/her lab areas, procedure rooms, portable carts, etc. Principal Investigators (PIs) and laboratory staff are responsible for ensuring that expired drugs, medical supplies and/or devices are properly disposed of by their expiration date.

3. **Expiration Date:** Expired materials found in the vivarium may be discarded by Animal Care or the IACUC at any time, unless the materials are clearly labeled “Not for use in animals” and are stored separately from materials for use in animals.

The expiration date is the date printed on the label/package for materials with a manufacturer’s expiration. For dilutions, preparations, reconstitutions or mixtures of drugs or fluids prepared using aseptic technique and under proper storage conditions the expiration date is no more than thirty (30) days from the date of preparation. Such materials should be labeled by name, drug concentration, and include the new expiration date as soon as they are prepared. Secondary containers which hold an unadulterated solution (i.e. a drug or material from an original stock to which no drug has been added) should be clearly labeled with the name of the drug or material and the expiration date of the original stock. An item is considered expired the day after the month or date indicated on the label (i.e. an item labeled January 2016 would be considered expired on February 1, 2016).

Powdered forms of drugs or compounds (e.g., chemical grade substances ordered from Sigma) that do not bear an expiration date should be labeled with an expiration date of one (1) year from the date of receipt provided that they are stored aseptically in an air tight, light protective container. For drugs or solutions that are reconstituted for use, the expiration date may vary from the labeled expiration date. Reconstituted drugs and compounds that do not contain expiration or efficacy guidance in the labeled directions are to be labeled for expiration thirty (30) days after reconstitution.

4. **Discard Date:** All chemicals used on or in animals must have a discard date clearly labeled on the container. If an expiration date is not indicated by the manufacturer, or if the chemical is compounded/adulterated and the discard date is not detailed in the approved IACUC protocol, follow these guidelines:

- Whenever possible, items should be compounded for the project the day of use and discarded immediately after use.
- Sterile diluents without a manufacturer expiration date: When investigators wish to access sterile diluents multiple times (i.e. to obtain small volumes for administration and drug mixing), the investigators can do so only if they do not add any chemical to the fluid, they access the fluid(s) aseptically and they store the fluid(s) as recommended by the manufacturer. Under these conditions, the investigator can use the sterile fluid(s) for up to thirty (30) days after initial opening.
- If a drug is diluted or mixed with another compatible drug and put into a sealed, sterile container, it may be used for up to thirty (30) days (or at the earlier expiration date of the component drugs, whichever comes first).

For example, a ketamine-xylazine rodent anesthetic cocktail (10 mg/mL of ketamine + 2 mg/mL of xylazine in saline), when stored in a sterile sealed glass container, may

be kept up to thirty (30) days without refrigeration. If one or the other of the drugs reaches its original date of expiration prior to the thirty (30) days, then the solution must be discarded on or before the date of expiration.

- Upon dilution, the container must be marked with the thirty (30) day expiration date, for example: “Discard after <insert the date thirty (30) days in the future>”
- If a drug is diluted or mixed it must be inspected for precipitate prior to use and discarded if visible precipitate has formed.

5. **Exceptions:** The USDA's Animal Care Policy Manual allows for the use of expired materials (**except** anesthetics, sedative analgesics, and euthanasia solutions) in acute terminal procedures. Expired medical devices or materials (such as sutures, wound clips, catheters, etc.) and expired fluids (such as saline) may be used in non-survival animals without IACUC review. Expired materials must be kept in **a separate location** and must be labeled **“For Acute Studies Only”**.
6. **Record Keeping:** All records of any IACUC actions with regard to expired drugs, medical supplies and/or devices will be maintained in the IACUC files for a period of three (3) years from the conclusion of the matter in accordance with USDA standards or longer if required by applicable Rhode Island Hospital approved policies and procedures for records retention. The use of all controlled substances will be tracked and maintained within laboratories as specified by the Animal Care Facility.

7. **References:**

The Animal Welfare Act is posted on the USDA website at:
<https://www.nal.usda.gov/awic/animal-welfare-act>

The Animal Welfare Regulations are posted on the USDA website at:
https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf

The AVMA Guidelines on Euthanasia, 2013, are posted on the AVMA website at:
https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm_source=prettyurl&utm_medium=web&utm_campaign=redirect&utm_term=issues-animal_welfare-euthanasia-pdf

The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources is available from National Academy Press and posted on the web at:
http://www.nap.edu/catalog.php?record_id=12910

The Public Health Service Policy on Humane Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare is posted on the DHHS website at:
<http://grants.nih.gov/grants/olaw/references/phspol.htm>

K. IACUC Policy for the Humane Euthanasia of Laboratory Animals

1. General Background

a. Definition

The NIH *Guide for the Care and Use of Laboratory Animals* defines euthanasia as “the procedure of killing animals rapidly and painlessly”. The *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* describes euthanasia by a method that minimizes

or eliminates pain and distress. A good death is tantamount to the humane termination of an animal's life. Techniques used for euthanasia must be chosen to assure that a rapid loss of consciousness will occur followed shortly by death without pain or significant distress being perceived by the animal.

b. Humane Considerations

There is a wide variety of animal species used in biomedical research, and specific methods used for each species must be considered based on their anatomy and physiology. However, the general principles for humane euthanasia in all species have been summarized by the International Council for Laboratory Animal Science (2006):

c. Principles for Animal Euthanasia

1. Whenever an animal's life is to be taken, it should be treated with the utmost respect.
2. Euthanasia should place emphasis on making the animal's death painless and distress-free. The method likely to cause the least pain and distress to the animals should be used whenever possible.
3. Euthanasia techniques should result in rapid loss of consciousness, followed by cardiac or respiratory arrest and ultimate loss of brain function.
4. Techniques should require minimum restraint of the animal and should minimize distress and anxiety experienced by the animal, before loss of consciousness.
5. Techniques used should be appropriate for the species, age, and health of the animal.
6. Death must be verified following euthanasia and before disposal of the animal.
7. Personnel responsible for carrying out the euthanasia techniques should be trained:
 - to carry out euthanasia in the most effective and humane manner;
 - to recognize signs of pain, fear, and distress in relevant species;
 - to recognize and confirm death in relevant species.
8. Human psychological responses to euthanasia should be taken into account when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.
9. Ethics committees should be responsible for approval of the method of euthanasia (in line with any relevant legislation). This should include euthanasia as part of the experimental protocol, as well as euthanasia for animals experiencing unanticipated pain and distress.
10. A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little species-specific euthanasia research has been done. Gentle, careful handling of subject animals is of the utmost importance during the procedure in order to minimize distress to the animal. Measures should be taken to ensure that euthanasia is performed in a way that minimizes reactions among other animals that may be present. Euthanasia should be performed quickly and efficiently in a procedural area that is separate from rooms in which animals are housed. [Note: This is not always possible in a biohazard containment rodent room; in that case, euthanasia should take place in the room's Class II Biological Safety Cabinet.] When considering the impact of euthanasia on animal well-being, it is important to note that an unconscious animal does not perceive pain. Appropriately conducted procedures that render the cerebral cortex non-functional eliminate the perception of pain. Once this

initial unconscious state is reached, reflex motor activity may still be observed, but pain is not perceived. This concept can be utilized in two-step approaches that combine an initial anesthetic event (e.g., general anesthesia via isoflurane or tricaine) with a secondary physical method (e.g., decapitation or exsanguination).

2. Best Practice Information

The primary source document for appropriate euthanasia practices is the [American Veterinary Medical Association \(AVMA\) Guidelines for the Euthanasia of Animals: 2013 edition](#). However, the committee writing that report recognized that it cannot be considered an all-encompassing document, and the language allows the use of professional judgment based on other current literature sources. The following reference list includes some of the most useful and readily available sources to be used when euthanasia methods are being considered.

a. Guidance

- AVMA *Guidelines for the Euthanasia of Animals* (2013)
American Veterinary Medical Association
https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm_source=prettyurl&utm_medium=web&utm_campaign=redirect&utm_term=issues-animal%20welfare-euthanasia-pdf
- *Guide for the Care and Use of Laboratory Animals* (2011)
Institute for Laboratory Animal Research
<https://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth>

b. Species-Specific Information

- *Report of the ACLAM Task Force on Rodent Euthanasia* (2005)
American College of Laboratory Animal Medicine
https://www.research.fsu.edu/media/1854/aclam_report_rodent_euth.pdf
https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b5-2017_rodent_euthanasia-adult_final_1-25-17.pdf

3. IACUC Requirements

a. Protocol Requirements

Euthanasia is generally performed at the end of a project or, in some cases, at a point where animals would otherwise experience severe or chronic pain or distress that cannot be relieved. Because euthanasia may be needed as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments, protocols should include criteria for monitoring and initiating an early endpoint. This type of pre-planning for potential adverse outcomes will enable a prompt decision to be made by the research staff in conjunction with the veterinarian to ensure that the studies are humane and the objective of the protocol is achieved. Even when the planned experiment does not include euthanasia, there may be a need to humanely euthanize animals for unanticipated reasons. For this reason, at least one method must be documented for each species used in a protocol. Euthanasia techniques must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) during review and approval of the submitted protocol application form.

Any subsequent change in euthanasia techniques must also be reviewed and pre-approved by the IACUC. The Office for Laboratory Animal Welfare (OLAW) characterizes the method of euthanasia as a significant component of the animal use protocol. Use of a euthanasia technique that is not described in the approved protocol may be considered significant noncompliance, which can result in protocol suspension and mandatory reporting to the federal funding agencies that support the Principal Investigator.

b. Training and Personnel Requirements

Euthanasia must be carried out by personnel properly trained in the procedure being used. This is especially important when physical methods such as decapitation or cervical dislocation are used as the primary methods, since these techniques require a certain amount of expertise to assure a humane outcome. It is the PI's responsibility to ensure that all persons performing euthanasia are properly trained and supervised. All individuals performing euthanasia as part of a research project must be listed on the approved protocol. The Veterinary Services staff of the Central Research Facilities (CRF) is available to demonstrate and/or discuss euthanasia techniques. Training forms must reflect species-specific euthanasia training. CRF personnel may provide euthanasia service for a nominal charge.

The CAF has refrigerators/freezers for disposal of small animals. The RIH Central Transport Department assists in the transportation of large animals to be disposed of by RIH. Radioactive animal carcasses should be disposed of in accordance with the guidelines in the *RIH Radiation Safety Guide*.

c. Verification of Death

Proper euthanasia technique will include a physical examination or close observation to assure that the animal is dead prior to disposal. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanized and/or a secondary method must be used. Whenever possible, the best method is to confirm the absence of a heartbeat, which is a reliable indicator of death in most species. Monitoring respiration by observing chest movement is less valuable, because a heartbeat may continue after visible respiration has ceased.

Verification of death in animals can present special challenges. Unless total exsanguination or radical postmortem tissue harvesting (such as in the complete removal of the brain, heart, or lungs, or complete removal of a vital organ and/or transection of the vena cava or aorta) can be performed, thus vital internal organs with major blood supply such as the liver or both kidneys) will be certain to cause death, a secondary physical method such as decapitation, cervical dislocation or bilateral thoracotomy must be used to ensure death.

d. Equipment Used for Physical Methods

Physical methods of euthanasia are approved with conditions per the AVMA Guidelines with the conditions being that the operator demonstrates competence in the technique and that the instruments used are appropriate. The Principal Investigator (PI) must ensure that all personnel that perform euthanasia are appropriately trained and have demonstrated competence in the technique. The PI must also ensure that the choice of instrument is appropriate for the size and the

anatomical conformation of the animal involved, with input from the Attending Veterinarian as needed. In many cases the use of specialized equipment such as a guillotine is required for use. Disposable blades (razor blades or scalpel blades) may be used for neonatal rodent decapitation. When using scissors for decapitation, each lab must provide for the proper periodic evaluation and sharpening or replacement of equipment to assure proper function and document the regular maintenance of the equipment. Cervical dislocation on rodents can be used for mice and rats <200g. Demonstrated proficiency in these techniques is required if used in awake, non-anesthetized animals.

e. Study Considerations and Alternatives

It must be recognized that it is extremely important for experiments be planned and performed in a way that ensures the validity of the data produced. If the euthanasia method used interferes with the ultimate goals of the research study and makes the data unusable, then the lives of the animals may have been wasted. Careful consideration of the possible adverse effects of the various options available must occur. There may occasionally be special circumstances or situations in which options that are not listed in this document might be considered acceptable. These exceptions must be carefully considered by the investigator and the IACUC to assure the best outcome for the animals as well as the study.

f. Disposal of Carcasses

Prior to placing the carcass in a cooler or freezer, put it into a bag and label it with the name of the PI, IACUC Committee number, initials and the date and method of euthanasia (both primary and secondary if applicable). This applies to all species.

4. Recommended Agents and Methods of Euthanasia Listed By Species

The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol. Generally, inhalant or non-inhalant chemical agents (such as barbiturates, inhalant anesthesia, or CO₂) are preferable to physical methods (such as cervical dislocation or decapitation). However, scientific considerations might preclude the use of chemical agents for some experimental studies. All methods of euthanasia must be reviewed and approved by the IACUC.

The table at the end of this section provides information about Lifespan IACUC approved methods of euthanasia for various animal species and ages.

a. Rats, Mice, and other Small Mammals

- Inhalant anesthesia (isoflurane) except in animals under two weeks of age. Note: Must be followed by a secondary physical method, such as cervical dislocation, decapitation or bilateral thoracotomy.
- Carbon dioxide (CO₂) except in animals under two weeks of age. Note: Must be followed by a secondary physical method, such as cervical dislocation. Decapitation or bilateral thoracotomy.
- Neonatal rodents are considered resistant to hypoxia and thus must be exposed to prolonged CO₂ or isoflurane. Please consult the veterinarian for the best method of euthanasia of neonatal rodents <14 days of age.
- Barbiturates (given intraperitoneally or intravascularly) at any age.

- Exsanguination (under general anesthesia)
 - Physical methods such as decapitation (especially in mice and rats less than one week of age) or cervical dislocation performed by a trained individual with demonstrated competence in the technique being used.
- b. Rabbits**
- Barbiturates (given intravascularly)
 - Exsanguination (under general anesthesia)
- c. Swine**
- Barbiturates (given intravascularly)
 - Exsanguination (under general anesthesia)

5. Technical Comments on Agents and Methods

a. *Inhalant Anesthesia*

Because most inhalant anesthetics act as topical irritants in their liquid state, animals should be exposed to the vapors of the anesthetic only. Chambers must be designed to assure the animals don't come into contact with the wicking material that may be saturated with the liquid phase of the anesthetic. Sufficient air or oxygen must be provided during the induction period to avoid hypoxia prior to unconsciousness. All agents are given "to effect" until respiratory and cardiac arrest occurs. ***In order to assure mortality after inhalant anesthesia in those circumstances where death is not always a certainty (see Verification of Death in Section 3.c., above), a secondary physical method must be employed prior to disposal.*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity). Isoflurane is the only inhalant anesthetic approved for animal euthanasia at Lifespan.

b. Non-Anesthetic Gas

(NOTE: The Lifespan Policy on Carbon Dioxide Euthanasia must be followed, and the use of special equipment is required. See Required Use of Flow Regulators for CO₂ Euthanasia of Rodents below)

Carbon dioxide has long been the preferred technique for euthanizing rodents over two weeks of age and other small laboratory animals. Use of a sealed chamber filled by a compressed gas cylinder is required. CO₂ generated by other methods, e.g., dry ice, is unacceptable. Chambers must not be overcrowded to avoid distress during the procedure. Because CO₂ can act as a reversible anesthetic, it is imperative that the animals be kept in the chamber for at least one minute following the cessation of respiration. ***In order to ensure mortality after CO₂ exposure, a secondary physical method must be employed prior to disposal (see Verification of Death in Section 3.c. above).*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity).

Carbon Dioxide Euthanasia Procedures for Mice and Rats

(Lifespan IACUC Recommended)

**Note: these procedures are not suitable
for neonatal (< 2 weeks of age) animals.**

1. Use compressed CO₂ from a cylinder affixed with a regulator. (The use of dry ice or other sources of CO₂ is prohibited.)
2. Overcrowding or combining of cages and unfamiliar or incompatible animals is prohibited.
3. Do not pre-fill the euthanasia chamber with CO₂ prior to placing animal(s) in the chamber. Residual CO₂ (e.g., when the chamber contains CO₂ from recent use) is not acceptable. Care must be taken to empty and clean the chamber between uses.
4. In Middle House and Claverick, use a flow rate of 1.5 L/min for a mouse sized cage and 5 L/min for a rat sized cage. In Coro East and Coro West, use the CO₂ tubing labeled as “mouse” for a mouse sized cage and the CO₂ tubing labeled as “rat” for a rat sized cage.
5. Allow 5 minutes to pass.
6. Verify euthanasia (death) by cessation of breath and loss of heart beat. Perform a secondary method prior to disposal.

Required Use of Flow Regulators for CO₂ Euthanasia of Rodents

The American Veterinary Medical Association (AVMA) [Guidelines for the Euthanasia of Animals: 2013 Edition](#) mandate that pressure-reducing regulators and flow meters (or equivalent equipment) be used during CO₂ euthanasia of rodents, to provide an environment of controlled, gradually increasing CO₂ concentration.

The rationale for the use of controlled, gradually increasing CO₂ concentrations is that CO₂ euthanasia can cause distress via: (1) triggering pain due to the formation of carbonic acid on respiratory and ocular membranes, (2) the production of so-called air hunger and a feeling of breathlessness, and (3) direct stimulation of ion channels within the amygdala associated with the fear response. **Without flow regulators it is impossible to adequately control CO₂ chamber filling to the level required by the new Guidelines.** The optimal flow rate for CO₂ euthanasia systems is one that yields a displacement rate of 10% to 30% of the chamber or cage volume per minute.

Accordingly, **regulator/flow meter systems are required for all CO₂ euthanasia stations on the Rhode Island Hospital Campus and in the Lifespan-affiliated research facilities (CORO, Claverick, and Kilguss).** New regulators and flow meters have been installed in the public CO₂ euthanasia stations within the CRF facilities. **Investigators who desire the convenience of performing CO₂ euthanasia in their laboratories must purchase and install appropriate CO₂ regulator and flow systems.**

c. Pharmacological Agents

Use of pharmacological agents requires adequate appropriate physical restraint and mastery of appropriate injection techniques. Barbiturates are acceptable for all species, but are most commonly used for mammalian species and birds. These drugs

should be administered intravenously (IV) whenever possible, but intraperitoneal (IP) administration is acceptable for rodents. Sodium pentobarbital is the most common barbiturate agent for euthanasia, used either alone or in commercially available euthanasia mixtures. The dosage is usually at least twice that required for anesthesia. Fatal-Plus at 1ml/10lbs (i.e. >86 mg/kg) of the recipient animal is most often recommended. Investigators using this agent are required to store the drug in a double locked location and maintain detailed use records. An overdose with non-barbiturate injectable anesthetic (e.g., ketamine/dexmedetomidine or ketamine/xylazine) is not acceptable as a sole method, but such drugs can be used to sedate or anesthetize animals prior to the use of a physical method in a two-step procedure. ***In order to ensure death after the use of pharmacological agents a secondary physical method must be employed prior to disposal (see Verification of Death in section 3.c., above).*** Examples of acceptable secondary physical methods include cervical dislocation (for mice or rats no larger than 200 grams), decapitation or thoracotomy (making a stab incision into the chest to open up the thoracic cavity).

d. Physical Methods - (NOTE: Physical methods require that the user have experience and skill in the techniques to be used.)

- **Exsanguination** is acceptable for all species under general anesthesia. Rapid removal of blood can be accomplished by severing major vessels or (in smaller animals) by cardiac venipuncture.
- **Cervical dislocation** is acceptable for mice and rats weighing less than 200 gm, but proper technique is essential. Individuals performing this technique must receive prior training and have demonstrated competence in its use.
- **Decapitation** with proper equipment may be performed on mice and rats. Decapitation using sharp scissors or a blade is a preferred method for mice and rats less than one week of age. Individuals performing this technique must receive prior training and have demonstrated competence in its use. Many species react adversely to the smell of blood, so animals should not be decapitated in the presence of other animals and the person performing decapitation should change gloves and/or wash hands between animals.

Unintended recovery of animals after apparent death (e.g., found alive in morgue) constitutes a SERIOUS NONCOMPLIANCE with the PHS Policy and serious deviation from the provisions of the Guide for the Care and Use of Laboratory Animals. Any incidents of unintended recovery must be reported to the IACUC and OLAW.

IACUC Recommended Euthanasia Methods			
Species	Age/Wt	Method/Route/Dose	Comments
Mice/Rats	≥ 14 days	CO ₂ /Inhalation/to effect	Follow Lifespan Procedures (above) Secondary physical method required
Mice/Rats	All	Fatal Plus (or other barbiturate) /IP/≥ 86 mg/kg	

Mice/Rats	All	Isoflurane/Inhalation/to effect	Secondary physical method required
Mice/Rats	≤ 7 days	Decapitation	Sharp blade or scissors; demonstrated competency
Mice/Rats	All	Decapitation	General anesthesia or justification with demonstrated competency
Mice/Rats	≤ 200 g	Cervical dislocation	Demonstrated competency
Mice/Rats Rabbits	All	Exsanguination	General anesthesia
Rabbits	All	Fatal Plus (or other barbiturate) /IV/ ≥ 86 mg/kg	Ear vein or other suitable vessel
Swine	All	Fatal Plus (or other barbiturate) /IV/ ≥ 86 mg/kg	Ear vein or other suitable vessel Typically sedated
Dogs/Cats	All	Fatal Plus (or other barbiturate) /IV/ ≥ 86 mg/kg	Cephalic vein or other suitable vessel

L. Animal Health Program

Lifespan maintains an animal health program physical examinations and assessments. Refer to *Appendix 6 Animal Health Program* for a list of normally administered vaccinations for large animals.

M. Animal Health Surveillance

- Diseases in rodents are known to alter research results. Several bacterial and mycoplasmal diseases manifest themselves clinically after long incubation periods or only after experimental stress. Inapparent viral diseases have been shown to have immunomodulatory effects. Therefore, the veterinary staff recommends that investigators utilizing rodents as animal subjects purchase them from vendors that maintain stocks and strains free from murine pathogens.
- Surveillance programs are instituted to monitor in-house colonies of these animals to ensure that their microbiological integrity has remained unchanged.
- The veterinary personnel, in conjunction with commercial laboratories, provide surveillance under the animal health program protocol. Periodic submission of sera for virus and mycoplasmal antibody testing and parasitological exams is recommended (*Appendix 6 Animal Health Program*).

N. Rodent Health Monitoring Program

1. Overview

The health status of the rodent colonies, which include all rodent holding rooms in Middle House, Coro West, Coro East and Claverick, are monitored on a quarterly basis for the early detection of viral and/or parasitic infections that could compromise animal health and/or the interpretation of research results. The program utilizes sentinel rodents which

have been exposed to soiled bedding from the study animals housed in the same location. The sentinel mice then undergo quarterly testing for endoparasites, ectoparasites via PCR testing and viral pathogens via serology testing. These quarterly results are available to all Rhode Island Hospital investigators conducting animal research and are also available to external facilities wishing to import/export rodents from our facility. If a potential contamination is detected, researchers are promptly informed as described below.

2. Response to Positive Murine Pathogen Findings in Lifespan Facilities

CRF Management Team: CRF Director, Veterinarians, CRF Managers

CRF Administration: Vice President, Research Administration, IACUC Chairperson and Vice Chairperson, IACUC Manager/Coordinator

General Practice

- The Veterinary Services Supervisor will forward all laboratory results to the veterinarians the same day of receipt.
- Within 12 hours of receipt the veterinarians will review the reports and make a determination as to whether the findings warrant action.
- *If the findings do not warrant action*, the veterinarians will inform the CRF Director and CRF Managers via email of the results and their interpretation.

Unanticipated Findings

- 1) *If the findings do warrant action*, the veterinarians will immediately:
 - Inform the CRF Administration via email,
 - Inform the CRF Director via email or telephone call, and
 - Contact the Veterinary Services Supervisor to initiate Confirmatory Testing
- 2) Upon receipt of the veterinarian's notice of actionable unanticipated findings, the CRF Director will immediately:
 - a. Instruct the Managers to increase the level of containment in the affected and any "at risk" rooms, including:
 - Cessation of movement of animals into or out of the affected room(s),
 - Posting of a notice on the door(s) to the affected rooms describing the results of the pathogen testing, the need for heightened containment measures, and any special sanitary precautions. The notice will include contact information (desk extension and/or cell phone numbers) for the Attending Veterinarian and the CRF Director
 - b. The CRF Director will open a Pathogen Outbreak file and begin documenting the outbreak and CRF response. The file will remain open and be updated regularly until the outbreak is eradicated.
 - A brief note will be written in the file each week day by the CRF Director or veterinarians to document progress, assess compliance with procedural issues and make recommendations for modifications.

- c. Notify all affected Principal Investigators via email
- d. Organize a meeting of the full CFR Management Team to review the unanticipated findings. The meeting will be held within 48 business hours of the veterinarian's notice to CRF. The purpose of the meeting will be to discuss the unanticipated findings, the timeframe and potential outcomes of the confirmatory testing, and potential modifications of the heightened containment measures. The goals of the meeting will be to:

Define and Contain the Outbreak

- Define the areas of “presumed” contamination and areas of “likely” contamination (based on the characteristics and transmissibility of the infectious agent).
- Identify potential cross-over areas that may require additional disinfection procedures, and define disinfection procedures.
- Define modifications to required garbing/protective gowning practices

Develop a Plan for Continued Surveillance

- Review the status of the Confirmatory Testing
- Develop recommendations for additional confirmatory and surveillance testing throughout the facility for all three possible confirmatory test outcomes: negative, positive or equivocal

Modify the Standard Animal Husbandry Plan

- Document the number of affected Investigators, IACUC Protocols, and animals in the presumed and likely contaminated areas
- Define standard husbandry procedures in the affected areas
- Generate recommendations for the modification of the standard husbandry procedures to minimize the risk to other areas of the facility and Institution

Generate a Tentative Plan for Animal Disposition

- Develop recommendations for disposition of animals in the affected rooms (and facility, if necessary), which may include depopulation.

- e. Coordinate individual or group meetings between any affected Principal Investigators, the veterinarians and CRF Director

(Note: It is expected that the CRF Director and Managers will contribute to the discussions outlined in 2d., above. However, the ultimate responsibility for the final Containment, Surveillance, Husbandry and Depopulation Plans rests with the Attending Veterinarian.)

- 3) By the time the results of the Confirmatory Testing are received (typically within 7 days) the veterinarians will have generated a brief report of the outbreak and a Tentative Management Plan, with contingency recommendations for negative, equivocal and positive findings (Table 1, below).

The veterinarians will forward their tentative management plan and recommendations to the members of the CRF Administration and the CRF Management Team as soon as they are completed.

Table 1: Options for Tentative Management Plan

Confirmatory Testing Results	Potential Courses of Action
Negative Results (veterinarian Interpretation)	1) No Additional Surveillance <ul style="list-style-type: none">• Return to normal operations 2) Continued Surveillance <ul style="list-style-type: none">• Recommend testing interval and duration of follow-up confirmatory testing• Recommend modifications to level of containment
Equivocal Result (veterinarian Interpretation)	Continued Surveillance <ul style="list-style-type: none">• Recommend testing interval and duration of follow-up testing• Recommend modifications to level of containment• Perform additional testing (i.e. environmental) or pooled or individual cage testing
Positive Result (veterinarian Interpretation)	1) Continued Surveillance <ul style="list-style-type: none">• Recommend testing interval and duration of follow-up testing• Recommend modifications to level of containment• Perform additional testing (i.e. environmental) or pooled or individual cage testing 2) Colony Disposition <ul style="list-style-type: none">• Recommend plan for colony disposition/depopulation

Results of Confirmatory Testing

- 1) Upon receipt of the results from the confirmatory testing the veterinarians will immediately:
 - Inform the CRF Administration and Director via email
- 2) Upon receipt of the veterinarians' notice, the CRF Director will immediately:
 - a. Notify all affected Principal Investigators
 - b. Coordinate an open meeting for all interested researchers to convey the results of testing and the Management Plan.
 - The meeting will be scheduled within 72 hours of receipt of the Confirmatory Testing results.
 - Researcher invitations will be via email and will include a description of the outbreak, the results of the confirmatory testing, and the tentative plan for containment or eradication.
 - Presenters at the open meeting will include, at minimum, the veterinarians, the CRF Director, the Administrative Director of Research Administration, and the IACUC Chairperson/Vice Chairperson.
- 3) **If the confirmatory testing results are negative or equivocal**, the veterinarians will contact the CRF Director to begin execution of the appropriate sections of the Management Plan.
 - If additional surveillance is necessary, the plan will be modified using the process outlined in item 2c, as necessary.
- 4) **If the confirmatory testing results are positive**, the veterinarians will contact the CRF Director to begin execution of the appropriate sections of the Management Plan.

- If additional surveillance is necessary, the plan will be modified using the process outlined in item 2c, as necessary.
- 5) The CRF Director and/or Attending Veterinarian will send progress updates to all PI's weekly until the offending pathogen has been eradicated.

O. **IACUC Policy for Tumor Implantation**

1. **Purpose**

- To provide guidelines for a tumor implantation and monitoring for mice or rats inoculated with neoplastic cells or toxic agents or animals that are genetically predisposed to develop tumors. This guideline is relevant to all investigators using models of neoplasia, including all subcutaneous, liquid, or non-palpable tumors; in addition, it applies to naturally occurring tumors. Humane interventions and endpoints should be determined and specified in the Animal Care and Use Protocol (ACUP) for all animals that will undergo tumor development as an expected part of the experimental protocol.
- To describe the procedures for monitoring and documenting animals on protocols involving experimentally induced tumors.
- To provide guidelines for evaluating the overall health of the animal and applying humane endpoint criteria.

2. **Tumor Implantation Sites**

Tumor implantation sites should be chosen to minimize adjacent tissue damage or disrupting normal physiology. **The IACUC recommends implanting tumors on the dorsum or flank of an animal**, as these areas will likely have the least amount of site-related morbidity. If other sites are to be used, describe and justify in the ACUP.

- Sites involving the face, limbs or perineum should be avoided as there is little to no space for tumor growth and expansion, and they may interfere with eating and drinking.
- Intramuscular implantation should be avoided to prevent inhibiting normal movement
- Tumor implantation on the abdominal surface of the body should also be avoided due to the risk of irritation to the tumor site in contact with the bedding and floor of the cage.

3. **Tumor/Clinical Evaluation**

Evaluating tumor burden based only on a percentage of body weight is generally not accurate while the growing tumor(s) may cause an increase in body weight, the general condition of the rodent may be decreased (loss of lean body mass), resulting in a relatively stable body weight but an unhealthy animal. Tumor burden should be determined by evaluating the following:

- Body Condition Score (BCS); see below. Alternatively, for liquid tumors body weights may be used.
- Objective dimensional criteria (size)
- Anatomical location
- Incidence of multiple tumors

e. Tumor ulceration

[The following guidance assumes that a normally sized adult rodent will be studied (a ~25 g mouse or a ≥250 g rat). The allowable sizes of tumors will be decreased if the tumors are injected into immature or genetically small mice.]

4. Tumor Size and Location

The concern of size for individual tumors is related to central necrosis, ulceration of skin overlying tumors, and abrasions. When on the dorsum or flank of adult rodent, tumors may be allowed to grow to the following volumes as long as the rodent remains otherwise healthy.

- Mice: 2000 mm³ in size (which is roughly 10% baseline body weight),
- Rats: 5000 mm³ in size

(For the basis of this policy, tumors may be measured using the following formula:
TV = [(Width)² X Length] / 2)

5. Multiple Tumors

Multiple tumors that are individually smaller than the single tumor limit may not have the same negative sequelae as a single tumor. Multiple tumors may be allowed to grow up 150% (or 3000 mm³) of the volume compared with the volume of a single tumor. Please note that the limitation on any single tumor (2000 mm³ volume in mice) will still be valid.

6. Tumor Ulceration

Ulceration (overt open lesion or scabbed area) of a tumor typically requires euthanasia UNLESS justified in the protocol and in consultation with the veterinarian, and will require at least daily monitoring.

7. Non-palpable or liquid tumors

Evaluating liquid tumors (e.g. leukemia) and tumors in central areas of the rodent's body (e.g. bone, brain and lungs) can be challenging. Tumor size will likely not be useful due to inability to measure size or because of the sensitivity of areas to compressive lesions. For these models, the BCS AND/OR body weight along with clinical evaluation of the animals take priority regarding decisions on humane endpoints. The expected clinical signs and the humane endpoints of those signs must be clearly described in the protocol. A scoring system (as mentioned above in this document) may be most helpful in this scenario. The evaluation of clinical signs in an animal with a tumor burden of this type should include consultation with a veterinarian.

8. Tumor Monitoring Procedures

a. Principal investigator or designated lab member

- 1) Identify each cage at the time of injection of tumor cells, cage cards must be identified with an identifying tag. Tumor monitoring must begin at this time per protocol specific frequency (**or at least once per week, whichever is more frequent**). After a visible or palpable tumor is evident, the animals must be monitored at least twice weekly. More frequent observations may be necessary as determined by the veterinarian, based on tumor growth rate, study parameters, and

general condition of the animal (possibly including weekends and holidays.) The overall wellbeing of the animal will take priority over precise tumor measurements in decisions regarding euthanasia or other interventions.

- 2) Provide each cage with a unique cage number on the identifying tag using a permanent marker. (This is intended to facilitate communication between the research laboratory and the animal care staff and veterinarians.)
- 3) A tumor monitoring sheet must be filled out for each protocol endpoint. The monitoring sheet must be filled out completely indicating:
 - protocol specific endpoints
 - monitoring frequency
 - contact information for the person who is directly working with the animals

For each observation, fill in date, observation code, cage identification numbers, and initials. For observations (U) ulcerated, (D) found dead and (E) euthanized, record number of animals with the observation code.

b. **Veterinary Services Staff**

- 1) Inspect the tumor monitoring sheet at least once a week (same day each week).
- 2) Notify the laboratory, in writing, that “tumor monitoring sheet upkeep” is required if not adequately completed and needs to be completed in the next 24 hours.
- 3) Examine any animal of concern during the standard daily animal health checks and report at least the following:
 - any tumor reaches the size of a dime (18 mm)
 - any tumor which inhibits mobility
 - skin ulceration noticed at the tumor location
 - clinical signs including loss of body condition
- 4) Verify the tumor monitoring sheet for completeness and consistency with the protocol for the following:
 - laboratory contact
 - protocol number
 - cage identification number
 - tumor monitoring frequency
 - protocol endpoint
- 5) Contact the responsible laboratory member as needed.
- 6) Report any communication issues to the veterinarian.

9. Animal Assessments

a. **Body Condition Score (BCS)**

The general physical condition of the animal is an important factor in effectively following the progression of tumors in rodents. Scoring systems from “1” (emaciated/wasted) to “5” (obese) are often used. BCS is a helpful adjunct to assessment of overall health of the animal. It is important to note that treatments designed to affect tumor growth (such as chemotherapeutics) which are often part of

tumor load studies, can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumor load experiments.

- b. General clinical signs should be assessed. Any evidence of lethargy or other change in behavior, change in ambulation, diarrhea, neurological signs (e.g. circling, head tilt) or increased respiratory effort need to be reported to the veterinary staff.
- c. The known biology and effects of any individual tumor model will be described in the ACUP, including expected clinical signs, anticipated moribundity/mortality, interventions for the relief of pain and suffering, and objective criteria for the assessment of humane endpoints.
- d. Any animal which is found to be at protocol endpoint or which meets the guidelines for end stage illness must be euthanized.
- e. The professional judgment and decision of the Attending Veterinarian is final.
- f. Reference: Wallace J. *Humane endpoints and cancer research*. ILAR J 2000; 41:87-93.



BC 1

Mouse is emaciated.

- **Skeletal structure extremely prominent;**
- little or no flesh cover.**
- Vertebrae distinctly segmented.**



BC 2

Mouse is underconditioned.

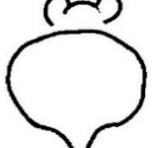
- **Segmentation of vertebral column evident.**
- Dorsal pelvic bones are readily palpable.**



BC 3

Mouse is well-conditioned.

- **Vertebrae and dorsal pelvis not prominent;**
- palpable with slight pressure.**



BC 4

Mouse is overconditioned.

- **Spine is a continuous column.**
- Vertebrae palpable only with firm pressure.**



BC 5

Mouse is obese.

- **Mouse is smooth and bulky.**
- Bone structure disappears under flesh and subcutaneous fat.**

Rodents must be euthanized if:

- The body condition score is 1/5
- The body condition score is 2/5 and the mouse has decreased activity/ responsiveness
- The tumor affects the rodent's gait or normal posture, ability to eat, urinate, or defecate independent of the size of the tumor
- The veterinarian determines that the animal should be euthanized for humane concerns

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

10. Utilization of transplantable tumors, cell lines and other biologics

Transplantable tumors, cell lines, and biologicals which have been passaged in animals may be contaminated with viable pathogens present in those animals. Murine viruses have inadvertently contaminated rodent colonies in this way and there is a potential for pathogen transfer in all species. All transplantable tumors, cell lines, and other biologicals with previous passage in animals must be tested for adventitious pathogens prior to use at Rhode Island Hospital. The CRF Director or veterinarians can provide additional information on testing options.

Biologicals posing special hazards to humans must also be approved by the Institutional Biosafety Committee. Organizations that provide biological materials, e.g. ATCC, typically do not test for these agents. Biologicals typically require additional testing in order to detect possible infectious contaminants before passage occurs in animals at Rhode Island Hospital.

In addition to obtaining IACUC approval, Investigators must obtain approval from the Institutional Biosafety Committee (IBC) to utilize particular biologics in animals within the facility.

P. Policy on Use of Human Source Tissues and Cells in Immunodeficient Animals

Human source tissues and cell lines may carry human or zoonotic pathogenic or adventitious agents. When placed in immunodeficient animals, such as nude or SCID mice, these agents have the opportunity to replicate and may present a risk to scientific and animal care staff.

ATCC does not test all cell lines for human pathogens, in fact, some are known to be positive for human pathogens. The organization recommends that viral testing should be performed on their cell lines, especially when culturing cell lines in an animal facility or *in vivo* conditions. ATCC recommends: "Please keep in mind that all adventitious agents may not be detected through viral testing. For this reason we strongly recommend that all human and other primate cell lines be handled at the same biosafety level as a cell line known to carry HIV or hepatitis virus."

Immunodeficient mice and rats carrying human cells or tumors will be housed at Animal Biosafety Level 2 (ABSL-2).

See supporting references: [CDC Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)](#) and [CDC Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories](#)

Q. Prolonged Restraint

In general, restraint for all animals should be the least restrictive and for the shortest time necessary to complete research objectives. Prolonged restraint should be avoided unless it is essential for achieving research objectives. Examples of prolonged restraint include primate chairing, rodent restraint in inhalation chambers, and swine and dogs restrained in slings. Consider the following guidelines:

1. Restraint devices are not to be considered normal methods of housing and must be justified in the animal use protocol.
2. Restraint devices should not be used simply as a convenience in handling or managing animals.

3. Alternatives to physical restraint should be considered
4. The period of restraint should be the minimum required to accomplish the research objectives.
5. Animals to be placed in restraint devices should be given training to adapt to the equipment and personnel.
6. Animals that fail to adapt should be removed from the study
7. Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.
8. Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates temporary or permanent removal of the animal from restraint.
9. The purpose of the restraint and its duration should be clearly explained to personnel involved with the study.

R. Environmental Enrichment Program for Laboratory Animals

1. Objectives

The objective of the Environmental Enrichment program is to provide the research animals housed in Rhode Island Hospital research facilities with living environments which allow for expression of non-injurious species-typical activities. This is required by the USDA Animal Welfare Act (AWA), the *Guide for the Care and Use of Laboratory Animals* (the *Guide*), and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Enrichment is a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animal and drawing out their species-appropriate behaviors and abilities. Environmental and Behavioral enrichment provides animals with the opportunity to do things that seem they seem to find enjoyable all while promoting physical and mental health. *The Guide for the Care and Use of Laboratory Animals* states providing laboratory animals with enrichment has shown to better the animal welfare and behavior, improve the handling and restraint of animals, as well as, improve the outcome of results and data. It also states animals should be housed with the goal of maximizing species specific behaviors and minimizing stress-induced behaviors. By providing certain species specific behavior, such as nesting, hiding, and gnawing, we can lessen the effect of the variables we are unable to control.

2. Definitions

*Manipulanda – Any objects that can be manipulated by an animal or encourage it to engage in fine motor movements, such as wooden blocks or prefabricated plastic chew toys.

3. Details of Procedures

a. General

- 1) All animals will be provided environmental enrichment, which is considered beneficial for that species.
- 2) When exemptions to this SOP are required due to study restrictions they must be justified by the Principal Investigator to the IACUC who will evaluate the request

based on scientific grounds. The IACUC has sole authority to grant exemptions. The PI can request the exemption in the ACUP or by an amendment. An alternative enrichment will be proposed whenever possible.

- 3) The Attending Veterinarian is charged by the IACUC for overseeing the Environmental Enrichment program as described in this SOP, and does have the authority to restrict environmental enrichment for medical reasons. Restrictions must be in writing and renewed monthly. Veterinary exemption will be noted in the animal's record.
- 4) The CRF management is charged with ensuring the implementation of all procedures. The animal care staff will be responsible for carrying out this program.
- 5) The CRF Supervisors will be responsible for periodically evaluating the condition of all environmental enrichment devices (manipulanda) and disposing of any items that are severely chewed, contain sharp edges or are otherwise broken or unsanitizable. Manipulanda will be changed, sanitized, or discarded at least every 2 weeks at the time of cage cleaning. Reusable manipulanda will be cleaned and disinfected in the cage washer.
- 6) Toys/devices will be selected and maintained with respect to the safety of the animals. The animal care staff will notify the CRF Supervisor of any problems or potential problems with enrichment items.
- 7) The environmental enrichment program will be re-evaluated periodically based on investigator and CRF staff feedback.
- 8) An enrichment program will be developed for new species prior to the species being received in the CRF. The IACUC is responsible for notifying the Attending Veterinarian and CRF Director of plans for adding the new species to the program. The Attending Veterinarian and Director will decide on the best items and methods to use to provide enrichment and will amend this SOP.

b. Enrichment Details

At least one enrichment method is always used from one of the following enrichment groups (comprising Manipulanda, Nutritional, and Socialization/Environmental) listed below.

1) Rodents

Laboratory mice show a diverse behavioral repertoire: they seek a wide variety of foods, are very physically active, form complex social organizations, build tunnels and construct nests. Mice experience chronic frustration when placed in conventional non-enriched cages. Mice that are placed in conventional enriched cages show improved breeding, larger litter sizes, minimize fighting, and lessen aggression.

Rats are naturally very social creatures that are curious and acceptable of new types of enrichment and socialization. Rats play frequently with each other which serves as a form of vigorous exercise that is essential for the well-being and

normal social and sexual development of the species. Individually caged rats are more susceptible to stress which jeopardizes the validity and outcome of research.

a) Manipulanda

- Chew Toys (rats): e.g. Nylabone®, wood blocks
- Nesting material (mice): e.g. Nestlets®, Enviro-Dri for hairless mice
- (rats): Alpha-twist, wood blocks

b) Nutritional

- Food: N/A

c) Socialization/Environmental

- Group housing

2) Rabbits

Rabbits are naturally prey creatures that prefer a quiet and calm environment with abilities to hide and exercise. Single caged rabbits that have access to hay and other enrichment objects show a reduction in stereotypical behaviors and a marked increase in overall activity. Rabbits who receive special attention from personal which includes handling, petting, gentle vocalization, show an increased resistance to certain pathological processes than subjects who receive no extra time. Providing treats helps win the rabbit's confidence and trust and allows the technician and rabbit to bond.

a) Manipulanda

- Small hard plastic balls
- Metal rings on a chain
- Plastic dumbbell
- Metal Rattles

b) Nutritional

- Timothy Hay
- Carrots (alternating schedule with Cheerios and/or Fruity Bites)
- Cheerios(alternating schedule with carrots and/or Fruity Bites)
- Fruity Bites(alternating schedule with Cheerios and/or carrots)

c) Socialization/Environmental

- Animals are group housed until 4 months of age or when fighting or mounting is observed in the cage. Animals are also separated after a sedated procedure/surgery.
- Once separated, animals socialize by touching noses through a 1 inch cage in our Allentown banked cages. If fighting occurs, a see through fighting barrier is placed.

3) Pigs

Pigs are conspicuously sensitive animals who require special attention to guarantee their physical and behavioral well-being in the often stress environment in the research institution. Pigs are naturally shy and quite aware of their surroundings, but will become very curious and smart with repeated enrichment. They can be trained to a variety of simple tasks such as sitting, lying down, and

walking onto a scale. The largest type of enrichment we can provide for swine is to promote their species-specific need for foraging. All pigs will be socially housed unless otherwise noted by veterinarian or by protocol reasoning.

- a) Manipulanda
 - Large, hard plastic balls
 - Suspended chain
- b) Nutritional
 - Food: fruit, cereals, marshmallows, carrots
- c) Socialization/Environmental
 - Pair housing in room when possible
 - Petting and grooming
 - Contact bedding (pine shavings) with small treats for foraging

S. **Mouse Tail Biopsy**

Tissue for genetic analysis of mice may be obtained by tail biopsy (tail snip) when scientifically justified and approved by the IACUC.

The following guidelines have been approved by the IACUC for the collection of mouse tail tissue. Note: tail biopsy must be described in the protocol/amendment and any proposed deviations from these guidelines require additional scientific justification

1. The genotype of a mouse is typically determined by Polymerase Chain Reaction (PCR) or Southern Blot analysis.
 - PCR analysis requires a minimal amount of tissue which can be obtained from tail biopsy. PCR provides genotyping results quickly and cheaply allowing for efficient colony management.
 - Southern Blot analysis requires larger amounts of DNA which is typically obtained by the excision of the distal tail.
2. The tail is composed of bone, cartilage, blood vessels, nerves and skin. The extent of mature vertebrae is related to the age of animals and the location along the length of the tail. A tail biopsy (2-5 mm at the distal end of the tail) that severs coccygeal vertebrae prior to completion of mineralization, which occurs when the mouse reaches 3 weeks of age, causes only minimal pain.
 - Tail amputation in mice >3 weeks of age may be a painful procedure with the potential to produce significant hemorrhage and will require anesthesia or analgesics.
 - A mouse's tail is important physiologically and behaviorally. Minimizing the amount of tail tissue removed will benefit the animal and its use in research.

3. **Procedure**

- Limit the amount of tail to be amputated to 2-5 mm; 2 mm would be preferable and will minimize cutting bone. If an additional testing is anticipated, section the original tissue and freeze a segment. A second biopsy is permissible but must be done under anesthesia (see #5).
- Gently restrain the mouse.

- Obtain tail biopsies, using clean procedures, by cutting the tip of the tail perpendicular to the long axis with very sharp scissors. Alternatively, use a scalpel or razor blade.
- Assure hemostasis. In mice <3 weeks, hemostasis is easily achieved by light, direct digital pressure around the tip of the tail. When necessary, hemorrhage can be controlled by cautery; a medical-grade, non-toxic, styptic powder (Kwik Stop®) or surgical adhesives. Consult the veterinarians if problems with hemostasis are encountered or expected (e.g., mutant mice with clotting disorders).
- If required, use a short acting inhalant anesthetic, such as Isoflurane: an open-drop technique, conducted in a fume hood while avoiding direct contact with the animal, would be acceptable. Closely monitor the animal's recovery from anesthesia, which should be transient, and avoid co-housing sedated and active animals.

T. Rodent Toe Clipping for Biopsy and Genotyping

This protocol outlines a set of guidelines for the use of toe clipping as an alternate method for rodent identification and biopsy for genotyping.

1. General

- a. This method should only be used when other identification methods (e.g. ear notching, tattooing, ear tags or microchip transponders.) are not feasible. This method is typically restricted to situations where young neonates need to be identified.
- b. This method is covered in the “Guide for the Care and Use of Laboratory Animals” and will follow the guidelines put forth.
- c. The use of this method must be outlined on the protocol and submitted to the IACUC for review. The IACUC will require justification for the use of this method over other methods.
- d. Toe clipping involves the removal of the last phalangeal (toe) bone of the digit, excluding the pollex. This method should only be performed by well-trained personnel, using a sharp, clean instrument. The removal of the distal phalange could interfere with research testing, although there is evidence that grip strength is not compromised and that the procedure did not cause hyperalgesia at the amputation stump e.g. In addition, neonates with clipped digits did not suffer rejection by their mothers.
- e. If at all possible, genotyping should be completed at the same times as this procedure, and in fact should provide adequate tissue for the PCR genotyping.

2. Procedure

- a. This procedure does not require anesthesia when restricted to neonatal rodents, up to seven days of age. Toe clipping of animals older than seven days is discouraged and would require anesthesia/analgesia and a literature search for alternatives to this painful and/or distressful procedure.
- b. The cut should remove only the distal portion of the toe but should include the entire nail bed

- c. Minimize the number of toes amputated. By policy, no more than two toes on one foot should be clipped and typically a numbering system that includes no more than two feet should be used.
- d. Use a very sharp, clean microsurgery scissors. The instrument should be cleaned in between each animal with 70% alcohol and chlorhexidine.
- e. Bleeding should not be a problem, but if it occurs, use gentle pressure with clean gauze.

U. Separating and Weaning Rodents

1. Overview:

The objective of this policy is to inform CRF and research personnel of the system of identifying overcrowded cages and newly split cages of rodents. Breeding cages must be identified with a unique number, code or LabTracks cage number that can be used by CRF staff in identifying these cages and will follow the rodents when weaning pups or when dividing cages. CRF staff must comply with all governmental regulations and guidelines. These guidelines, used by OLAW, are based on performance indices related to animal well-being and research with due consideration of the Animal Welfare Regulations and PHS Policy set forth by the most current edition of the NRC *Guide for the Care and Use of Laboratory Animals*. ***Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.***

On detection of an overcrowded cage, the PI/Lab will be contacted. At that point, the overcrowded cage must be separated **within 24 hours**. If the PI/Lab does not rectify the problem within a 24 hour period, the CRF staff will separate the animals and the cost center **will be charged a processing fee in addition to the per diem fee**.

Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

2. Details of Procedures:

- a. On detection of an overcrowded cage such as weaning required and/or with multiple litters, the PI/Lab will be contacted via the Vet Services rounds report, Vet Services staff or CRF Supervisor/Manager.
- b. CRF or Vet services will place a “Cage Overcrowded” sign on the cage. The top of the card will be filled out and the breeding cage bar code number will be noted.
- c. At that point, the overcrowded cage must be separated within 24 hours. If the PI/Lab does not rectify the problem within a 24 hour period, the CRF staff will separate the animals and the cost center will be charged a processing fee in addition to the per diem fee by Veterinary Services in the monthly Veterinary Services billing.
- d. Animals that just gave birth should not be moved for a minimum of 3 days. This includes weaning and cage change. If animal cages could become overcrowded due to the animal giving birth Vet services/ CRF Supervisor/Manager will notify the PI/Lab that the caging could become overcrowded.
- e. If after 24 hours and the PI/Lab has not separated the overcrowded cage, CRF staff will wean/split the animals by moving them into a new cage.

- f. The “Cage Overcrowded” card will be completed and the (separated by) line will be filled out by the person conducting the split/weaning.
- g. A yellow “Cage Split Notification” card will be placed on the breeder cage by the person conducting the split/weaning.
- h. A second “Cage Split Notification” card will be placed on the (weaned/split) cage/s and be filled out with the date weaned, initials and breeder cage number. This will notify the lab that the cage has been split and assure that food and water has been added to the new cage/s. This card also acts as a flag to the next CRF person that enters the room. This person will double check the cage/s to ensure that they have enough food and water and that the number of animals indicated on the card is correct.
- i. When weaning litters, separate males and females. Follow the space requirements to prevent overcrowding of cages.
- j. When writing out cards, use the original codes that are on the breeder’s cage. Normally these codes can be found on the top of the parent’s cage cards. For example:

When weaning pups from Cage #167, Pair 13-OB
 Mark the top of the pup’s cage with:
 “From cage # 167” or “From Pair 13-OB”

This will help the PIs with their record keeping and help keep track of where the pups originated.

- k. On the “Date In” line of the cage card, write the **weaning date**. This will help keep track of how old the pups were when they were weaned.
- l. The researcher is responsible for all overcrowded cages. Once notified of an overcrowded cage, the researcher will find the “Cage Overcrowded” cage card, separate the animals accordingly and initial and date the (separated by) line of the “Cage Overcrowded” card.
- m. A yellow “Cage Split Notification” card will be placed on the (weaned/split) cage(s) and be filled out with the date weaned, initials and breeder cage number. This will notify CRF staff that the cage has been split and assure that food and water has been added to the new cage(s). This card also acts as a flag to the CRF person that enters the room. This person will double check the cage/s to ensure that they have enough food and water and that the number of animals indicated on the card is correct.
- n. The cards are left on the cage for the CRF Supervisor to remove and file.

3. Recommended Practices:

- a. Pregnant females should be separated prior to **parturition** if the litter will create an overcrowded cage. When the litter is born, the cage is overcrowded, is non-compliant and needs to be rectified immediately.
- b. If the female becomes pregnant in addition to the current litter, culling or separating will be necessary if and when the second litter is born.
- c. Breeding animals will require more space, particularly if neonatal animals will be raised together with their mother or as a breeding group until weaning age. Other

considerations may include culling one of the litters or separation of litters from the breeding group to allow for the safety and well-being of the breeding group.

Please contact the CRF Veterinary Services if you have any questions.

V. Social Housing

Social housing is the default method of housing in all Lifespan animal facilities unless otherwise justified based on social incompatibility as a result of behavior, standard agricultural husbandry practices, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC. In general, social animals must be housed in stable pairs or groups of compatible individuals.

If single housing of animals is deemed necessary, the duration should be limited to the minimum time period necessary and, where possible, animals should be rehoused with appropriate conspecifics. When animals are singly housed, attempts should be made to facilitate visual, auditory, olfactory and protected tactile contact with compatible conspecifics as appropriate for the species.

In situations where animals are housed alone in rooms without conspecifics, additional enrichment should be offered, such as positive interaction with humans, supplemental enrichment items, and/or the addition of a companion animal in the room or housing area.

Exceptions

Social animals may need to be singly housed for a variety of reasons. The following are the known general categories of exceptions to social housing and the IACUC approval requirements for each:

1. **Social incompatibility, standard animal husbandry and management practices:** The IACUC approves single housing of social animals for standard agricultural husbandry practices or situations where attempts to socially house the animals could jeopardize animal welfare. When animals are singly housed for one or more of such reasons, *specific justification in the animal use protocol and case by case approval by the IACUC is not required.* Examples of such situations include, but are not limited to:

- separation of aggressive or incompatible conspecifics (for example adult males of certain species such as rabbits where aggression is a documented issue)
- individual housing due to attrition of cage/pen mates or uneven number of animals
- pregnant females separated to prior to or at the time of parturition to prevent overcrowding following birth of offspring
- quarantine prior to entering or reentering a facility or herd
- separation of littermates at weaning when the number of offspring does not allow for all animals in a litter to be placed with a compatible cage mate (for example, single male weanlings)
- animals housed singly for short term recovery post-operatively; single housing must be for the minimum amount of time post-operatively necessary for recovery and/or healing as determined by the PI in consultation with the veterinarians

- individual housing when an animal is considered a danger to other animals, to itself or personnel
2. **Clinical Necessity:** Veterinary staff may require individual housing of animals due to medical concerns. In such cases, **IACUC approval is not required.** The responsible Veterinarian will record the period of single housing and the frequency of reevaluation in the animals' medical record, will monitor the animal as. These cases will be reported to the IACUC at the discretion of the Attending Veterinarian.
 3. **Scientific Necessity:** When the single housing of social species (other than short term recovery from experimental manipulation) is required for scientific reasons, specific justification must be described in the animal use protocol or an amendment. Social housing for scientific purposes must be reviewed and approved by the IACUC, and single housing cannot begin until **approval is granted by the IACUC for that protocol.**

VII. General Information

A. Animal Procurement

Hospital policy requires that all-vertebrate animals intended for teaching and/or research be purchased or transferred by the CRF office only. No animals will be purchased unless the Institutional Animal Care and Use Committee (IACUC) has granted prior protocol approval.

Animal orders may be placed by facsimile or email. A copy of the animal order form can be found on the [Core Research Services](#) webpage. The deadline for placing animal orders is 3:30 PM Thursday for deliveries to be made the following week.

The CRF Management must be consulted in advance of any requests for animal procurement through a non-commercial vendor. The CRF makes an effort to use vendors who maintain strict animal health programs that include monitoring for infectious agents by serologic and other diagnostic procedures. Also, the Attending Veterinarian must be consulted for new vendor requests. In general, the CRF tries to avoid mixing animals from sources, which might have different microbiological backgrounds.

B. Conditioning Period

The conditioning periods required for incoming animals are dependent on the species, the vendor/source of the animals, and their intended use. Experimental studies indicate that all animals should be allowed seventy-two hours to acclimate to their new environment and recover from the stress of shipping. Experimental results may vary considerably in the post-shipment period. Animals may carry agents that are communicable to man and other animals. The veterinary personnel may perform various diagnostic tests dependent upon species to ensure that animals are free of such agents (*Appendix 6 Animal Health Program* contains routine tests performed by species). Animals are usually conditioned in conventional animal rooms.

Vendors supplying rodents perform in-house surveillance on their colonies. The following chart provides the recommended conditioning periods for commonly used species.

Recommended Minimum conditioning period:

Rats/Mice	3 days
Rabbits	3-7 * days
Ducks	3 days
Pigs	7 days

*Note: rabbits being used in research with a surgical component will have a 7-day acclimatization.

Quarantining of animals received from non-conventional vendors/sources is mandatory. The animals coming from non-conventional vendors/sources must be quarantined for up to sixty days. All rodents imported from non-commercial vendors/sources to the Coro East Barrier must be rederived. See Section VII.G.

C. Animal Transfer Policy

When an Investigator has surplus animals that they wish to donate or transfer to another Investigator within Lifespan, they must use an Animal Transfer Form. This form can be obtained from the intranet through the [Core Research Services webpage](#).

All fields must be completed – the form will be validated by the IACUC Coordinator before the transfer is accepted. The signature of the donating and receiving Investigators must be on the form.

Submit the completed and signed form to the IACUC Coordinator or the CRF main office for verification. No animals are to be transferred or used on any protocol until the IACUC Coordinator has verified the number of animals, protocol and cost center. The IACUC Coordinator will return a signed copy of the form by email indicating that the transfer has been accepted. Once the transfer has been accepted, it is the labs' responsibility to change the PI name or protocol number and cost center. The CRF will change the information in the database.

D. Quarantine (Importation) Requirements

Laboratory animal facilities are now being asked to receive rodents from many more different sources than was the case just a few years ago. Moreover, many of these are transgenic or genetically altered animals supplied by research investigators from other institutions. Although health status information is usually available to the Central Research Facilities office before animals are shipped, the confidence level that animals are free of significant murine parasites or pathogens is much lower than it is when they are purchased from reputable commercial suppliers. The trend toward sourcing from multiple non-commercial institutions will probably increase in the future. The RIH animal facilities have established the following quarantine program in order to help protect all investigators using rodents from the incursion of variables which could confound research results.

Disposition for Importing Rodents – by Risk Level	
Risk Level¹	Disposition

Approved (Commercial) ²	Direct into Animal Room
Low Risk	60 Day Quarantine and Testing
Low to Moderate Risk	Quarantine or Rederivation at Vet's Discretion ³
Moderate to High Risk	Requires Rederivation ³

¹ Exporting facilities that have evidence of adventitious rodent infections either in the animal room or in close proximity will be considered moderate to high risk.

² These approved commercial sources maintain barrier facilities and rigorous health monitoring programs which are frequently reviewed by the veterinarians.

Examples of approved commercial sources include Charles River Laboratories, Taconic, Jackson Laboratories, and Harlan.

³ The Attending Veterinarian (AV) is available to assist the Principal Investigator in getting the animals rederived.

1. Rodents will only be directly imported from facilities designated as low-risk.

- The Principal Investigator (PI) requesting to Import rodents from an unapproved (non-commercial) source is responsible for providing CRF with the necessary contact information at the Exporting facility. Forms have been developed and are available from the ***CRF Import/Export Coordinator (IEC)***.
- The CRF IEC is responsible for contacting the Exporting facility to obtain the rodent health information and to arrange for shipment to the Quarantine Facility. The PI will be notified and requested to assist in the event the CRF IEC is experiencing difficulties in making the contact.
- The CRF IEC is responsible for coordinating receipt of the imported animals into the Quarantine Facility, notifying the PI of their receipt, obtaining progress reports of the Quarantine, and receiving the imported animals into the CRF animal facility once Quarantine is complete.

2. Import Procedures

- (PI) Contact the CRF IEC to initiate the importation procedures. The CRF IEC will provide form (Rodent Import Request) requesting contact information concerning the Exporting facility. The information requested will include:

Exporting Facility Information

- | | |
|---------------------------------|-----------------------------|
| - Supplying institution | - Species/strain |
| - Contact (phone #, email) | - Zygosity |
| - Veterinarian (phone #, email) | - Number of animals and sex |
| - Investigator | - Coat color |
| - Building and room number | - Special requirements |

- (PI) Return the Rodent Import Request form to the CRF IEC. The form can be returned electronically to maria.veliz@lifespan.org.
- (CRF Import/Export Coordinator/IEC) Contact the Exporting facility to obtain pertinent health monitoring data. The typical information requested will include:
 - General description of their rodent health monitoring program
 - Panel of selected adventitious agents for testing
 - Testing schedule (routine, frequency)
 - Recent test results from room/building (viral, parasitic, and bacterial)
 - Historic (1 year) test results from room/building (viral, parasitic, and bacterial)
 - (CRF IEC) Provide the PI and AVs with progress updates. Two weeks will be allotted to obtain this information. In the event of problems, including lack of response, the PI will be promptly notified in writing that the IEC requires additional assistance to proceed.
 - (CRF IEC - AVs) Assess the Export facility's rodent health monitoring program and designate the risk level. Notify the PI and discuss the disposition for the animals. At the discretion of the AV, animals at moderate risk may be approved for shipment to the Quarantine Facility. (PI understands that a "positive" quarantine test results will preclude the release of the shipment from the Quarantine Facility.) Typically, animals from moderate to high risk facilities will need to be rederived.

NOTE: All rodents imported to the Coro East Barrier from a non-commercial source must be rederived at a vendor/facility approved by the Attending Veterinarian.

- (CRF IEC) Provide PI with appropriate Quarantine Facility paperwork to be filled out and returned to IEC.
- (CRF IEC) Obtain Purchase Order for Quarantine Facility service charges. All charges will be charged back to the PI by the CRF.
- (CRF IEC) Arrange for shipping the animals and e-mail the Exporting facility an Authorization for Shipment form. This authorization will include any discussed shipping details (some of this may be done by the Quarantine Facility receiving department):
 - Strain, coat color, number, sex
 - Animal room identification
 - Common carrier
 - Shipping lading number
 - Date of shipment and receipt
 - Special requirements

3. Receipt Procedures (Quarantine Facility procedures)

- Each approved Quarantine Facility has their own approved receipt procedures.
- If breeding is required in Quarantine, PIs may provide instructions for pairing or otherwise housing the animals. (not allowed during quarantine at Brown University)

- The CRF IEC or Quarantine Facility Manager will notify the PI of the receipt including the specific caging arrangements (sex and coat color) and other remarkable findings.
- Imported animals are under Quarantine conditions until released.

4. Receipt of Animals from the Quarantine Facility into Lifespan CRF

- The CRF IEC will coordinate the shipping of the imported animals from the Quarantine Facility into Lifespan CRF animal facilities.
- The CRF IEC will notify the PI and the AV of the status of the shipment and delivery date. If the quarantine test results are “positive,” this will preclude the release of the shipment from the Quarantine Facility. The AV is available to assist the PI in getting the animals rederived.
- The CRF IEC will notify the PI and AV when the shipment has arrived so they may be inspected.

5. Records, Forms and Reports:

- Rodent Import Request (from PI to CRF IEC)
- Rodent Importation Procedures (from CRF IEC to PI)
- Rodent Donation and Health Report Request Form (from CRF IEC to Exporting Facility)
- Progress and Status Reports (from CRF IEC to PI)
- Health Reports (from Exporting Facility to CRF IEC and AV)
- Quarantine Facility Services Request forms (CRF IEC to PI to CRF IEC to Quarantine Facility)
- Authorization for Shipment (from CRF IEC to Exporting Facility, AV, and PI)

6. Resources for Quarantine:

- Brown University Quarantine
*Note: shipments will be scheduled into Brown Quarantine on a “space available” basis
- Charles River Laboratories
- Jackson Labs

E. Transportation of Animals

1. Between Buildings on Campus:

Animals must be conveyed in appropriate transport cages when moving within or between buildings or laboratories. No animals are to be moved without proper containment. Rodent cages must have micro isolator tops in place while being transported. All cages must be covered during transport using a towel, surgical drape or another opaque material. The CRF has a limited supply of transparent cages available for short term loan. Transportation devices should provide safety, adequate ventilation for the animals and should be able to withstand sanitation procedures. “Veri kennels” are provided for larger animals. Animals transported from the animal facility cannot be housed in research laboratories or procedure rooms overnight.

Used transport cages and “Veri kennels” must be returned to the facility cage wash area so the facility technical staff may properly sanitize them before reuse.

Rodents leaving the Coro East Barrier will not be allowed to return to the Barrier. They will be placed in disposable containers for transport, as cages cannot be returned to the Barrier Facility once removed.

2. Between Main Campus and Off Sites

No animals may be transported from the main campus and the off sites (and vice versa) without the express knowledge and consent of the CRF management.

3. Between Lifespan and Brown University Facilities

No animals may be transported between Lifespan and Brown University facilities without the express knowledge and consent of the CRF management.

4. Between Institutions

The CRF recognizes the need to transfer animals from one institution to another. All requests for animal transfer or receipt of animals by RIH, other than those procured through CRF purchasing services must receive approval in advance from the CRF management and the Attending Veterinarian. All arrangements for said shipping or receipt of animals will be processed by the CRF office. Once animal health status has been discussed between institutional veterinarians the animals will be cleared for shipping or receipt. A certificate of health signed by the veterinarian must accompany interstate shipping of animals. A USDA “record of requisition, disposition or transport of animals” form may be required and must accompany that species in transit.

5. Patient Areas

Transport of animals into patient areas needs to be authorized by the IACUC, the department head and in some cases, the Biohazard and Laboratory Safety Committee and the Department of Epidemiology, Infection Control Management.

On a rare occasion testing may be conducted on animals within a diagnostic area of the hospital. The use of diagnostic procedural areas and equipment may only be conducted with prior approval of the Department of Epidemiology and Infection Control. See [Section J below; Clinical Area Use Sanitation Procedures.](#)

6. Miscellaneous

Other types of transport not herein expressly mentioned will be considered on a case by case basis by the CRF management and the attending veterinarian.

F. Per Diem and Other Billable Expenses

A partial cost recovery program (per diem) for boarding and housing charges has been established. Per Diem helps cover the cost of procurement, processing paperwork, and care of animals used in research and education. Per Diem rates are reviewed, established and published yearly by CRF management.

The PI or designee sends the animal order to the CRF office with the cost center that will be utilized and cross checks with the information in the LabTracks database program. The cost center is given to the animal vendor and will be specified on the invoice. CRF approves the invoice and sends it to Accounts Payable. Fees are charged to the cost center by CRF for

receiving the animals and for the daily housing. Any extra costs such as for technician assistance, use of anesthesia equipment, etc. are also charged to the designated cost center.

Monthly bills are sent to the PI by the CRF Office with copies of the animal invoices. The PI has 10 days to get back to the CRF with any concerns about the bill.

The charges are then posted to the PI's cost center. The LabTracks database program tracks daily cage census and calculates the monthly invoices. When animals are received, the PI, protocol, cost center and animal information are entered into the database. Barcoded cards are printed out with the PI's assigned color and detailed information. The cage is assigned the per diem rate in the database. It is critical to return the card to the CRF Supervisor after euthanasia so the cage can be removed from the system and stop charging per diems. Per Diem rates can be requested from the CRF office or found on the CRF page at [Core Research Services webpage](#).

G. Identification of Animals

Animals must be clearly identified at all times with cage cards bearing the standard information (*Appendix 7 Cage Card*).

CRF personnel prepare cage cards when the animals are received into the facilities. However, any investigator subdividing animals or otherwise altering cage arrangements must complete all data requested on each new cage card. CRF Staff will enter the new cage information into the LabTracks database and print out new cards with the barcode. An investigator may add data to the card, as desired, but the basic information must be legible. All investigators have color-coded cage cards assigned to them.

If animals will not be returning, the cage card needs to be initialed and dated under euthanasia and the card placed on the clipboard in the respective animal room. **If the cage card is not returned as euthanized, per diem charges will continue.** If only one of several animals will be taken, subtract one from the number on the cage card, initial and date. Cage cards must never be discarded. If an entire rack of animals will not be returning, please notify the CRF Supervisor.

USDA covered animals must carry individual numbers either as a tattoo or ear tag. Cage cards for chronic animals should be kept with the animal(s) at all times.

Please notify the CRF office immediately if cage cards are missing.

H. Husbandry

1. Food

Natural ingredient diets are utilized in the animal care facilities. These diets are manufactured in environments which do not handle pesticides, insecticides, growth promoters, antibiotics, etc., using closely controlled processing techniques to ensure consistent nutrient content; the approximate nutritional compositions are provided. All shipments are monitored for the date of manufacture. All diets are utilized within 180 days of milling. All feed within a shipment is checked for damage or improper packaging and refused if unsatisfactory. All feed bags are sprayed with a germicidal compound prior to being placed in the feed room. Rodent diets are purchased irradiated

or, for the Coro East Barrier, extruded sterilizable diets are autoclaved in the facility. Please contact the CRF Office for a list of specific diets used within the facility.

If investigators require food of the same milling lot for the duration of their studies, CRF staff should be consulted in advance. Specialized diets, including semi-purified and chemically defined diets, are available from several vendors. The CRF office can be consulted for details.

Animals are fed daily by Animal Care Technicians except for special diets.

Note: Any special diets are to be acquired and dispensed by individual laboratories unless special arrangements have been made with the CRF.

2. Water

Water is available to the animals at all times – exceptions must receive IACUC approval. All laboratory animals are provided with tap water (except in the Coro East Barrier).

Automatic watering systems are available for large animal housing pens. No bottles, stoppers, sipper tubes, waterers, or bowls are re-used before being properly sanitized. Water bottles are changed a minimum of once per week.

The Coro Barrier automatic watering system provides reverse osmosis water which is chlorinated to 2.0 to 4.0 ppm. This water is provided through valves at each cage in ventilated racks. The same high quality water can be provided in bottles as needed.

3. Environmental Conditions

The light / dark cycles in the animal rooms are 12 hr. / 12 hr.: 6:00 AM – 6:00 PM in all animal facilities, unless noted otherwise. Timers do allow for other time cycles. PI's requesting accommodations for light sensitive studies should contact CRF management.

The relative humidity target in the animal rooms is 30-70 %. See the list of species below for specific room temperatures. All personnel should be aware that the rodent cage temperature and humidity for ventilated and non-ventilated cages may differ.

The generation of noise and vibration from humans and machinery is minimized as much as possible. Loud animal species are housed away from quieter ones. The animal rooms are remotely situated from the cage wash areas in all CRF animal facilities. Voices must be kept to a minimum in the animal rooms. Unless prior approval has been granted by the AWC, music may not be played in the animal rooms. Noisy cart casters must be repaired or replaced.

4. Animal Care by Species

Room conditions and cage cleaning tasks are documented on the Room Check Log.

- Mice**

Room Temperature: 70-74 °F (recommended range 68-79 °F)

Feed: Dry ration provided in wire lid feeders ad libitum.

Caging: Group housed in shoebox cages *or individually if justified.*

Bedding: Corn cob with nesting material

Cages in ventilated racks changed weekly. Static cages changed 2 times weekly.

- **Rats**

Room Temperature: 70 -74°F (recommended range 68-79 °F)

Feed: Dry ration provided in a wire lid feeder ad libitum.

Caging: Group housed in shoebox cages *or individually if justified*. Some may be housed in suspended wire cages or metabolic cages due to experimental design.

Bedding: Corn cob with enrichment

Cages changed 2-3 times weekly

- **Rabbits**

Room Temperature: 66 -70°F (recommended range 61-72 °F)

Feed: Rabbit diet provided in a stainless steel J feeder. Loose timothy hay is placed in a polycarbonate hay holder at least once per day.

Caging: Group housed in compatible pairs or groups in suspended stainless steel cages with either stainless grated flooring or plastic, or floor housed in pens.

Individual housing in cages if justified in the approved protocol or if fighting and/or other incompatibility occurs

Bedding: Plastic lined paper pads are placed in the pans under the flooring.

Liners replaced 3 times a week. Wood shavings as contact bedding if housed in pens.

- **Pigs**

Room Temperature: 70-74°F for adults over 15 Kg, 74-78°F for pigs under 15Kg (recommended range 61-81°F)

Feed: Dry ration provided in stainless steel J feeders or bowl once per day in the morning.

Caging: Group housed in a room with stainless steel lower walls containing pens divided by a chain-link fence. *Individual housing if justified in the approved protocol or if fighting and/or other incompatibility occurs*

Bedding: Wood shavings. Post-operative animals may be recovered on raised floor grates. Soiled bedding removed daily.

- **Ducks**

Room Temperature: 64-68 °F (recommended range 61-81 °F)

Ducklings: 80 – 85 °F, drop by 6 °F each week. (Agricultural Guide, page 44.)

Feed: Waterfowl diet provided in heavy gauge plastic fowl feeders ad libitum.

Caging: Group housed in a pen with a steel corral and a swimming pool with a non-slip steel access ramp.

Bedding: Wood shavings.

Soiled bedding removed daily.

5. Cage Cards

Animals are provided with cage cards at the time of receipt. After euthanasia, these cards are returned to CRF. Cards placed on cages to flag for problems by the CRF staff may only be removed by CRF staff.

Each rodent room has a number of instructional cage cards available to flag cages. Below is a list of the cards and meaning.

- Cage Overcrowded – Too many animals, animals must be separated
- Cage Split Notification – animals were separated, CRF to check

- CRF Available – animals transferred to CRF
- Hazards – agent used, precautions
- Health Check – medical issues noted, needs to be addressed
- H₂O – agent added to water, special instructions for changing or supplying water
- Malocclusion – overgrown teeth, trimming of teeth
- Noncompliant – missing or incorrect information listed on cage card
- Notes – reminder, notes for lab, general purpose
- NPO – do not feed, fasting (for large animals)
- Rodent NPO- do not feed and/or water (rodents)
- Please Check – issue noted, needs to be addressed
- Pregnant/DOB – animal breeding, due dates, birth dates, wean dates
- Special Food – study specific, provide supplemental items, provide wet pellets on cage floor
- Surgery Care – surgical and post-operative information
- Survival Study – study specific

6. Space Requirements

Rodents are preferably housed with more than one per cage. There are minimum space requirements for each species. A list of the appropriate number of mice, rats or rabbits by weight or age for each cage type is posted on the back of each animal room door. The space requirements for larger animals are listed in the CAF husbandry Standard Operating Procedures for each species.

- Space Requirements for Mice:
 - >25 g requires 15 in² floor space each adult
 - One mouse with litter under 21 days of age requires 51 in²

Cage with 5-6 in Height	Floor Space	# Adult Mice	# Adults w/# litters weaned at 21 days	# Adults w/# litter weaned at 28 days
Small Mouse	67 in ²	4	2 w/1 litter	1 w/1 litter
Allentown JAG 75	75 in ²	5	2 w/1 litter	1 w/1 litter
Large Mouse	153 in ²	10	3 w/2 litters	2 w/2 litters
Thoren duplex	51 in ²	3	1 w/1 litter	0
Thoren large mouse	112 in ²	7	2 w/2 litters	1 w/2 litters

- Space Requirements for Rats Based on Weight and Age:
 - <200 g (up to 6 weeks old) requires at least 23 in² floor space each
 - 200 - 400 g (6-12 weeks old) requires at least 40 in² floor space each
 - > 400 g (12 weeks or older) requires 70 in² floor space each
 - One adult with litter requires 124 in² or more as determined by the Attending Veterinarian.

Cage with 7-8 in Height	Floor Space In cage	# Rats < 6 wks or < 200 g	# Rats 6-12 wks or 200-400 g	# Rats >12 wks or >400 g	# Adult Rats w/litter
Standard Rat	143 in ²	6	3	2	1 w/1 litter

Cages must not be overcrowded. Care must be taken to keep the number of breeding mice and litters appropriate for the size of the cage. If a cage is found to be overcrowded, CRF staff will notify the investigator and mark the cage. The overcrowding must be corrected within **24 hours** or a fine and other charges may be imposed. Please see [Section VI, U: Policy for Separating and Weaning Rodents.](#)

7. **Cage and Equipment Sanitation Policy**

All non-disposable items in the Central Animal Facilities and procedural laboratories must be made of materials that are cleanable and sanitizable by high heat (cage washer or autoclave) or by chemical disinfectants. Plastic rodent cages must be replaced if cracked or crazed. Rusty equipment must be repaired or replaced. Items made of wood need clearance by CRF management. The CRF staff performs routine inspections of all animal facility and procedural laboratory areas and may cite non-sanitizable surfaces and equipment. Corrugated cardboard boxes are not sanitizable and may not be kept in animal housing rooms or be brought into the Coro East Barrier.

I. **Use of Image Capturing Devices**

The use of any image producing device is **strictly prohibited** in all areas of the Central Research Facility (CRF), without prior permission from the CRF Director. This includes, but is not limited to; the research operating rooms, procedure rooms, animal housing rooms, research areas, research personnel, and corridors of all animal facilities.

The following will be allowed only after permission is granted by the CRF Director.

1. **Research Operating Rooms**

Only image recording deemed necessary to document surgical instrumentation, technique, product application and results pertinent to the objectives of the research project will be allowed. Permission of the Attending Veterinarian and CRF Director must be obtained before any recording is approved.

Image recording devices must be openly displayed to the OR staff. Recorded images will be monitored by staff and only pertinent (as specified above) images/data will be allowed to be recorded. Video recording of laparoscopic procedures within the context of acceptable practice is allowed via the laparoscopic equipment tower.

2. **Procedure Rooms, Animal Housing Rooms and Corridors of all CAF facilities:**

Image recording of research animals, research animal housing areas, research laboratories and research personnel is **strictly prohibited**. Any recording of the above mentioned areas must have the approval of the CRF Director and the Attending Veterinarian.

J. Use of Animals in Clinical Areas- Sanitation Protocol

When animal procedures are scheduled in hospital clinical areas such as CT scan, MRI, or Gamma Knife, the following precautions will be followed to minimize any potential contamination of those areas.

1. Pre-Transport Equipment

The stainless steel gurney or hydraulic lift table will be used for animal transportation. It will be sanitized in the rack washer using a detergent and 180° rinse water just prior to use or it can be sprayed down thoroughly with a CRF approved disinfectant (i.e. Rescue H₂O₂ for 5 minutes). After the appropriate contact time, the table is then wiped down.

The animal transport sled is made of a hard plastic surrounded by a lip deep enough to contain any urine and/or feces. This sled will be disinfected using an approved disinfectant just prior to use. The sled will be lined with a plastic sheet and absorbent material prior to the animal being placed in it.

The animal anesthesia equipment will be sprayed and wiped down with the disinfectant just prior to transport from the animal facility. Scavenging of waste gas will be contained by an f/air canister. A container with supportive supplies will also be sprayed and wiped down with the disinfectant just prior to transport from the animal facility.

2. Transporting Animals

All the wheels of the equipment being used will be sprayed with disinfectant and left standing for the appropriate contact time prior to leaving the animal facility.

Just prior to leaving the animal facility, the animal will be anesthetized with injectable anesthetics, intubated, placed on isoflurane and positioned into the animal transport sled. The animal will then be completely covered by a sheet so that no part of the animal will be exposed, but can be monitored by CRF veterinary staff during the transportation.

The animal patient and equipment will be expeditiously transported to the intended location through the areas with the least patient/visitor traffic. This is the recommended route: leave the animal facility by the elevator on the fourth floor of CRF to the basement tunnel. Travel to the APC building and take the APC elevator. Go to the first floor of the APC building, travel through the public corridor toward the Southwest Pavilion, and then take a left to the Gamma Knife/MRI department.

3. Animals in Clinical Locations

Place plastic sheeting on the surface of the diagnostic equipment to be used within the patient area prior to placing the animal transport sled containing the animal on that surface. The animal will remain in the animal transport sled for the duration of the procedure. Personnel will wear all PPE required by both the department being visited and those required for working with the specific specie (i.e. shoe covers, procedure gowns, lab coats, etc.).

4. Post-Procedure

The animal in the animal transport sled will be removed from the diagnostic/treatment unit and placed back onto the gurney.

The plastic sheeting will be removed from the table/surface of the diagnostic equipment, placed into a red biohazard bag and tied. The surface of the diagnostic equipment will then be sanitized using a hospital approved disinfectant provided by the Department of Environmental Services. In addition, research personnel will comply with all requirements of the host department in the sanitization and restoration of the area to acceptable conditions for human use.

The animal will be prepared for transport back to the animal facility in the same manner in which it was prepared for transport to the clinical location. The animal will be evaluated for the depth and adequacy of anesthesia. The transport sled and animal will be again covered by a drape such that it can be evaluated by CRF veterinary staff during transport. The animal will continue to be maintained under inhalant isoflurane via the endotracheal tube. Emergency IV anesthetics will be located with the traveling emergency kit.

The route back to the animal facility will be the reverse of the original route.

K. Policy on the Review of Animal Cadavers or Animal Parts Used in Research

1. Background

The United States Department of Agriculture (USDA), in agreement with the Office of Laboratory Animal Welfare (OLAW) at the National Institutes of Health (NIH), suggests that each institution formulate a policy on how the IACUC manages use of animal cadaver tissue and/or recognizable parts.

Strictly speaking, IACUC review is not required for the use of animal cadaver tissue in research. While the Animal Welfare Act (AWA) defines “animal” as “any live or dead animal...intended for use, for research, testing, [or] experimentation,” it (and 9 CFR [part 1.1 and part 2.30 (a) (1)]) also defines a “research facility” as an entity that “uses or intends to use live animals.” In addition, the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals is only applicable to activities involving live vertebrate animals. Accordingly, USDA and OLAW have agreed that formal protocol review requirements do not apply to dead animals in the research setting.

Although there is no legal mandate to provide IACUC protocol review of the use of animal cadaver tissue, it is recognized as best practice to document a review of this kind of research at the institutional level. Review provides assurance that appropriate standards have been met regarding the acquisition, use, and disposal of the cadaver/animal parts. Providing standardized overview for this kind of research also serves the best interests of the institution for a variety of other regulatory and non-regulatory reasons (*e.g.*, biosafety, public relations, liability, occupational health and safety, etc.).

2. Policy

The use of cadaver tissue or animal parts for research and/or teaching must be reviewed by a program veterinarian ***where the animal carcass or tissue is being brought onto campus without prior Lifespan IACUC review***. Such sources may include, but are not

limited to, slaughterhouses; other academic, private-industry, government research facilities, or commercial vendors.

Notification and review will be via submission of the IACUC's *Animal Cadaver and/or Animal Parts Form* in Appendix 9. This form can be found on IRBnet. The form will be reviewed by a program veterinarian and will be filed with the IACUC Coordinator and Central Research Facility veterinary staff.

It is expected that all animal cadavers or parts obtained under this policy will meet the following requirements:

- The animal will have been ordered, used and euthanized in accordance with all applicable regulations at its source institution including IACUC review if applicable.
- The cadaver or animal tissues will not represent a hazard to those handling the tissues (this includes but is not limited to chemical, biological and radioactive hazards).
- The cadaver or tissues will be disposed of in accordance with all federal, state, local and institutional regulations and policies.

L. Use of Avian Embryos

All use of vertebrate animals in research, teaching and testing is regulated by the Institutional Animal Care and Use Committee (IACUC).

Avian embryos are not considered live animals by U.S. regulatory agencies and many universities do not regulate their use in research.¹ Nonetheless, there is a consensus in the scientific community that avian embryos that have attained > 50% incubation have developed a neural tube sufficient for pain perception.² Also, if avian embryos hatch, intentionally or unintentionally, they are live vertebrate animals and thus, are regulated by the IACUC.

Consequently, the Lifespan IACUC has adopted the following guidelines. These guidelines were developed based on recommendations of the Institute for Lab Animal Research (ILAR)³ and the AVMA Guidelines for the Euthanasia of Animals: 2013 edition.² Chicken embryos, which hatch after approximately 21 days of incubation, are considered the model species. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching.

1. Investigators using avian embryos must inform the IACUC by means of the "*Notice of Intent to Use Avian Embryos*" form (see Appendix 11). If embryos will be sacrificed prior to 3 days before hatching (i.e. day ≤ 18), the research is not subject to IACUC review unless specifically requested by the investigator. *Studies using embryos within three days of hatching (i.e. day ≥ 19), or using hatchlings, must be reviewed by the normal IACUC procedure for vertebrate animals.*
2. Chicken embryos **younger than embryonic day 10 (E10)** are assumed to be unable to experience pain. It is recommended that E10 or younger embryos be euthanized by hypothermia, typically by placing the eggs in a -20°C freezer for a minimum of 4 hours.
3. Chicken embryos from **E11 to E18** may perceive pain and therefore should be euthanized by rapid decapitation. Additional humane methods of euthanasia may be considered.²
4. Chicken embryos **E19 and older** must be euthanized by CO₂, decapitation or prolonged exposure to anesthetic agents through the air cell. Avian embryos are resistant to CO₂.

Therefore, embryonated eggs must be exposed to 90% CO₂ for a minimum of 20 minutes. Dry ice is unacceptable as a source of CO₂ for euthanasia.

5. The IACUC recognizes that inadvertent hatching may occur. Investigators are asked to describe their methods for humane euthanasia of hatchlings.

References:

1. OLAW FAQ. http://grants.nih.gov/grants/olaw/faqs.htm#App_4 (accessed November 4, 2014).
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. AVMA: Schaumburg, IL.
3. ILAR. 1991. ILAR News 33(4):68-70. Issues for Institutional Animal Care and Use Committees

M. Guidelines for Counting Animals Used in Research

Institutions are required to review and approve the use of animals in research. Tracking is essential to assure that only approved animals are used, and to fulfill federal obligations for reporting animal use and ensure compliance with IACUC-approved protocols. This policy defines the Lifespan Animal Care and Use Committee's position as to which animals must be counted, and when counting must be performed.

- Each IACUC protocol is approved with sufficient animals to achieve the project's scientific goals. The Principal Investigator must count and account for all animals used in association with a given protocol, and report those numbers to the IACUC during the annual and three year *de novo* review processes, for AAALAC reporting purposes, and when otherwise requested.

1. What must be counted?

All animals used in association with each approved protocol must be counted. This holds for research, testing, teaching, and holding protocols. Animals are reported as either:

- **Adults** – Defined as aged beyond weaning and/or able to reproduce.
- **Neonates** – Defined as young animals not yet weaned, requiring parental protection or nursing.
- **Embryos/fetal animals** – Defined in mammals as the period from implantation to birth.
Note: Embryos/fetal animals are counted only if they are manipulated before birth.
Note: Avian embryos (e.g. fertilized chicken eggs) are not considered live animals by U.S. regulatory agencies and the Lifespan IACUC does not require full protocol review and approval before use, rather Notification of Use of Avian Embryos (see Avian Embryo Use Policy).

2. When should counting occur?

Animals are counted upon receipt by CRF after purchase or importation; when born as part of a breeding program, and; when manipulated as part of a protocol involving *in utero* procedures.

- **Animals purchased from a vendor or imported from outside institution:** Each animal is counted as 'used' upon arrival at the research facility. (*Example: 10 female rats with day 3 litters are received for a study on lactation following parturition. Mammary gland tissue from the adult females is studied, while the pups are euthanized. All adult females and their pups must be counted.*)

- **Animals generated via in-house breeding colonies:** All animals produced (breeders and offspring) as part of a breeding program are counted at birth, even if only a subset of those animals are eventually used for actual experimentation. (*Example: 20 mice are produced from a selected mating, but genotyping reveals only 5 possess the correct genotype for the research project. All 20 mice must be counted.*)
- **Animals subjected to embryonic/fetal manipulation:** Fetal animals and embryos must be counted as ‘used’ if they are subject to experimental manipulation prior to birth. Where there is pre-term manipulation, all animals in the litter are counted as used. (*Example: Extraction of the uterus revealed 8 embryonic pups. Only 3 were needed for the research. All 8 embryonic pups should be counted.*)

3. **How should animals be reported if born at RIH or manipulated in utero?**

Animals received through CRF are immediately counted in the LabTracks database. *But the neonates born here and embryo/fetal animals that have been manipulated must be reported monthly to the IACUC Coordinator via the Monthly Breeding Report.* The numbers will be logged into the LabTracks database and compared with the numbers approved in the IACUC protocol.

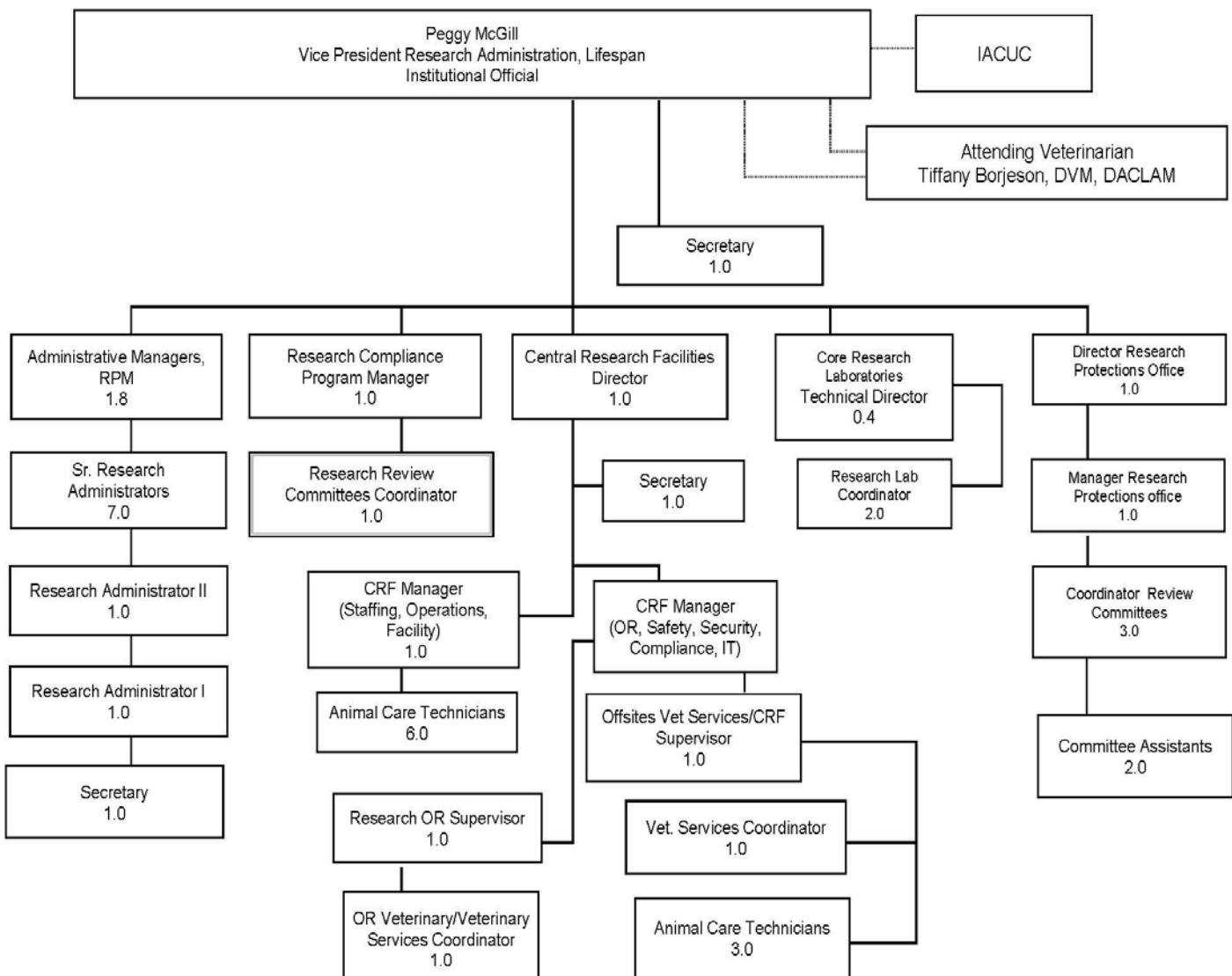
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RESEARCH ADMINISTRATION (LIFESPAN)

ORGANIZATIONAL CHART



ORA chart Rev 03/2018

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Appendix 2 – Zoonoses of Concern in Animal Care Facilities

Please refer to the CRF Policy and Procedure Manual, Section V. L. for a more detailed discussion of zoonotic disease transmission and prevention.

1. **Definition** – A zoonosis is a disease communicable from animals to humans under natural conditions.

2. **Several species**

a. **Salmonellosis**

Bacteria of the genus Salmonella can be present in any domestic or laboratory animal species. Outbreaks of animal disease characterized by diarrhea have occurred in most species, and human disease caused by transmission of the bacteria via direct contact with animals has been documented. Infection with Salmonella in humans is characterized by fever, myalgia, headache, malaise, abdominal pain, vomiting, and diarrhea. Prevention of salmonellosis is based on good personal hygiene practices. Gloves should be worn when cleaning animal cages.

b. **Leptospirosis**

Several species of the genus Leptospira are capable of producing disease in humans. These bacteria are most commonly associated with wild rodents, especially rats. Swine, cattle, and dogs are also host to the infection. Bacteria are excreted in the urine and enter humans through skin or mucous membranes. Commercially-bred laboratory rodents do not harbor these organisms. Dogs and swine are vaccinated for leptospirosis. Wild rodents are eliminated from the CRF. Personal hygiene and protective clothing are important methods of control.

c. **Campylobacteriosis**

Infection with bacteria of the genus Campylobacter is common in many species of domestic animals. While usually asymptomatic, the organism is capable of producing diarrheal disease in most species. Human infection is characterized by diarrhea. Direct contact with fecal material of infected animals has been implicated in transmission of the disease. Infection of humans with Campylobacter of animal origin is prevented by good hygiene practices and wearing gloves while cleaning animal cages.

d. **Hantavirus - Wild Rodents**

Rodents are the primary reservoir for all hantaviruses, shedding virus from saliva, urine, and feces. People acquire infection most often by inhalation of rodent excreta; person to person transmission has not been documented.

This virus is not present in laboratory rodents from commercial sources, but should be looked for via serological assays whenever wild-caught rodents are to be introduced to an animal facility.

Human deaths due to acute respiratory failure have been associated with Hantavirus infection. This condition presents clinically as a rapidly progressive buildup of fluid in the lungs, and has been called Hantavirus Pulmonary Syndrome (HPS). Cases have been confirmed from wild rodents in Indiana, Virginia, Florida, Rhode Island, and other states.

3. Mice

a. Lymphocytic Choriomeningitis (LCM)

Infection with the Arenavirus which causes LCM is usually inapparent in mice. The disease can be transmitted horizontally or vertically. In utero infection leads to tolerance and persistence of the virus. Transmission to humans can occur by aerosols, direct contact, or vectors.

The disease in humans is usually clinically inapparent, but severe cases of meningitis have been reported due to LCM. Rodent vendors maintain surveillance for LCM infection in their production stock. Wild rodents can harbor the disease and must be eliminated from the CRF.

b. Hymenolepiasis

Infection with the cestode parasite Hymenolepis nana occurs in mice, rats, and hamsters. This tapeworm has a direct life cycle and causes few if any complications in the animal host. Humans are infected by ingestion of materials contaminated with animal feces. Development of the cestode in the human intestines can cause abdominal pain, vomiting, and diarrhea. Rodents from reliable vendors are free of H. nana. Wild rodents are kept out of animal housing areas and feed supplies.

4. Rats

a. Rat Bite Fever

Two bacterial agents, Streptobacillus moniliformis and Spirillum minus, have been implicated in the disease known as rat bite fever. The rat is an inapparent carrier of these bacteria in its nasopharynx. During the incubation period of 2 to 14 days, the bite wound, inflicted by the rat will heal without complication. The affected human then experiences flu-like symptoms which may lead to polyarthritis and endocarditis in severe cases. Mortality in untreated cases is 10%. Proper handling techniques are the major means of prevention of rat bites and the associated disease.

b. Leptospirosis - See description under 1b.

c. Ringworm - Rats may exhibit white, crusty lesions on the head and body.

5. Rabbits

a. Salmonellosis - See description under 1a.

6. Pigs

a. Encephalomyocarditis - A picornavirus which primarily infects swine is the cause of encephalomyocarditis. Young pigs die suddenly due to cardiac lesions caused by the disease. Adult pigs show no symptoms. The natural reservoir of the virus is unknown, but may involve wild rodents which shed virus in feces and urine. Humans infected with encephalomyocarditis virus develop flu-like symptoms but show no evidence of cardiac pathology. No control measures for this disease are possible due to its unknown epidemiology.

b. Salmonellosis - See description under 1a.

c. Campylobacteriosis - See description under 1c.

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Appendix 3 – Selection and Use of Anesthesia and Analgesia

Mouse Anesthetic and Analgesics

Lifespan/Rhode Island Hospital

Inhaled Anesthetic Drugs				Mouse
Agent	Dosage	Route	Comments	
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	Inhaled (nose cone, intubation)	Administer inhalation via vaporizer and compressed O2.	

Injectable Anesthetetic Drugs/Combinations				Mouse
Agent(s)	Dosage	Route		
Ketamine/Xylazine	80-100 mg/kg (K) + 5-10 mg/kg (X)	IP	Anesthesia	
Ketamine/Xylazine/Acepromazine	100 mg/kg (K) + 2.5 mg/kg (X) + 2.5 mg/kg (A)	IP	Anesthesia	
Ketamine/Dexmedetomidine	75-100 mg/kg (K) + 0.5-1 mg/kg (D)	IP	Anesthesia	
Pentobarbital (note: not available as commercially available anesthetic product)	50-90 mg/kg	IP	Anesthesia	
Atipamezole	1 mg for every 10 mg of xylazine used	SQ	Reversal	

Local Anesthetics				Mouse
Agent(s)	Dosage	Route		
Lidocaine (1-2%)	2-4 mg/kg (max = 4 mg/kg)	SQ	Local block; line block	
Bupivacaine (0.25% Marcaine)	1 mg/kg (max = 2 mg/kg)	SQ	Local block; line block	

*note: dilute lidocaine, bupivacaine with sterile saline as the small volume of stock may be easily overdosed (ex: 0.25% Marcaine to 0.125% Marcaine then give SQ)

Analgesics (pain relief)				Mouse
Agent(s)	Dosage	Route		
Buprenorphine	0.05-0.1 mg/kg	SQ	MUST be dosed every 8-12 hours, minimum	
Meloxicam	1-2 mg/kg	SQ	Dosed every 24 hrs	
Carprofen	2.5-5 mg/kg	SQ	Dosed every 24 hrs	

Rat Anesthetic and Analgesics

Lifespan/Rhode Island Hospital

Inhaled Anesthetic Drugs				Rat
Agent	Dosage	Route	Comments	Rat
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	Inhaled (nose cone, intubation)	Administer inhalation via vaporizer and compressed O2.	

Injectable Anesthetics/Combinations				Rat
Agent(s)	Dosage	Route		Rat
Ketamine/Xylazine	50-80 mg/kg (K) + 5-10 mg/kg (X)	IP	Anesthesia	
Ketamine/Dexmedetomidine	75 mg/kg (K) + 0.15 mg/kg (D)	IP	Anesthesia	
Pentobarbital (note: not available as commercially available anesthetic product)	30-60 mg/kg	IP	Anesthesia	
Atipamezole	1 mg for every 10 mg of xylazine used	SQ	Reversal	

Local Anesthetics				Rat
Agent(s)	Dosage	Route		Rat
Lidocaine (1-2%)	2-4 mg/kg (max = 4 mg/kg)	SQ	Local block; line block	
Bupivacaine (0.25% Marcaine)	1-2 mg/kg (max = 2 mg/kg)	SQ	Local block; line block	

*note: dilute lidocaine, bupivacaine with sterile saline as the small volume of stock may be easily overdosed (ex: 0.25% Marcaine to 0.125% Marcaine then give SQ)

Analgesics (pain relief)				Rat
Agent(s)	Dosage	Route		Rat
Buprenorphine	0.01-0.05 mg/kg	SQ	MUST be dosed every 8-12 hours, minimum (note: higher doses may cause pica)	
Meloxicam	1-2 mg/kg	SQ	Dosed every 24 hrs	
Carprofen	2.5-5 mg/kg	SQ	Dosed every 24 hrs	

Rabbit Anesthetic and Analgesics

Lifespan/Rhode Island Hospital

Drugs for Sedation				Rabbit
Agent	Dosage	Route	Comments	
Acepromazine	1-2 mg/kg	SQ, IM	Useful to reduce handling stress.	
Diazepam	1-3 mg/kg	IM		
Midazolam	1-2 mg/kg	IM		

Pre-medication Drugs				Rabbit
Agent	Dosage	Route	Comments	
Glycopyrrolate	0.01-0.02 mg/kg	IV, IM	Some rabbits produce atropinesterase, which inactivates atropine. Glycopyrrolate is suggested in lieu of atropine.	

Inhaled Anesthetic Drugs				Rabbit
Agent	Dosage	Route	Comments	
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	Inhaled (nose cone, intubation)	Administer inhalation via vaporizer and compressed O ₂ .	

Injectable Anesthetic Drugs/Combinations				Rabbit
Agent(s)	Dosage	Route		
Ketamine/Xylazine	25-35 mg/kg (K) + 5 mg/kg (X)	IP, IM	Anesthesia	
Ketamine/Xylazine/Acepromazine	35 mg/kg (K) + 5 mg/kg (X) + 0.75 mg/kg (A)	IP, IM	Anesthesia	
Ketamine/Dexmedetomidine	15 mg/kg (K) + 0.12 mg/kg (D)	IP, IM	Anesthesia	
Pentobarbital (note: not available as commercially available anesthetic product)	20-60 mg/kg	IP, IV	Anesthesia	
Propofol	10 mg/kg	IV	Anesthesia. Respiratory support should be available.	
Atipamezole	1 mg for every 10 mg of xylazine used	IM, SQ	Reversal	

Local Anesthetics				Rabbit
Agent(s)	Dosage	Route		
Lidocaine (1-2%)	2 mg/kg (max = 4 mg/kg)	SQ	Local block; line block	
Bupivacaine (0.25% Marcaine)	1 mg/kg (max = 2 mg/kg)	SQ	Local block; line block	

*Note: recommended to dilute lidocaine and bupivacaine with sterile saline to obtain usable volumes

Analgesics (pain relief) Rabbit			
Agent(s)	Dosage	Route	
Buprenorphine	0.01-0.05 mg/kg	SQ	MUST be dosed every 8-12 hours, minimum
Fentanyl Trans-Dermal patch	½ of 25 mcg/hr per 3 kg BW		Patch lasts ~72 hrs
Meloxicam	0.1-0.2 mg/kg	SQ	Dosed every 24 hrs
Carprofen	5 mg/kg	SQ	Dosed every 24 hrs

Swine Anesthetic and Analgesics
Lifespan/Rhode Island Hospital

Drugs for Sedation Swine			
Agent	Dosage	Route	Comments
Acepromazine	0.1-0.2 mg/kg	SQ, IM	Mild sedation
	0.1-1 mg/kg	SQ, IM	For use before ketamine

Pre-medication Drugs Swine			
Agent	Dosage	Route	Comments
Glycopyrrolate	0.01-0.02 mg/kg	IM	Once before induction

Inhaled Anesthetic Drugs Swine			
Agent	Dosage	Route	Comments
Isoflurane	Induction: 3-5% Maintenance: 1.5-3%	Inhaled (nose cone, intubation)	Administer inhalation via vaporizer and compressed O2.

Analgesics (pain relief) Swine			
Agent(s)	Dosage	Route	
Buprenorphine	0.01-0.1 mg/kg	SQ, IP	MUST be dosed every 8-12 hours, minimum
Fentanyl Trans-Dermal patch	2.5 mcg/kg/hr		Patch lasts ~72 hrs; variable absorption
Meloxicam	0.2-0.4 mg/kg	PO, SQ, IM	Dosed every 24 hrs
Carprofen	2-4 mg/kg	SQ, IM	Dosed every 24 hrs

*note: > 3 days of NSAIDs in swine may cause gastric ulceration

Local Anesthetics Swine			
Agent(s)	Dosage	Route	
Lidocaine (1-2%)	2-4 mg/kg (max = 6 mg/kg)	SQ	Local block; line block
Bupivacaine (0.5% Marcaine)	1-2 mg/kg (max = 4 mg/kg)	SQ	Local block; line block

Injectable Sedation & Anesthetic Drugs/Combinations				Swine
Agent(s)	Dosage	Route		
Ketamine/Xylazine	20 mg/kg (K) + 2 mg/kg (X)	IM	Anesthesia	
Ketamine/Xylazine/Acepromazine	20 mg/kg (K) + 2 mg/kg (X) + 0.2 mg/kg (A)	SQ, IM	Anesthesia: non-survival surgery	
Ketamine/Xylazine/Acepromazine	10-15 mg/kg (K) + 2 mg/kg (X) + 0.2 mg/kg (A)	SQ, IM	Anesthesia: survival surgery	
Ketamine/Dexmedetomidine	10 mg/kg (K) + 0.05 mg/kg (D)	IP, IM	Anesthesia	
Ketamine/Midazolam	33 mg/kg (K) + 0.5 mg/kg (M)		Anesthesia	
Pentobarbital (note: not available as commercially available anesthetic product)	20-40 mg/kg	IV	Anesthesia	
Propofol	5-6 mg/kg 0.6 mg/kg/min as CRI	IV IV	Anesthesia. Respiratory support should be available.	
Telazol	5-8 mg/kg	IM	20 min immobilization	
TKX	Reconstitute Telazol with 250 mg ketamine and 250 mg xylazine. <u>Dose 1mL per 25 kg of pig</u>	IM	Anesthesia ~ 30 min	
Atipamezole	1 mg for every 10 mg of xylazine used	IM, SQ	Reversal	

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Appendix 4 - Resources for Rodent Survival Surgery

This appendix includes definitions, tables of information, and references as a resource for investigators. Please refer to the CRF Policy and Procedure Manual, Section VI.G.2. for procedural details.

DEFINITIONS:

ASEPTIC SURGICAL PROCEDURES: Surgery performed using procedures that limit microbial contamination so that significant infection or suppuration does not occur.

MAJOR SURGERY: Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for producing permanent or significant physical or physiological impairment; and/or any procedure associated with orthopedics or extensive tissue dissection.

MINOR SURGERY: Any surgical intervention that neither penetrates and exposes a body cavity nor produces permanent or significant impairment of physical or physiologic function. Examples are superficial vascular cut down, and percutaneous biopsy.

STERILIZATION: The process whereby all viable microorganisms are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if a growth supporting medium is supplied.

DISINFECTION: The chemical or physical process that involves the destruction of pathogenic organisms. Disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

* Note: The use of common brand names as examples does not indicate a product endorsement.

Table 1 - RECOMMENDED HARD SURFACE DISINFECTANTS (e.g., table tops, equipment)

* Always follow manufacturer's instructions for dilution and expiration periods.

AGENT	EXAMPLES *	COMMENTS
Hydrogen Peroxide	Rescue®	Contact time required is 1 minute. Remove soil first.
Alcohols	70% ethyl alcohol 85% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using.
Quaternary Ammonium	Roccal®, Quatricide®, Tec-Surf II®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.
Glutaraldehydes	Glutaraldehydes (Cidex®, Cetylicide®)	Rapidly disinfects surfaces.
Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants. (do not use in a cat's environment)
Chlorhexidine	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.

Table 2 - SKIN DISINFECTANTS

*Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub (with soap) can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution (without soap). Alcohol, by itself, is not an adequate skin disinfectant. Since the evaporation of alcohol can induce hypothermia in small animals, avoid exposing excessively large areas.

AGENT	EXAMPLES*	COMMENTS
Iodophors	Betadine®, Prepodyne®, Wescodyne®	Reduced activity in presence of organic matter. Wide range of microbicidal action. Works best in pH 6-7.
Cholorhexidine	Nolvasan®, Hibiclen®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.

Table 3 - RECOMMENDED PROCEDURES FOR STERILIZING SURGICAL INSTRUMENTS

*Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

AGENT	EXAMPLES**	COMMENTS
Steam sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (e.g., 121°C for 15 min. vs 131°C for 3 min).
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue. <i>Only tips of instruments are sterilized with hot beads.</i>
Gas sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue; all materials require safe airing time.
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Glutaraldehyde (Cidex®, Cetylicide®, Metricide®)	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
Hydrogen peroxide-acetic acid	Actril®, Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.

Table 4 - RECOMMENDED INSTRUMENT DISINFECTANTS

*Always follow manufacturer's instructions for dilution, exposure times and expiration periods.

AGENT	EXAMPLES**	COMMENTS
Alcohols	70% ethyl, 70% isopropyl alcohol	Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using.
Chlorine	Sodium hypochlorite (Clorox® 10% sol.), Chlorine dioxide (Clidox®, Alcide®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Chlorhexidine	Nolvasan®, Hibiclen®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

Table 5 - WOUND CLOSURE SELECTION

* The use of common brand names as examples does not indicate a product endorsement.

MATERIAL*	CHARACTERISTICS AND FREQUENT USES
Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)	Absorbable; 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydioxanone (PDS®) or, Polyglyconate (Maxon®)	Absorbable; 6 months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable
Polypropylene (Prolene®)	Nonabsorbable. Inert.
Nylon (Ethilon®)	Nonabsorbable. Inert. General skin closure.
Silk	Nonabsorbable. Restrict the use of silk to cardiovascular procedures or where silk's excellent handling properties are critical. Avoid for such purposes as routine skin closure since it may wick microorganisms into the wound, and cause tissue reactive.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel Wound Clips, Staples	Nonabsorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®)	Skin glue. For non-tension bearing wounds. Note: use only products labeled for surgical use, super glue is not acceptable for surgery.

- Suture gauge selection: Use the smallest gauge suture material that will perform adequately.
- Cutting and reverse cutting needles: Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
- Non-cutting, taper point or round needles: Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

Table 6 - ANESTHESIA

See CRF Manual Appendix 3 – Selection and Use of Anesthesia and Analgesia

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Appendix 5 – Post-Op Animal Treatment Form (Rodents)

RHODE ISLAND HOSPITAL RODENT POST OPERATIVE CARE FORM

Date & Time of surgery: _____

Investigator: _____ Phone #: _____ Protocol #: _____

Emergency Contact (name & phone #): _____

Species: _____ Stock/ Strain: _____ Sex: _____

Animal ID(s)/Cage ID(s): _____

Procedure: _____

Anesthetic agent(s): _____

Analgesic: _____ Dose: _____ Frequency given: _____ # of Days: _____

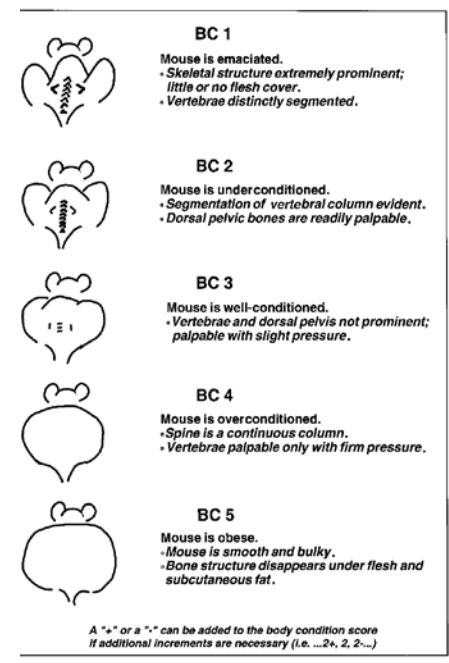
Other Medication(s): _____ Dose: _____ Frequency/Days: _____

Date	Time	Behavior, Appearance & Activity Assessment (Description)	Treatment/Medication	Initials

Some important post-operative parameters to consider:

- Assessment of Behavior, Appearance & Activity – are the animals bright, alert well-groomed and walking around the cage or are they quiet, scruffy and hunched in the corner? Do any post-op rodents have squinted eyes? Visual inspection of the cage before handling animals is important. Complete evaluation of animals in the hood can confirm your assessment.
- Body Condition Scores (BCS) – ideal scores fall within a range of 2+ to a 4- when palpation over the tail head.
- Weight – weight is a valuable tool when assessing the condition of your animals. Weight loss >15% from the pre-operative weight is considered significant and may be criteria for euthanasia.
- Fecal and Urine output – are there fecal pellets present in the cage?
- Incision site – is the surgical area clean and dry, is there discharge? Are all the sutures or wound clips present and intact?

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Appendix 6 – Animal Health Program

Swine

Vendor	Description	Upon Arrival	Maintenance
Tufts University, Cummings School of Veterinary Medicine	<p>Health reports are approved by the Veterinarian before arrival.</p> <p>Tufts health monitoring includes:</p> <ul style="list-style-type: none"> Encephalomyocarditis virus, Leptospira, Mycoplasma hypopneumoniae, Porcine Respiratory and Reproductive Syndrome (PRRS), Porcine Parvovirus, Pseudorabies, Swine Influenza Virus, Transmissible Gastroenteritis Virus, Brucellosis, and Toxoplasmosis before shipping. Tufts vaccines for Parvo, Erysipelas, Swine Influenza H1N1 and H3N2, Leptospirosis. Doramectin is used for parasite control. Pigs under 4 weeks old are additionally vaccinated for Bordetella, Pasteurella, Erysipelas, Mycoplasma, and Circovirus Type 2. 	<p>Identification is confirmed by ear tag or tattoo.</p> <p>Animals are examined and assessed by Veterinary Services. Any health issues that are found are brought to the attention of the Veterinarian.</p> <p>Animals are co-housed as space allows. Separation of animals may occur when fighting is observed or when under study if the approved IACUC protocol states as such.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>
EM Parsons and Sons, Inc.	<p>Health reports are approved by the Veterinarian before arrival.</p> <p>Parsons pigs are blood tested quarterly for Brucellosis, Pseudorabies, African Swine Fever, and Hog Cholera</p> <p>Parsons vaccines for Bordetella Bronchiseptica, Clostridium Perfringens, Erysipelethrix Rhusiopathiae, E. coli, Pasteurella Multocida, Porcine Parvovirus and Leptospira (Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaimorrhagiae, Pomona)</p>	<p>Identification is confirmed by ear tag or tattoo.</p> <p>Animals are examined and assessed by Veterinary Services. Any health issues that are found are brought to the attention of the Veterinarian.</p> <p>Animals are co-housed as space allows. Separation of animals may occur when fighting is observed or when under study if the approved IACUC protocol states as such.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>

Rabbits

Vendor	Description	Upon Arrival	Maintenance
Charles River Labs	<p>Health monitoring data is routinely reviewed by the Veterinarian.</p> <p>Specific Antibody Free.</p>	<p>Identification is confirmed by ear tag or tattoo.</p> <p>Animals are examined and assessed by Veterinary Services. Any health issues that are found are brought to the attention of the Veterinarian.</p> <p>Animals are group or co-housed as space allows. Separation of animals may occur when fighting is observed or when under study if the approved IACUC protocol states as such.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>
Penn State University	<p>Health reports are approved by Veterinarian before arrival.</p> <p>Must have proof that that colony has tested negative for: Clostridium piliforme, CAR Bacillus, B. bronchiseptica, Pasteurella sp., E. caniculi, Rotavirus, Treponema, external/internal parasites and pinworms.</p>	<p>Identification is confirmed by ear tag or tattoo.</p> <p>Animals are examined and assessed by Veterinary Services. Any health issues that are found are brought to the attention of the Veterinarian.</p> <p>Animals are group or co-housed as space allows. Separation of animals may occur when fighting or mounting is observed or when under study if the approved IACUC protocol states as such.</p> <p>Animals are placed in quarantine for 2 weeks with last room entry.</p> <p>Animals are acclimated to RIH pelleted rabbit feed over 4-5 days.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p> <p>Animals are fed a gradual diet of $\frac{1}{4}$ cup, $\frac{1}{2}$ cup, $\frac{3}{4}$ cup, up to 2 cups of pellets over a course of the first 4-5 days to acclimate to RIH purchased rabbit pelleted feed. Rabbits are fed 2 cups of pellets/day until 6 months of age, where it will be decreased to 1 cup/day.</p>
Robinson Services Inc.	Health reports are approved by Veterinarian before arrival.	<p>Identification is confirmed by ear tag or tattoo.</p> <p>Animals are examined and assessed by Veterinary Services. Any health issues</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any</p>

	<p>Must have proof that that colony has tested negative for:</p> <p>Clostridium piliforme, CAR</p> <p>Bacillus, B.</p> <p>bronchiseptica,</p> <p>Pasturella sp., E. caniculi, Rotavirus,</p> <p>Treponema,</p> <p>external/internal parasites and pinworms.</p>	<p>that are found are brought to the attention of the Veterinarian.</p> <p>Animals are group or co-housed as space allows. Separation of animals may occur when fighting or mounting is observed or when under study if the approved IACUC protocol states as such.</p> <p>Animals are tested for on arrival for the following via Charles River PCR: B. bronchiseptica, CAR bacillus, C. piliforme, Cryptosporidium app., E. cuniculi, enteric protozoa, Giardia spp., EDIM, P. ambiguous, P. multocida, Pinworms, and Salmonella spp.</p> <p>Animals are separated once they recover from a survival surgery.</p>	<p>health issues are brought to the attention of the Veterinarian.</p> <p>Animals receive two rounds of Fenbendazole dosing PO 10-14 days apart at 20mg/kg while in quarantine.</p> <p>Animals are fed a gradual diet of $\frac{1}{4}$ cup, $\frac{1}{2}$ cup, $\frac{3}{4}$ cup, up to 2 cups of pellets over a course of the first 4-5 days to acclimate to RIH purchased rabbit pelleted feed. Rabbits are fed 2 cups of pellets/day until 6 months of age, where it will be decreased to 1 cup/day.</p>
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Rat

Vendor	Description	Upon Arrival	Maintenance
Charles River Labs, Jackson Labs, Envigo	<p>Health monitoring data is routinely reviewed by the Veterinarian.</p> <p>Specific Antibody Free.</p>	<p>Animals are examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p> <p>Sentinel Testing is performed quarterly via MFIA and PCR for SEND, PVM, SDAV, KRV, H-1, RPV, RMV, NS-1, REO, RTV, MPUL, PCAR, LCMV, HANT, Fur mites, and Pinworms. A more extensive test is done once a year which includes CARB, ECUN, HANT, H-1, IDIR (ROTA-B), KRV, LCMV, MAV1 and 2, MPUL, NS-1, PCAR (RRV), PVM, REO, RMV, RPV, SDAV, SEND, Fur mites, Pinworms, RCV/SDAV, H. genus, P.bn-Heyl, P.bn-Jawetz, and S. muris.</p> <p>The Barrier Facility does the more extensive testing twice a year. Only rats that are directly from an approved vendor and/or rederived may enter the Barrier.</p>
Collaborating Universities (i.e. unapproved or atypical vendor sources)	<p>A minimum of 12 months of health reports and a facility description are reviewed by the Veterinarian. Explicit approval must be given by the Veterinarian before shipment</p> <p>Animals receive a 60-day quarantine at Brown University, Charles River, or Jackson Labs.</p>	<p>Animals are examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services (Brown University, others). Any health issues are brought to the attention of the Veterinarian.</p> <p>Sentinel Testing is performed quarterly via MFIA and PCR. This tests for SEND, PVM, SDAV, KRV, H-1, RPV, RMV, NS-1, REO, RTV, MPUL, PCAR, LCMV,</p>

			HANT, Fur mites, and Pinworms. A more extensive test is done once a year which includes CARB, ECUN, HANT, H-1, IDIR (ROTA-B), KRV, LCMV, MAV1 and 2, MPUL, NS-1, PCAR (RRV), PVM, REO, RMV, RPV, SDAV, SEND, Fur mites, Pinworms, RCV/SDAV, H. genus, P.bn-Heyl, P.bn-Jawetz, and S. muris.
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Mouse

Vendor	Description	Upon Arrival	Maintenance
Charles River, Jackson Labs, Envigo	Health monitoring data is routinely reviewed by the Veterinarian. Specific Antibody Free.	Animals are examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.	Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian. Sentinel Testing is performed quarterly via MFIA and PCR. This tests for EDIM (ROTA-A), GDVII, MHV, NS-1, MVM, MPV-1, MOV-2, MNV, PVM, REO, SEND, MPUL, LCMV, HANT, LDV, Fur mites and Pinworms. More extensive testing is done once a year which includes SEND, PVM, MHV, MVM, MPV-1, MPV-2, NS-1, MNV, GDV11, REO, EDIM (ROTA-A), LCMV, ECTRO, MAV 1 AND 2, MCMV, K, MTLV, POLY, HANT, MPUL, ECUN, CARB, PHV, LDV, Furmite, Pinworm, TMEV, P, bn-Heyl, P.bn Jawetz,

			<p>Entamoeba, and S. muris.</p> <p>The Barrier Facility does the more extensive testing twice a year. Only mice that are directly from an approved vendor and/or rederived may enter the Barrier.</p>
Collaborating Universities (i.e. unapproved or atypical vendor sources)	<p>A minimum of 12 months of health reports and a facility description are reviewed by the Veterinarian. Explicit approval must be given by the Veterinarian before shipment.</p> <p>Animals receive a 60-day quarantine at Brown University, Charles River, or Jackson Labs.</p>	<p>Animals are examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p>	<p>Animals are checked a minimum of once per day by CAF technicians and/or examined and assessed by Veterinary Services. Any health issues are brought to the attention of the Veterinarian.</p> <p>Sentinel Testing is performed quarterly via MFIA and PCR. This tests for EDIM (ROTA-A), GDVII, MHV, NS-1, MVM, MPV-1, MOV-2, MNV, PVM, REO, SEND, MPUL, LCMV, HANT, LDV, Fur mites and Pinworms.</p> <p>More extensive testing is done once a year which includes SEND, PVM, MHV, MVM, MPV-1, MPV-2, NS-1, MNV, GDV11, REO, EDIM (ROTA-A), LCMV, ECTRO, MAV 1 AND 2, MCMV, K, MTLV, POLY, HANT, MPUL, ECUN, CARB, PHV, LDV, Furmite, Pinworm, TMEV, P, pn-Heyl, P.bn Jawetz, Entamoeba, and S. muris.</p>

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Appendix 7 - Cage Card Sample



1

Dept:

Date in:

Investigator:

Committee#:

Date Euth./Initials: _____

Cost Center:

Treatments: _____

Vendor:

of Anl/cage:

Species:

Comments:

Strain:

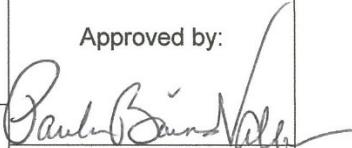
For Investigator Use:

DOB:

Sex:	_____
Age:	Weight: _____

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Appendix 8 – Hazardous Containment Procedures

Lifespan Central Research Facilities	Subject: Procedures for the Care and Handling of Rodents on Animal Biosafety Level 2 (ABSL-2) and Chemical Containment Protocols	Policy Number: ORA-CAF Animal Care 18
Issuing Department: Central Animal Facilities		Approved by:  (CRF Director)
Original Policy Date: 11/24/10 Latest Revision Date: 08/3/16 pab	Page 1 of 5	 (CRE Veterinarian)

1. Purpose

Research projects that involve biohazardous agents (e.g. bacteria, viruses, parasites and human derived materials) and chemical hazards (e.g. carcinogens, mutagens, teratogens, anti-neoplastic agents) must be classified by risk level, in accordance with the CDC *Biosafety in Microbiological and Biomedical Laboratories*. The IACUC, in conjunction with the Biohazard and Lab Safety Committee (BLSC), will determine the hazard level for studies involving animals at the time of the protocol request. The nature of the hazard and Biosafety Level classification will be communicated to the Research and CRF staff through training meetings.

Rodents and other species involved in biohazard research will be handled and housed in a manner to contain the hazard. BSL-2 animals will be dosed and housed in an animal room separate from non-BSL-2 animals. The animal handling and husbandry procedures for these animals are contained in the following description.

2. Biohazard/Hazard Signage

A hazard warning sign, incorporating the universal biohazard symbol or chemical symbol, as appropriate, will be posted on the access door to the animal holding room. The hazard warning sign will identify the agents in use, the biosafety level, and will list the names and telephone numbers of the Principal Investigator and other key laboratory personnel. It will also indicate the special requirements for entering the animal holding room. Another sign will be posted for exiting the room. Contact information for the Safety Office and CRF personnel will be posted within the animal facility.

3. Access to Animal Housing Areas

Access to the animal housing facility is limited. Only those persons required for the experimental project or support purposes are authorized to enter the animal facility and the areas where animals that have been dosed with infectious materials and/or hazardous chemicals are housed or manipulated.

4. Animal Housing

Animals which have been treated with BSL-2 agents will be kept in a room under negative pressure, and either housed in ventilated cage units that are under negative pressure, in a Modular Air Displacement (MAD) unit or in cages with microisolator filter tops on a static rack. The MAD units must be kept at a negative pressure to the room at all times. These animals will be grouped together on a rack or racks separate from unexposed animals.

Animals which have been treated with hazardous chemical agents will be kept in a room under negative pressure, and may be housed in ventilated cage units that are under negative pressure, in a negative pressure Modular Air Displacement (MAD) unit or in cages with microisolator filter tops on a static rack (as decided by CRF Management).

a. **Biological Hazards – the time for each hazard must be specifically determined by the BLSC.**

- rDNA (recombinant DNA) - Cages of animals injected with Viral Vector Agents classified as BSL-2, such as Adenovirus or Lentivirus, will not be touched for a period of 3 days (72 hours) after viral vector treatment unless required for emergent husbandry purposes (such as spilled water bottle or an escaped animal). In this event, the animal care staff will immediately contact the Supervisor(s) and the Principal Investigator or their designee for direction in the care of the animal(s). The animal care staff will provide routine husbandry care for the life of the animal after the initial (3) day waiting period and cage change.
- Human source tissues - Cages of immunodeficient animals (nude or SCID) injected with human source tissues or cell lines, will receive sterile cages and supplies, and will also be housed under ABSL-2 conditions for the life of the project.
- Live bacteria – Cages are considered BSL-2 for the life of the project.

b. **Chemical Hazards – the amount of time for each hazard must be specifically determined by the BLSC.**

- Chemotherapy drugs and other hazardous chemicals - Cages of animals dosed with a hazardous chemical will be handled as recommended by the BLSC, as the number of days to wait before changing cages may differ. (In general, three days is adequate.) After the chemical is out of the system of the animal and the cage has been changed, the card/signage may be removed and cage handled normally.
- PCBs are hazardous chemicals and must be administered to animals in a fume hood or biosafety cabinet. PCB agents do not excrete in urine or feces, but stay in the animal tissues. Cages may be handled as normal, non-contaminated.

5. Animal Identification

Animals which have been treated with BSL-2 and/or chemical agents will be kept in cages with special card identification. The cage card must identify the hazard (with a label), the agent(s) dosed into the animals and the date of the procedure.

6. Work Environment

- a. Work with animals exposed to BSL-2 agents, including the dosing and cage changes, must be done in a Class II biosafety cabinet or as specified by the BLSC. Procedures must be carefully performed to minimize the creation of aerosols or splatter of infectious materials and waste.
- b. Animals must be dosed with hazardous chemical agents in a biosafety cabinet or fume hood, as specified by the BLSC. Cages must be changed in a Class II biosafety cabinet. Procedures must be carefully performed to minimize the creation of aerosols or splatter of chemical materials and waste.
- c. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible. Devices must be thoroughly cleaned to remove residual chemical or biological hazards before leaving the room. If the device is autoclavable, place in double autoclavable bags and send to be autoclaved.

7. Personal Protective Equipment

- a. All personnel entering the animal BSL-2 holding room must wear double shoe covers, disposable gowns, appropriate respirator, typically N95 or PAPR (powered air purifying respirator), caps and one pair of chemo gloves or 2 pairs of regular gloves. This level of protection is sufficient if treated animals are to be handled in the holding room.
- b. Before leaving the holding room, personnel will place disposable apparel items in a biohazardous or chemotherapy waste container, as appropriate. Gloves and PPE should be removed in a manner that prevents transfer of infectious materials.
- c. Personnel must also wear safety glasses if treated animals are to be experimentally manipulated. Eye protection, such as face shield or goggles, is required for anyone injecting hazardous agents into animals outside of a hood and is required for any procedure with a high potential for creating aerosols, such as necropsy of infected animals, harvesting of infected tissues or fluids and intranasal inoculations.
- d. For animals housed in the normal housing room, follow standard PPE instructions, but wear a second pair of gloves. Dispose of the outer pair of gloves before touching surfaces in the room or before leaving the room.

8. Appropriate Disinfecting Agents and Methods

- a. For biohazardous agents, Clidox® diluted at 1:18:1 will be provided as the disinfecting agent and to be used as a spray or to wipe on surfaces, or as a dip to decontaminate items. The contact time is a minimum of 5 minutes.
- b. An alternate disinfectant would be Hydrogen Peroxide (H₂O₂) as approved for use by CRF Management.
- c. Care must be taken to observe the recommended expiration dates for the solutions.
- d. Unless specified, an alcohol-based hand sanitizer (e.g. Purell foam or liquid) is acceptable for decontamination of hands.
- e. Other disinfecting agents must be approved by CRF Management prior to use.
- f. Chemicals must be diluted/removed with water and placed in chemo waste.

9. Work Surface Decontamination

Any work area(s) where animals treated with a BSL-2 or hazardous agent have been handled, either for project or cage changing purposes, must be decontaminated immediately after the termination of the activity. Note: These animals should always be the last ones worked with or changed by people entering the holding room. (See section 8. above.) Paper or cloth products used for these applications will be placed in biohazardous waste.

Hazardous chemical work surfaces should be first wiped with water and paper towels to neutralize and remove the chemical, and then disinfected.

10. Personal Hygiene

If other mice (with a different hazard) must be handled in the same Biohazard room after working with a group of treated animals, remove and dispose of gloves, cleanse hands or use hand sanitizer, then re-glove before proceeding to the other animals. Discarded protective items will be disposed of as biohazardous waste.

Before exiting the animal holding room, personnel who have handled any animal(s) dosed with a BSL-2 agent or hazardous chemical will use the proper method to cleanse their hands. Hand sanitizer may be used, unless specified. If hands are visibly soiled (e.g. blood, urine, feces), proceed directly to the nearest non-food area sink to wash hands with the provided soap and water. Showering is recommended, but optional, after leaving the room.

11. Sharp Items

The use of syringes, needles, and sharps in the animal facility is limited to situations where there is no alternative to parenteral injection, blood collection, or aspiration of fluids from laboratory animals. Any sharp items used in the animal facility must be disposed of in a sharps container. Such containers will be readily available in the animal holding room. Only needle-locking syringes or disposable syringe-needle units are to be used for these purposes. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal in the sharps container. Chemotherapy needles and syringes are placed in a yellow sharps bin.

12. Spills

Any spill and/or accident which results in overt exposure to biohazardous or hazardous material will be immediately reported to the appropriate Safety Officer, key laboratory personnel and animal care staff in the affected area, as well as and Central Research Facilities management. Contact information for the Safety Officer and CRF management is posted within the animal facility. The Safety Officer will report the incident to the appropriate safety committee(s) at the next scheduled meeting.

13. Transporting Rodents and Tissues Treated with a BSL-2 or Hazardous Chemical Agents

Rodents which have been treated with a BSL-2 or hazardous chemical agents must be moved within the animal facility or between the animal facility and the laboratory in a clean shoebox cage fitted with a microisolator filter top. The shoebox cage lid must be secured either by wrapping a large elastic band around the container or using a binder clip to secure the filter top; wiped with appropriate disinfectant, and then placed inside a secondary transport container (tote box or enclosed cart). The container must be dedicated to the transport of hazardous animals, be labeled as such, and approved by CRF Management. BSL- 2 precautions must be observed wherever filter tops are removed from cages or animals are handled for any purpose.

Biological material removed from animals which have been treated with a BSL-2 agent will be transported to the investigator's laboratory for analysis in a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility.

14. Cage Changing (Shoe Box Type)

- a. Surface decontamination procedures (see above) will be carried out before and after rodent cages are changed.
- b. For BSL-2 animals, all cage components and bedding will be autoclaved before being dumped/washed. Water bottles are first emptied into the dirty bedding. Stainless steel wire cage lids and microisolator cage tops or vent rack lids will be changed every two weeks. All dirty caging will be securely wrapped or double bagged in an autoclavable plastic bag to prevent exposure during transport to the cage processing area. The outside of the bags are disinfected before leaving the room. Bags of caging are autoclaved before washing. Alternately, disposable cages may be used.
- c. For chemical hazard cages, bedding may be emptied into doubled red plastic biohazard bags in a Class II biosafety cabinet or Class I dump station in the cage wash area by personnel wearing gowns, gloves and appropriate respiratory protection. Cages will be wiped clean with water and paper towels to remove debris. Cages may be washed in the cage washer. Disposable cages or cage liners may be used as approved by CRF

Management. These items would be bagged and disposed in the same way as the bedding.

- d. If cages contain mice exposed to both BSL-2 and chemical hazards, disposable cages must be used (exceptions only with approval by CRF Director).

(If exception is made with hazardous chemicals and chemotherapy agents in a non-disposable cage: bedding may be emptied into doubled red plastic biohazard bags in a Class II biosafety cabinet or Class I dump station in the cage wash area by personnel wearing gowns, gloves and appropriate respiratory protection. Cages will be wiped clean with water and paper towels to remove debris, then thoroughly wetted with hydrogen peroxide or Clidox® disinfectant solution for at least five minutes. Wire lids and filter tops will be treated in a similar manner with disinfectant and mechanical washing. Waste disinfectant solution may be washed down the drain with excess water.)

- e. If disposable cages are used, bag the cages in doubled red bags, place in biohazard boxes. Mark the boxes for INCINERATION if only biohazardous, but CHEMO if chemicals are present.

15. Disposal of Animal Carcasses

The carcasses of mice treated with a BSL-2 agent or current with a hazardous chemical are to be double bagged in plastic, with the outer bag being a biohazard bag. The outer bag will be sprayed or wiped with appropriate disinfectant prior to leaving the room. Bags are stored in the animal facility freezer until removed and transported for incineration. For rDNA projects, investigators are responsible for maintaining a permanent record of animal use and disposition for each animal or group of animals (NIH Guidelines, Appendix Q-1-B-2)

16. Waste

Trash containers in the BSL-2 room will be marked with a biohazard sticker and lined with a red bag. Containers for chemotherapy waste are puncture proof yellow plastic and identified as chemo waste only. All waste, including disposable apparel, will leave the room as biohazardous or chemotherapy waste.

17. Records, Forms and Reports

Biohazard or hazardous chemical sign on door and on cage cards

18. Reference Sources

- a. Centers for Disease Control and Prevention; The National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC. 2009.
<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>
- b. Department of Health and Human Services, The National Institutes of Health.
NIH Guidelines for Research Involving Recombinant DNA Molecules
http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

Appendix 9 - Cadaver and/or Animal Parts Form

Cadaver and/or Animal Parts Form

Instructions: Completion of this form is only required for those cadavers or animal parts that are obtained from sources outside of Rhode Island Hospital/Lifespan Corporation. Such sources may include but are not limited to slaughterhouses; other academic, private-industry or government research facilities or commercial vendors. Please refer to the Rhode Island Hospital IACUC Policy on Review of Cadavers or Animal Parts used in Research for additional information.

Today's Date: _____

Name: _____

Address: _____

Phone Number: _____

E-mail Address: _____

Species: _____

Tissue(s) required: _____

Date Needed: _____

Source of cadaver/parts: _____

Is there a known or potential hazard/infectious diseases associated with this tissue? Yes No, If yes, please specify

Biohazard

Recombinant DNA

Chemical Hazard

Radioactive Hazard

Additional review by EH&S may be required if hazardous tissues are to be utilized for research purposes.

Intended use:

Disposal method:

For CRF Veterinary Staff Use Only

Reviewer:

Approval Date:

EH&S Reviewer (if applicable):

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Appendix 10 - Tumor Monitoring Form

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Appendix 11 - Notice of Intent to Use Avian Embryos Form

Notice of Intent to Use Avian Embryos

Project Title:

Principal Investigator:

Department:

Email:

Phone:

Avian Embryo Use Summary

1. Avian Species to be Used.

(Specify all species, typical incubation for each, and incubation at planned use)

Species	Length of Normal Incubation	Embryo Age(s) at Planned Use
<input type="checkbox"/> Chicken	21 days	
Other - Specify		

(Note: Add or delete rows as necessary)

2. Building and room number where avian embryo use will occur

3. Method of euthanasia of embryos < 50% incubation (<10 days for chickens)

Not applicable. Embryos will be used after 50% incubation

4. Method of euthanasia of embryos > 50% incubation (≥11 days for chickens)

(Specify for all species, in the event planned use is delayed for some reason)

5. Procedure for euthanasia of inadvertently hatched chicks

(See AVMA Guidelines for the Euthanasia of Animals: 2013 and/or consult veterinarians)

Investigator Assurance

I have read the Lifespan IACUC "Policy for Use of Avian Embryos" and agree to abide by it. (See CRF Policy & Procedure Manual, Section VII.M)

Signature

Date

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