THE LUNDQUIST INSTITUTE

Investigator's Guide to the Care and Use of Laboratory Animals

C.W. Steers Biological Resources Center

January 2021 (Revised)

This manual is dedicated to
the hope of refining, reducing, and replacing
the use of animals in research, testing and teaching.

Note:
All technical support staff, physicians and post-doctoral trainees working with animals will be expected to review this MANUAL, with special attention given to those aspects of animal care policy and procedure relevant to their work environment. It is the direct responsibility of the principal investigator to ensure that personnel working in animal research have reviewed this MANUAL.

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I. Essential Information About the Use of Animals

A. Faculty and Investigator Responsibilities

Individual faculty members who use animals in their teaching or research are, by law, accountable for conforming to the federal regulations and local policies governing animal use at The Lundquist Institute. These regulations and policies cover: (a) the acquisition, care and use of animals, (b) efforts to minimize animal pain and distress, (c) the training of personnel using animals, and (d) consideration of alternatives to animal use.

B. Regulations and Policies

1. Animal Welfare Act

The Animal Welfare Act (AWA) and its amendments regulate the transportation, purchase, care, and treatment of animals used in research. The AWA specifically includes dogs, cats, nonhuman primates, guinea pigs, hamsters, rabbits, and wild animal species intended for use in research. To date, the AWA does not cover farm animals or laboratory rats and mice.

Recent amendments address such issues as exercise for dogs; care of nonhuman primates to ensure their psychological well-being; the composition and duties of the Institutional Animal Care and Use Review Committee (IACUC); responsibilities of the attending veterinarian; and training of all personnel using laboratory animals in experimentation. They also require the IACUC to review all protocols using animals to make certain that they meet criteria listed in the amendments, and to conduct semiannual inspections of all animal study areas and animal facilities.

The AWA is administered by the United State Department of Agriculture (USDA). Research facilities are subject to unannounced inspections by USDA veterinarians, and are required to file an annual report listing the species and numbers of animals used in research, and certifying that anesthetic, analgesic, and tranquilizing drugs were used appropriately during research and testing.

Failure to comply with USDA standards may result in civil or criminal prosecution and suspension of animal research activities. A full copy of the AWA and its amendments can be found in the following links:

Animal Welfare Act as of February 1, 2010
Animal Welfare Regulations

Or you can visit the USDA's Animal Welfare Information Center page by

following this link:

USDA Animal Welfare Information Center

2. Public Health Service Policy (NIH Policy)

The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, otherwise known as the NIH Policy, requires each institution which receives PHS funds for research involving animals to file an approved Animal Welfare Assurance Statement with NIH (see attachment). This statement commits the institution to compliance with the Animal Welfare Act, the NIH "Guide for the Care and Use of Laboratory Animals", the "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training", and other applicable laws and regulations. The statement must describe in detail the institution's program for the care and use of animals (including mice and rats) and its program for assuring compliance with the NIH policy.

The NIH policy requires IACUC's to approve the care and use of animals as proposed in PHS grant applications before funds will be awarded. IACUC's also are required to conduct semiannual assessments of the institution's program, using the "Guide" as a basis for evaluation. Significant deficiencies in the institution's program must be identified, and the institution must adhere to an approved plan and schedule for correction of the deficiencies.

An institution's failure to comply with these policies may lead to various actions, including the termination of PHS support for all projects involving animals.

For complete information regarding PHS policies, you can visit the Office of Laboratory Animal Welfare (OLAW) following the link bellow:

OLAW – Office of Laboratory Animal Welfare

Or to read the PHS Policy on Humane Care and Use of Laboratory Animals or the “Guide” follow the links below:

Public Health Service Policy on Humane Care and Use of Laboratory Animals

Guide for the Care and Use of Laboratory Animals

3. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

The following principles were developed by the U.S. Government's Interagency Research Animal Committee. Both PHS policy and The

Lundquist Institute policy require that all research and teaching uses of animals conform to these Principles, which are:

a. The transportation, care and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seg.) and other applicable federal laws, guidelines and policies.
b. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
c. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in-vitro biological systems should be considered.
d. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
e. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on un-anesthetized animals paralyzed by chemical agents.
f. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedures, or, if appropriate, during the procedure.
g. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
h. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in service training, including the proper and humane care and use of laboratory animals.
i. Where exceptions are required in relation to the provisions of these principles, the decisions should not rest with the investigators directly concerned but should be made by an appropriate review group such as the IACUC. Such exceptions should not be made solely for the purposes of teaching or demonstration.

4. Other Regulations and Regulatory Agencies

The use of specific animals (e.g., non-human primates) and certain

procedures (e.g., drug testing) may be subject to additional regulation at the federal, state, or institutional level.

5. Accreditation

The C. W. Steers Biological Resources Center on The Lundquist Institute campus has full accreditation of its animal care and use program by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). AAALAC is a non-profit organization established by scientific and educational organizations to ensure high standards of laboratory animal care and use. The accreditation process involves periodic inspections in which the animal program and facilities are evaluated for compliance with the requirements and recommendations of the NIH "Guide".

C. Oversight of Animal Care and Use

1. Administrative Line of Authority

The institutional responsibilities for compliance with animal welfare regulations and policies lie with the Board of Directors of The Lundquist Institute. This authority passes through the President/CEO directly to the Institutional Animal Care and Use Review Committee and the Director of the C. W. Steers Biological Resources Center.

2. C. W. Steers Biological Resources Center (BRC)

The BRC is responsible for administering all activities related to the care and use of animals. BRC's functions include procurement of all live vertebrates for research and teaching, supervision of animal technicians, control of animal holding facilities and provision of veterinary care.

3. Institutional Animal Care and Use Review Committee (IACUC)

Both the Animal Welfare Act and the NIH Policy require the IACUC to oversee the animal care and use program. This committee must be composed of at least five members and include an individual unaffiliated with the institution, a veterinarian with program responsibilities, a practicing scientist experienced in research involving animals, and a member whose concerns are in a non-scientific area.

The IACUC is the institutional animal care and use committee at The Lundquist Institute. It currently has 13 members, including the Director of the BRC. The present composition of this committee is listed in Appendix A.

The IACUC's functions are clearly defined by NIH and by the Animal Welfare Act. They include:

a. Semiannual review of the institution's program for the humane care and use of animals.
b. Semiannual inspection of all institutional animal facilities.
c. Review of all proposed uses of live vertebrate for research and teaching.
d. Review and development of institutional policy on care and use of laboratory animals.
e. Review of specific concerns or complaints about animal care or use.
f. Provision of recommendations to the responsible administrator regarding all aspects of the campus animal care and use program.
g. Authority to suspend any activity involving the use of animals not being conducted in accordance with NIH "Guide" standards, or applicable laws, regulations and/or institutional policies.

In all of these functions, the NIH "Guide" provides the primary standard for evaluating the campus animal care and use program.

D. The Lundquist Institute Training Program

In order to meet legal requirements while also serving broad educational objectives, The Lundquist Institute has instituted a three-tiered training program on animal use in teaching and research.

1. The Core Program

All faculty at The Lundquist Institute will be provided this MANUAL which outlines general information on applicable regulations and policies, preparation of animal use protocols, the use of animal models, and perspectives on ethical and humane considerations. All faculty are expected to review this material and to sign a statement which appears on the Animal Use Review Form acknowledging responsibility for knowing applicable rules and institutional policies and for ensuring that all staff under their supervision also know and comply with these rules and policies. This signed statement will be required for full approval of Animal Use Review Forms by the IACUC.

All technical support staff, physicians and post-doctoral trainees working with animals will be expected to review this MANUAL, with special attention given to those aspects of animal care policy and procedure relevant to their work environment. It is the direct responsibility of the principal investigator to ensure that personnel working in animal research have reviewed this MANUAL.

2. Animal or Use Specific Certification

Animal users are required to obtain additional certification on

recommended biomethodology for each species used. This certification can be achieved by participating in workshops and wet labs, and/or viewing web videos. Documentation of these sessions in the training log is required for certification. The conference room in the C. W. Steers Biological Resources Center (BRC) is available for viewing videos and reviewing other reference materials. Teaching materials and other reference materials available in the BRC are listed in Appendix D.

3. Special Procedures

Animal users performing invasive procedures and/or administering general anesthesia will be required to submit appropriate forms, maintain accurate medical records, and provide post-operative care in accordance with BRC recommendations (Appendices O and Q). In some cases, attendance at specialized training sessions provided by the BRC may be required. Documentation of these sessions is also required.

E. Animal Use Protocols

1. Who Must Submit a Protocol

Any research or instructional use of live vertebrates by faculty, staff or industry client, requires the submission and approval of an animal use form (AUF). The AUF must be fully approved before an investigator may acquire, house, or use animals. Access to the iRIS system that hosts the IACUC application forms can be obtained by contacting the Lundquist Institute's Compliance Office (complianceac@Lundquist.org).

2. Information Required for the Protocol

The AUF requires a non-technical description of the research, a justification for the use of animals, a description of all procedures to be performed on animals, a database search for alternatives to the use of animals. The AUF also requests information on the numbers of animals to be used, types of animals, special housing requirements, and information on anesthesia and/or euthanasia, if applicable. Investigators with questions regarding protocol preparation are encouraged to contact the Compliance Office. Thorough preparation of protocols facilitates the review process and reduces the chance of delay in initiating projects and in renewal of applications with extramural funding agencies.

Once approved, the protocol is a public document. Accordingly, investigators should anticipate that the protocol will be reviewed by the general public and should make an effort to prepare protocols that are appropriately non-technical and clear.

3. Timetable for Protocol Submission

a. NIH and NSF Applications

Both NIH and NSF require verification that an applicant requesting funds for animal research has an approved animal use protocol for the proposed project. This verification must be received within 60 days after the agency's application receipt deadline. To allow sufficient time for protocol review and verification of approval, applicants should submit protocols to the Compliance Office for review by the IACUC no later than the funding agency's proposal submission deadline. If verification of IACUC approval is not received by funding agencies within the allotted 60 days, the application will be considered incomplete and may be deferred to the next review cycle.

b. Other Research and Teaching Protocols

All other protocols should be submitted at least 8 weeks before the projected use of animals is to begin. This lead-time is required because the IACUC meets only once per month, and protocols frequently require revisions before they can be submitted to the full membership of the IACUC for review.

4. The Protocol Review Process

a. Protocols are submitted the Compliance Office and the Veterinarian. This first review ensures that the information on the form is accurate and complete, that the species and number of animals to be used are justifiable, and that drugs, dosages, and the animal use procedures meet current standards.

b. Following this preliminary review, the Compliance Office will contact the applicant by email if clarifications or revisions are necessary.

c. The protocol is then distributed to all IACUC members for review.

d. The protocol is voted upon by the full Committee. The Committee can approve, require modifications in (to secure approval), or withhold approval the protocol.

F. Animal Acquisition and Housing

1. Arrangements for Acquisition and Housing

Animals may not be purchased or otherwise acquired until a fully approved protocol is on file and funding is available. All arrangements for

acquiring and housing live vertebrates from any source must be made through the BRC. Approved housing in the central facility must be available before an order will be placed by the BRC. If wild animals are to be used, special arrangements (quarantine, housing, etc.) must be made through the BRC before animals are acquired. The investigator is responsible for contacting appropriate agencies (such as U.S. Fish and Wildlife or California Fish and Game) to inquire about the need for permits. All necessary permits must be obtained before animals are acquired. Users requiring special care, equipment, or supplies for their animals or exemptions from standard animal care procedures should submit special husbandry request to the BRC so that appropriate arrangements can be made (BRC@Lundquist.org). Users must notify the BRC, in writing, if their animals will be exposed to materials or procedures which may be hazardous to personnel or to other animals.

Animals that are or will become contaminated with biohazardous agents must be housed in appropriate areas and cared for accordingly. Information concerning Q-fever in sheep is included as Attached Document.

Costs for animals maintained in the BRC facilities will be charged to the investigator. Per diem charges are billed monthly and include room and board, routine veterinary care, and administrative costs. The current prices of routine housing of animals in the BRC are listed in Appendix F.

2. Animal Identification and Recordkeeping

The AWA and the "Guide" require appropriate identification of animals and maintenance of animal records. Accepted methods of animal identification include room, rack and cage cards; collars and bands; ear notches and tags; implantable microchips and tattoos.

Individual clinical records must be maintained for all rabbits and higher mammals. These records serve as a means of communication between research and veterinary personnel. All animal manipulations and drug use as well as objective observations on health status must be recorded by both research and veterinary personnel. Animal records will be maintained on file by the BRC for 3 years after the animal is terminated. They are subject to inspection by the USDA, NIH site visitors and other regulatory agencies. Forms to be used for these purposes are attached.

II. C.W. Steers Biological Resources Center (BRC)

A. Overview

The BRC office is responsible for managing and administering a centralized program of laboratory animal care which is in compliance with the AWA, the "Guide", the Public Health Service Policy on Human Care and Use of Laboratory Animals, and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. This section describes the functions and services of the BRC and provides basic information about animal husbandry, veterinary care, animal surgery, and euthanasia.

The BRC staff provide the following services: daily checks and routine veterinary care for resident animals, animal husbandry, environmental monitoring, and maintenance of safe and sanitary facilities; ordering, receiving, identifying incoming animals and monitoring for signs of infectious diseases. In addition, training research and technical personnel in state-of-the-art techniques and consultation on animal selection and use is available through the BRC. Other services are available on a recharge basis (Appendix G). BRC personnel and their titles are listed in Appendix H.

B. Animal Husbandry

1. Housing

Current regulations require that animal housing meet certain minimum standards. The central animal facility has been designed to meet these requirements. Therefore, the central facility is the only approved animal housing area. Animals may not be housed in research laboratories without prior approval by the IACUC and must be coordinated with the BRC.

2. Caging

The BRC is responsible for selection of appropriate cages for laboratory animals, and for ensuring that housing complies with the "Guide's" standards and AWA requirements while meeting research needs (Appendix I). The BRC is responsible for maintaining cages in good condition. Investigators who have special housing requests must contact the BRC in advance to ensure adequate preparation time. Exceptions to established standards must be scientifically justified and approved by the IACUC.

3. Environmental Factors

a. Micro- and Macro-environments

The design of the cage or primary enclosure can greatly influence the animal's environment. The environment in the cage (the microenvironment) may differ from the environment of the animal room (the macro-environment). Some of the newer caging systems for rodents, for example, incorporate a microbiological barrier. This may result in substantially higher temperature, humidity, carbon dioxide, and ammonia in the cage than in the room. Since such factors may adversely affect research as well as animal health, they should be considered in experimental design and animal housing.

b. Temperature and Ventilation

The "Guide" contains information on requirements for the proper maintenance of laboratory animals, as species may thrive under different environmental conditions. Thus, environmental factors must be carefully controlled because they affect metabolism and behavior, and therefore, may adversely affect research results. Appendix J gives recommended housing temperature for commonly used research animals.

Heating, ventilation, humidity and air conditioning systems in animal facilities require constant monitoring to assure proper ventilation and appropriate temperature. The BRC is responsible for maintaining and monitoring these conditions in the animal facilities. Any departures from appropriate levels must be reported to the BRC office immediately.

c. Illumination

The lighting in an animal room must meet several needs. It must meet the animal's biological needs with regard to quantity and periodicity, and must also provide adequate illumination for daily observation and care of the animals. In addition, lighting should be sufficient to ensure safe working conditions for animal care personnel.

The BRC is responsible for establishing and maintaining light cycles in animal housing areas. Regular 12-hour diurnal light/dark cycles are provided by time controlled lighting systems in most facilities. Special research needs which require departures from normal light cycles can be arranged through written request to the BRC by filling the special husbandry request form attached.

4. Feed

Standardized commercial diets are available for most laboratory species.

The BRC is responsible for providing appropriate diets and for ensuring that food is fresh and free from contaminants. For special research needs, certified diets are available. The BRC can assist with selection of specialized diets and provide information on their availability. Special diets are the responsibility of each investigator and need to be ordered by the investigator through the Purchasing Department and the BRC notified of the arrival of these diets. In many instances these diets will require special storage conditions, please consult with the BRC director before administering a special diet to any animal.

5. Bedding

Currently cellulose pellets with or without enrichment (BioFresh Performance Bedding) is the standard bedding material provided by the BRC. Other types of bedding are available by special request, please contact the Director of the BRC for guidance on the appropriate bedding for your animals.

6. Automatic Watering System

Many of the animals, rabbits, pigs, and dogs are on automatic watering. This system is maintained by the BRC. Investigators should be aware of the proper use of the system to avoid problems that may arise. Please refer to Appendix K for details on these systems.

7. Sanitation

a. Cleanliness

The AWA and the NIH "Guide" have established schedules for frequency of cleaning animal rooms and changing cages. In some cases frequent cage cleaning may be disruptive to research objectives, as in the case of reproductive studies where frequent changes may eliminate pheromones necessary for the stimulation of reproductive behavior. Schedules can be altered to accommodate special research needs by written request to the BRC.

b. Waste Disposal

Radioactive or biohazardous carcasses and animal wastes must be disposed of according to procedures established by the campus Office of Environmental Health and Safety (EH&S). All other animal carcasses and wastes should be disposed of in refrigerators that are designated for that purpose only. No food, supplies or materials other than animal carcasses or tissues should be placed in these refrigerators. The policy for carcass disposal is included as Appendix L.

c. Vermin Control

The presence of pests in animal colonies can result in contamination of feed and bedding, and the introduction of disease. All vermin infestation should be reported to the BRC immediately.

C. Veterinary Care

The BRC provides a centralized program of veterinary care including: assessment of animal health; prevention, control, diagnosis and treatment of animal disease and injury; consultation with researchers on handling, restraint, anesthesia, analgesia and euthanasia; training of research personnel in appropriate surgical techniques and procedures; and monitoring of surgical procedures and post-surgical care.

1. Preventive Medicine

a. Animal Procurement, Quarantine and Stabilization

Newly acquired animals can introduce disease into established colonies. In addition, production colonies maintained by suppliers occasionally experience outbreaks of disease. Therefore, only animals from approved vendors with acceptable quality control programs can be admitted to our facilities. The BRC monitors animal health quality from different suppliers and maintains quality control data provided by approved vendors. This information can be provided to investigators to assist in choosing appropriate sources of animals.

To minimize the possibility of introducing disease into campus animal facilities, all arrangements for acquiring and housing live vertebrates must be made through the BRC.

All arriving animals are delivered to BRC receiving area. Each shipment of animals is inspected to verify that order specifications have been met, and that the animals have arrived in good health. At the time of this inspection a decision is made to either introduce the arriving animals into established colonies or to quarantine them. If specifications are not met, all requests for replacement animals are coordinated through the BRC office.

With some species of laboratory animals, quarantine is necessary to minimize the introduction of disease into established colonies. The extent of the quarantine period is determined by the species and by knowledge of the animal's source and previous history. Regardless of source, incoming animals should be allowed a stabilization period of at least 2 days before use. Such a period

allows the animal to recover from shipping stress, adapt to its new surroundings, and become physiologically stable. Terminal procedures do not usually require a stabilization period.

b. Surveillance and Control of Disease

Physical separation of animals by species is required to reduce the possibility of transmission of latent diseases. This separation is accomplished by housing different species in separate rooms. When animals of the same species are obtained from multiple sources, their microbiological status may differ and separate housing is provided.

Since health status can significantly alter research results, programs for the surveillance and control of disease have been established. For example, quality control data from approved vendors is maintained on file in the BRC. This information is updated regularly and is used to assist in the control of disease throughout the facility. The BRC has an established murine health surveillance program where tissues are submitted on both incoming and resident animals. Specifically, extra animals sent to investigators and others purchased by the BRC are used to determine health status on arrival to our facility and after being housed at The Lundquist Institute in designated areas. Results of these studies are available to investigators upon request.

In addition, necropsies should be performed on animals that die of unknown cause, as well as animals suffering from diseases of unknown etiology. Necropsies should include both gross and histologic examination and can be performed by or with the assistance of the veterinary staff in the BRC.

2. Diagnosis and Treatment of Disease

The BRC personnel check all animals daily, including weekends and holidays, for signs of illness, injury or abnormal behavior problems are noted and the responsible person for the animal is notified, if appropriate. Investigators should consult with the BRC to develop a plan for any ongoing animal health problems.

3. Emergency Care

Any animal health emergency noted by any animal user at any time, including evenings, weekends and holidays, should be reported immediately to BRC personnel at (310) 222-1262, or after hours, weekends and holidays contact the BRC Veterinarian on call; the contact number will be available on the voice message on the BRC main office line (310) 222-1262. For facilities emergencies contact the The Lundquist

Institute Administrator on Duty (AOD) (714) 287-5804.

4. Weekend and Holiday Coverage

Weekend and holiday coverage is provided by the BRC. In the event that an animal-related emergency is noted by BRC staff, every effort is made to contact the investigator. The BRC maintains a weekend and holiday book which includes a list of investigator names and telephone numbers. If you wish to be contacted, complete the form attached and submit to the BRC office. In any situation where an investigator cannot be reached, the BRC staff is authorized to take appropriate action, including euthanasia, when deemed necessary.

5. Anesthesia and Analgesia

Animal procedures are reviewed by the IACUC to ensure that proposed anesthetics and analgesics are appropriate for the species and research objectives. The BRC veterinary staff is available to provide training in the proper administration and use of anesthetics. A list of anesthetics and analgesics is provided in the BRC office in manuals for each specie. These manuals also contain physiologic measurements for these species. Muscle relaxants and paralytics are not anesthetics and cannot be used alone for surgical restraint; they can be used in conjunction with other drugs which produce adequate anesthesia. The NIH "Guide" requires that any proposal to conduct painful procedures without anesthesia or analgesia must be approved by the IACUC. Such procedures must be supervised directly by the responsible investigator.

6. Medical Recordkeeping Requirements

The Lundquist Institute policy requires written documentation of all survival surgical procedures, and the types and amounts of anesthetic, analgesic or tranquilizing drugs used, and objective data on the health status of the animals. Specific recommendations and sample forms are in attached. The appropriate information should be recorded in the charts located in each animal room. At the time of animal termination, the forms are removed from the animal room and filed in the C. W. Steers Biological Resources Center for a minimum of 3 years.

Controlled substance records must be maintained in accordance with the Code of Federal Regulations on Food and Drugs, Title 21. These records must be kept with controlled substances in a secured area (Attached sample form).

All records are subject to inspection without notice by AAALAC inspectors, members of Drug Enforcement Agency (DEA), the IACUC, the USDA, the FDA and the BRC staff or any other regulatory agency conducting an inspection.

For a complete copy of the Controlled Substance Act follow the link bellow:

Title 21 United States Code (USC) Controlled Substances Act

7. Surgery and Postsurgical Care

a. Survival surgery

Survival surgery is defined as any surgery from which the animal regains consciousness.

Major surgery is defined as any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover. Minor surgery is any operative procedure in which only skin or mucous membrane is incised (e.g., vascular cutdown for catheter placement or implanting pumps in subcutaneous tissue). Also included are procedures involving biopsies or placement of probes or catheters requiring entry into a body cavity through a needle or trocar in combination with a minor surgical procedure. Because they are minimally invasive, gonadectomies on male rodents are usually considered minor surgical procedures. Multiple major survival surgery is defined as two or more major survival surgical procedures on a single animal. Multiple major survival surgery is discouraged. The IACUC will consider special approval, however, when the surgeries are essential and related components of a single study. Investigators must submit a justification for review and approval by the IACUC. Cost alone is not an adequate reason for performing multiple survival surgeries on one animal.

Major surgical procedures on mammals other than rodents must be conducted in surgical facilities intended for that purpose, using aseptic techniques. These techniques include wearing sterile surgical gloves, gowns, caps and face masks; using sterile supplies and instruments; and maintaining an aseptically prepared surgical field. See Appendix O.

Minor surgical procedures on mammals other than rodents may be performed in a suitable location or equipped laboratory area, subject to approval by the IACUC. Appropriate aseptic technique for these procedures includes a clean uncluttered work area, preparation of the surgical site including clipping of the hair, disinfection of the skin and draping of the surgical site with sterile drapes; the use of sterile supplies and instruments; and the use of sterile gloves and a surgical mask by the surgeon and any assistants

working in the surgical field. For further information, contact the BRC.

Surgical procedures on rodent and non-mammalian species may be conducted under the same conditions as outlined for minor surgery on non-rodent mammalian species.

b. Pre- and Post-surgical Care

Prior to surgery, animals should be examined to ensure good health. Animals should be fasted overnight (rabbits, mice and rats are exempt) prior to anesthesia and surgery to prevent vomiting, aspiration, and problems associated with a distended intestinal tract during the surgical procedure.
Postsurgical care includes observation of the animal to ensure uneventful recovery from anesthesia and surgery. The animal should be returned to a recovery cage. No food or water should be left in the cage until the animal is fully conscious. The animal must be monitored until it regains sternal recumbency and is capable of holding its head up. The animal should be kept warm and dry, and fluids, analgesics and antibiotics administered as required. Surgical wounds should be kept clean, and bandages or wound dressings changed as frequently as necessary to keep them clean and dry.

Once consciousness is regained, return animals to their cage. Monitor ALL animals as deemed necessary to assure uneventful recovery from surgery. Use the Post-Op Progress Report to record information on attitude, appetite, hydration, body weight, body temperature (T), pulse (P), respiration (R), and the condition of the incision. Duration of post-surgical observation period will vary with type of surgery performed and condition of the animals; however, all animals should be closely monitored for a minimum of 24 hours after surgery. Monitor and record Temprature for at least 3 days post-op. See attached Post-Op Monitor Form.

Responsibility for experimentally manipulated animals lies with the investigator; however, care should be provided in accordance with recommendations made by the veterinarian**.** If the investigative team is unable to provide adequate care, such care will be provided by the veterinary staff for a fee. If an animal's health or wellbeing is in question and the investigator is not available and has not left specific instructions, the veterinarian shall act accordingly, including administration of euthanasia when necessary**.**

CAUTION: Use of heat lamps and electric heating pads can result in severe burns or hyperthermia in animals that are anesthetized or otherwise unable to escape from the heat. Close observation is

required, and use of circulating water blankets is preferred and recommended whenever possible. Arrangements with the BRC can be made to use the heating pump; however, investigators must furnish the heating pad.

c. Non-survival surgery

Non-survival surgery is defined as any surgery in which the animal does not regain consciousness. Such procedures may be performed in a suitably located and equipped laboratory, subject to IACUC evaluation and approval.

8. Euthanasia

The NIH "Guide" defines euthanasia as "the procedure of killing animals rapidly and painlessly". Euthanasia guidelines established by the American Veterinary Medical Association are attached for your review or can be found by following the link bellow.

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Proposed euthanasia techniques must be reviewed and approved by the IACUC during review and approval of animal use protocols.

Euthanasia should be carried out by personnel properly trained in the procedure being used. While decapitation and cervical dislocation may be humane when administered by properly trained personnel, animal use protocols proposing these techniques without sedation or anesthesia must include the rationale justifying such techniques. Measures should be taken to ensure that euthanasia is performed in a way that minimizes reactions among other animals that may be present.

Proper euthanasia technique includes a follow-up exam to confirm the absence of a heartbeat for several minutes, is a reliable indicator of death. Monitoring respiration is not considered sufficient since with some euthanasia techniques heartbeat may be maintained after visible respiration has ceased. Decapitation, cervical dislocation or laceration of major blood vessels should generally be performed with the animal under general anesthesia.

9. Drugs and Other Medications

The Federal Drug Enforcement Agency requires that all controlled substances be stored in a secure locked cabinet, and that an aliquot log be maintained by the user accounting for the entire volume of each drug received.

This log should be in a bound notebook and provide the following

information for each bottle of drug received:

- Name, size and concentration of drug;

- Date and volume of each aliquot dispensed;

- Name of person dispensing drug and purpose for which dispensed;

- Animal identification numbers or reference to data showing species and numbers of animals treated should be included if possible.

III. Selection of Animals and Animal Models for Use in Research and Training

A. General Consideration

The use of animals in research and instruction generally occurs in one of two contexts: (a) the animals serve as model systems for the investigation of phenomena and processes which cannot be studied directly, or (b) the animals are being studied to investigate a problem specific to the particular species. Most biomedical research falls into the former category and examples of the latter include field studies of behavioral processes which form an important part of the adaptations of one or more species.

This section reviews scientific, ethical, and humane considerations in experimental design and model selection for research involving animals. Ethical and humane considerations should be viewed as compatible with good scientific practice. There is a body of literature that supports the premise that animals which are humanely cared for are healthier both physically and psychologically, and therefore make better, more predictable, subjects. Similarly, unless a research project is intended to study pain itself, pain and suffering on the part of the animal subject will rarely, if ever, contribute anything positive to the experimental procedure. Thus, ethics and humane considerations can be viewed as integral parts of the process of experimental design and model selection.

B. Selecting a Model for Research

1. Types of Models

Selection of an appropriate model must be based on extensive familiarity with the problem or system to be studied, so that the researcher can determine the range of biological responses necessary to the experimental design. Once this familiarity is developed, either by extensive review of the published literature or from pilot studies, the researcher can proceed to select an appropriate model, whether a whole animal, an animal-derived model or a non-animal model.

Whole animal models are usually chosen when the system being studied can best (or only) be understood in the context of its interactions with other systems in the organism (e.g., sexual differentiation in embryonic development) or when it is the organism as a whole which is the system to be studied (e.g., the ontogeny of aggressive behavior). Some systems are better studied in isolation in animal cells, tissue and organs. For example, a number of biochemical and cellular processes can be studied in tissue or organ culture derived from animal material. For other kinds of studies, biostatistical or computer models may be appropriate. It should be obvious, however, that the data generated from such non-animal models are only as good as the data upon which the models are based; thus, animal

studies of some kind are prerequisite for developing and verifying all models.

2. Animal Models - Factors Affecting Choice of Species

An animal model is a living organism in which normal biological processes can be studied, or in which a spontaneous or induced pathological process can be investigated. To be effective, the process being modeled should closely resemble the analogous process in human beings or some other species in one or more ways (cf. Held, 1983). Some important criteria of animal models are: relevance to the problem being studied; the accuracy with which the model reflects all or some important aspects of the problem; the model's predictability; and the model's availability to researchers. In addition, general species characteristics such as life history parameters, behavior and diet can be as important as physiological parameters in species choice. Some ways in which these factors may affect a species' suitability as a model are discussed below.

a. Life History Parameters

Aspects of life history patterns which may influence species choice include developmental rate, age at first reproduction, frequency and time of reproduction, gestation length, litter size and life span. These parameters can be important in and of themselves: for example, species with relatively short life spans are sometimes the most useful choice for model of the long term effects of early ontogenetic factors. Life history parameters may also combine with other species' characteristics to influence appropriateness of model choice: for example, litter size is an important consideration when studying a naturally-occurring genetic condition since a large litter will increase the likelihood that some offspring will carry the trait.

b. Behavior

The normal behavior patterns of a species can be important to model choice whether or not the researcher is interested in the animal's behavior, per se. The normal social organization of a species affects such variables as how animals must be housed or fed and under what circumstances they will reproduce successfully. Social and individual behavioral characteristics may influence research variables in both obvious and subtle ways.

c. Diet

Knowledge of the normal nutrient requirements of a species is an important factor in selection of an appropriate model. Potential effects of diet on experimental variables can be readily evaluated

for common laboratory species whose nutrient requirements are well studied, but this may not be possible for rarely used or little studied species whose normal diet may be unknown. Use of poorly nourished animal subjects, or of subjects which lack some critical dietary component, may introduce extraneous, uncontrolled variability into experiments.

d. Genetics

Genetic factors are important in model selection in several respects. The species selected should have a well-known background, since some species may develop naturally occurring genetic disease or conditions which can provide useful spontaneous models. In research areas where no spontaneous animal model has been identified, extensive knowledge of a species' genetic properties is essential to selection of a likely candidate for an inducted model.

In some common laboratory species, mutant or inbred stains have been developed with well documented and often highly specific genetic properties. Catalogues and bibliographies of such models provide a useful source of information for researchers (e.g., the NIH Rodent Catalog, Hegreberg and Leathers, 1982a, b; Greenhouse, 1984; the National Research Council's Animals for Research, 1979).

C. Alternatives to the Use of Animals in Research

Critics of animal research have suggested that most, if not all, uses of animals in research and education could be eliminated by the use of alternatives such as tissue culture or computer models. In 1986, the U.S. Congress' Office of Technology Assessment produced an extensive study of the use of animal models and options for alternatives to animal use in research, education and testing. The general conclusion of the report was that the very nature of the research in many areas makes it highly unlikely that reasonable alternatives to animal use will be developed. In some areas, however, alternatives to use of animals exist or appear feasible to develop, and in some cases such alternatives may be more economical than the use of whole animals. Alternatives can be divided into four broad categories:

- Modification of existing use of animals;

- Use of animal-derived material in place of whole animals;

- Replacement of living systems with non-living ones;

- Use of mathematical or computer models.

Each of these categories is discussed in more detail below. It should be noted that many of these alternatives are not new. Indeed, the entire history of the use of animals in research can be viewed as an ongoing process of the refinement of use of animal models with many factors (ethical, humane, scientific and economic) driving refinement process.

1. Modification of Existing Animal Use

It is sometimes possible to substitute one species of animal for another. For example, lower vertebrates or invertebrates may be substituted for higher vertebrates, or so-called "laboratory species" (such as mice or rats) may be substituted for "companion animals" such as dogs or cats. Such substitutions are usually advocated on the grounds that species differ in their capacity to suffer pain or distress and it is assumed that invertebrates or lower vertebrates will suffer less than higher vertebrates, and laboratory species less than companion species.

If the research is to yield useful results, the animal species selected must be the one which will best fulfill the requirements of the model or most closely mimic the condition being studied. Substitutions, although they can and in some cases have yielded fruitful results, may raise as many difficulties as they resolve. The relative capacity of different species to suffer pain or distress is intensely debated by animal welfare advocates and scientists alike. In addition, selection of a particular species for a research project is constrained by many considerations and there may be few or no other species which satisfy all the requirements of the model. In biomedical research, where the models are often of human diseases, invertebrates or lower vertebrates may share so few relevant characteristics with humans that substitution is impossible. In basic biological research, the problem being studies may be specific to a particular species or group of species and may not even occur in other groups.

Plants and microorganisms have also been suggested as substitutes for animals. For example, Salmonella is used in mechanistic studies in genetics, and the active steroid hormones found in yeasts are used in some endocrinological and immunological studies. Use of plants and microorganisms as substitutes is limited by their evolutionary distance from humans and other higher vertebrates, and by their own unique characteristics.

Improvement in experimental design or in the statistical analyses of results may reduce the number of animals needed. Knowledge of statistics can improve experimental design by including consideration of such factors as randomization, confounding variables, sample size and statistical power, and the problems associated with testing multiple hypotheses (Geller, 1983).

Animals are expensive to use, so experimenters usually employ the

minimum number of subjects, raising the possibility that improved design or statistics may increase the number of animals used rather than decrease it. If too few animals are used, experimental results may be statistically invalid and therefore useless. Improved design and analysis could reduce animal use if more robust or clearer results reduced the total number of experiments, or eliminated unnecessary duplication of experiments (Still, 1982). Reproducibility of results is a key component of the scientific method, however, so that all repetition of experiments cannot and should not be eliminated.

Alternative designs may reduce the numbers of animal used. For example, a two-stage screening process may replace a single pass design, crossover designs may be able to replace parallel designs for studies of short-term effects, or group sequential controls can be used, in which subjects are divided into equal-sized groups and the experiment performed in stages with additional groups being added only until cumulative differences in treatment reach significance (Elashoff and Beal, 1976; Geller, 1983).
In some cases, experimental design can be modified to reduce or eliminate pain and distress of the animal subject. For example, non-invasive systems for sampling physiological parameters are sometimes used to replace invasive methods. In behavioral research, there is some evidence that allowing a subject increased control over painful or unpleasant stimuli reduces distress for some species (e.g., Lea, 1979).

2. Use of Animal-Derived Material

Although critics of animal research see alternatives as a way to eliminate animal use in research, many suggested "replacements" consist of animal-derived material. Examples of use of animal-derived material include cell, tissue and organ cultures. Working with culture specimens avoids potentially painful manipulations of live animals, although these materials must originate in a living animal. Use of such models may reduce the number of animals needed for research, as when several researchers needing small tissue samples or different organs are able to share a single animal. In some cases cell culture studies can utilize cell lines derived from propagation of relatively few cells. Tissue or cells can also be obtained from human donors, cadavers or placentas. Ultimately, however, hypotheses and data derived from these must be checked in the whole organism.

3. Chemical, Physical and Mechanical Models

In some instances it is possible to use physical or chemical models to study living systems. The study of many biochemical mechanisms, for example, use materials isolated from organs or tissues. Some physical and mechanical models have been developed mainly for educational uses, like Resusci-Dog, a canine cardio-pulmonary resuscitator training mannequin used for veterinary training.

4. Mathematical and Computer Models

Whenever a function or a relationship within a living system can be described mathematically, the possibility exists for developing a mathematical model. Scientists have long employed such models in biological and medical research because they provide the opportunity to vary the parameters involved and to predict what effects different parameters will have on the system. A complete inventory of information and parameters that should go into a model is not available until after extensive experimental work has been done on a living system, however, and the final stage of this process must be to go back to the organism to check the accuracy of the model's predictions.

It has been suggested that computer technology is now so advanced that computer-based models can completely substitute for the use of animals in research. Computers analyze data; they do not generate it. To make use of a computer model a researcher or instructor must supply the computer with whatever information will be needed for the model. If a living system is being modeled, the only source of this information is the living system itself. The more detailed the information supplied to the model, the better the model is likely to be.

Based on information derived from animal studies, computer models have been developed to analyze relationships within and between living systems. Computer models have been particularly useful in modeling feedback systems. In animal behavior, for example, game theory has been used to construct computer models which would predict how animals might behave during aggressive encounters.

Biomedical applications of computer models include aspects of kidney, cardiac and lung functions, sensory physiology, neurophysiology and developmental biology. Biochemical applications of computer models include recognition programs to identify toxic substances. Similar attempts to identify carcinogens by computer have, so far at least, been less successful.

5. Legislative Mandate for the Development of Alternatives to Animal Use

Congress has been urged to provide funds specifically to develop alternatives to animal use in research, testing and education. Legislation has included requirements that alternatives be considered (e.g., the Health Research Extension Act of 1985 and the amended Animal Welfare Act), and NIH has a funding program for the development of methods which reduce or replace animal use.

D. Ethical and Humane Considerations in the Use of Animals for Research and Teaching

1. Ethical Positions on Animal Use

Questions concerning the ethics of animal use in research and teaching have been debated by scientists, theologians, philosophers and the lay public since the use of animals for these purposes began. Even when consideration is restricted to recent discussions of the issue, there are almost as many ethical positions as there are writers on the subject. The prevailing view is that animals can and should be used in research which benefits humans and the ecosystem, and that there is no acceptable alternative to such use. Implicit in this view is the expectation that research animals will be treated humanely. Extreme views are held by small minorities. On the one hand are those who believe that humans have no responsibility to other animals and, therefore, any use of animals is permissible. On the other hand are those who believe that all animals, human and non-human, have the same rights and, therefore, humans have no right to use animals for any purpose. There are many variants of each of these views and even among those who hold that animals have legal rights, there is disagreement about whether all animals should be accorded the same moral or legal status.

Following the prevailing view, laws and regulations at many levels require the humane treatment of animals used in research and teaching. Essential elements of humane treatment include that animals be housed in clean, comfortable quarters, that they be fed an adequate diet and that they be maintained in good health. There is no general agreement as to what additional factors might be necessary for humane treatment. Most conscientious researchers and the agencies which regulate animal care accept that an animal's well-being is dependent on its mental state as well as its physical state. It is also recognized, however, that it is much more difficult to establish objective guidelines for the assessment of the psychological well-being of an animal than it is to monitor physical well-being. Some of the factors involved in assessing an animal's well-being are discussed in the sections which follow.

2. Subjective Experience in Animals

Early views on the capacity of animals to experience pain and other sensations were often predicated more on philosophical positions than on scientific observation. Followers of the mind-body dualism of Descartes denied the existence of mental states in non-human organisms. The Romantic tradition of the 19th century attributed elaborate anthropomorphic thoughts, feelings and intentions to animals. Behaviorists of the early 20th century sidestepped the issue: because psychological states were private, they could not be objectively character-ized, even in humans.

Evidence regarding subjective experience in animals comes from

neurophysiological studies and from ethological (behavioral) studies (e.g., Lorenz, 1971). Physiological evidence indicates that animals which possess a central nervous system or which show evidence of receptors for endogenous opiates have the potential, at least, to experience pain. Behavioral evidence of pain in many higher vertebrates is similar to its manifestations in humans, including screaming, squealing and struggling, for example. Behavioral evidence of pain in species more remotely related to humans (e.g., fish), and of less obvious forms of distress like fear, frustration, exhaustion and anxiety in all non-human animals is harder to identify.

3. Captivity and Suffering

Some opponents of the use of animals in research have suggested that captivity alone causes suffering for animals. They argue that distress is indicated whenever an animal shows behavior that deviates from the behavior exhibited by wild conspecifics. This concept of suffering is based on unfounded assumptions about the relationship between behavior of wild and captive animals. Behavior of wild and captive or domestic members of the same species may differ for a number of reasons. For example, many species commonly used in research have been subject to many years (in some cases, thousands of years) of artificial selection by humans. The genetics and the normal behavior of these animals may now be very different from those of their wild progenitors. In addition, many ethological studies, such as the pioneering work of Konrad Lorenz, have shown the importance of early experience on later behavior. Animals of wild parentage, which are born and raised under captive conditions, may behave differently than wild born and raised conspecifics. Such genetic and environmentally determined behavioral differences do not automatically indicate suffering.

Is it legitimate to interpret all behavioral differences between wild and captive animals as negative ones? Animals in captivity are free from the need to watch for and to escape from predators. It is difficult to interpret this alteration in the animal's environment as a negative one, or to conclude that animals suffer by not having to avoid predators (cf. Hediger, 1968). It has been argued that safety alone does not constitute psychological well-being, especially for normally social animals housed alone. This is an important consideration and here, knowledge of the behavior of wild animals may be useful in designing research environments which promote psychological well-being.

It is often assumed that wild animals live in a kind of natural paradise and that it is only the appearance and intervention of human agencies that bring about suffering. This essentially Rousseauian view is at odds with the wealth of information derived from field studies of animal populations. Scarcity of food and water, predation, disease and intraspecific aggression are some of the factors which have been identified as normal parts of a

wild environment and which cause suffering in wild animals on a regular basis.

4. Recognizing Signs of Distress in Laboratory Animals

As in the case of pain, physiological parameters and behavioral responses provide important cues to distress in animals, although distress is harder to define and identify than pain. Physiological parameters include hormonal responses (changes in the levels of adrenal hormones, for example), increased susceptibility to disease (which may indicate an impaired immune system) or weight changes. Any unusual behavior in an animal which shows physiological signs of stress may be such a cue. Some behavioral changes, however, may be normal adaptive responses which help the animal cope with a new environment or moderate stress, so behavioral changes should not automatically be considered pathological. Behavioral changes which occur in the absence of physiological signs of stress may also indicate suffering. For example, conflict behavior, in which an animal exhibits conflicting tendencies to perform different or incompatible behaviors, may indicate fear or frustration. Conflict behavior can, however, result from positive stimuli also, such as conflict between the desires to eat and mate. Finally, the appearance of abnormal or sterotypic behavior such as the pacing of big cats in zoo cages or the self-biting of monkeys housed alone in small cages can signal distress. Even clearly abnormal behaviors may help animals cope with the conditions of captivity. For instance, chimpanzees which throw feces at zoo visitors may be enhancing the psychological condition of their captivity; thus, this might be considered an adaptive behavior.

IV. Special Considerations

A. Physical Restraint

Protocols requiring prolonged physical restraint must be carefully justified to the IACUC by the investigator. The investigator must convince IACUC that such restraint is required, and make satisfactory arrangements for adequate veterinary care before such restraint may be approved.

The use of physical restraint in an animal protocol needs to be carefully considered before a decision is made to use it. Because the Guide for the Care and Use of Laboratory Animals summarizes this issue so well, the following discussion has been paraphrased from that source.

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices such as restraint stocks or squeeze cages. It is important that such devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, should be used when compatible with research objectives. The following are important guidelines for the use of restraint equipment:

Animals to be placed in restraint equipment should be conditioned to such equipment prior to initiation of the research.

The period of restraint should be the minimum required to accomplish the research objectives. Prolonged restraint for any reason must be approved by the committee.

Restraint chairs or similar devices are not considered "normal" methods of housing, although they may be required for specific research objectives. Restraint chairs or similar devices must not be used simply as a convenience to investigators in handling or managing animals. When such devices are used, their use must be specifically approved by the committee.

Attention must be paid to the possible development of lesions or illnesses associated with restraint, including contusions, decubital ulcers, dependent edema, and weight loss. If these or other problems occur, veterinary care must be provided to treat the animal which, if necessary, must be temporarily or permanently removed from the restraint device.

B. Multiple Major Surgical Procedures

Multiple major survival procedures on a single animal are allowed only when they are related components of a research project. The investigator must convince IACUC that the requested surgical procedures are necessary and that provisions for proper use of anesthetics, analgesics and tranquilizers, as well as proper treatment modalities have been discussed with and approved by the BRC Director.

C. Guidelines for Blood Collection in Laboratory Animals

Periorbital bleeding of rodents must be carried out under general anesthesia by highly experienced personnel. Frequency must not be greater than once every 2 weeks. Bleeding by cardiac puncture must also be carried out under anesthesia. Bleeding by cardiac puncture may result in such complications as cardiac tamponade, pneumothorax, excessive hemorrhage, or death of an animal.

1. Rabbits

   a. The total blood volume of rabbits is approximately equal to 6-8% of the lean body weight. Allowing for 5% in the capillary bed this translated to about 67 ml/kg (1 kg x 0.07 x 0.95 = 67 ml/kg).

   b. Acute loss of 25-30% of the blood volume is fatal in 50% of rabbits undergoing hemorrhage.

   c. Up to 15% of the blood volume may be removed at one bleeding. For example, for a 4 kg rabbit: 4 x 0.07 x 0.15 = 0.0399 liter (40 ml). This volume may be extracted weekly. For animals producing antibodies, however, such bleeding should be restricted to once every 2-4 weeks.

   d. If greater volumes are removed the hematocrit and serum proteins must be monitored.

2. Rodents

   Up to 20% of blood volume may be removed. For example, mouse of 30g - 0.4 ml; rat of 300g - 4.0 ml.

D. Guidelines for Use of Freund's Adjuvant in Laboratory Animals

Freund's complete adjuvant can cause inflammation, induration or necrosis. The following guidelines are intended to minimize animal discomfort. Departure from these guidelines requires adequate justification in the proposal.

1. Consider the use of incomplete Freund's adjuvant, or another adjuvant.

2. Complete Freund's adjuvant should be used only for the first antigen dose. The use of two or more doses of the complete adjuvant is rarely warranted and must be adequately justified.

3. Injections containing Freund's complete adjuvant should be subcutaneous or intraperitoneal. Intradermal injections can cause skin necrosis and sloughing. Intramuscular injections may lead to temporary or permanent lameness. Intravenous injections have been known to produce pulmonary lipid embolism.

4. Footpad injections are not recommended. Injection of the hind limb footpad in rodents, or intradermal injections in rabbits may be approved if injections at other sites are shown not to produce significant antibody titers to weak antigens. Footpad injections must be carried out under general anesthesia.

5. The injection should be divided into fractions so that no more than 0.1 ml is injected subcutaneously at any one site in rabbits or more than 0.05 ml in mice.

6. The inoculum should be free of extraneous microbial contamination. Millipore filtration of the antigen prior to mixing with adjuvant is recommended. Injection sites should be cleaned, but need not be aseptically prepared.

E. Guidelines for Monoclonal Antibody Production in Mice

1. Immunization for Hybridoma Production

a. The site of injection is wiped with a clean cotton ball saturated with 70% alcohol.

b. Volume of all inoculations should be limited to 0.05 ml or less per injection site.

c. On day 1, mice are immunized SQ with antigen in complete Freund's adjuvant.

d. On days 14 and 28, mice are boosted SQ with antigen in incomplete Freund's adjuvant. Blood samples may be taken after each booster to determine antibody levels. If necessary, on day 35, a third boost using incomplete Freund's can be given.

e. If antibody levels are sufficient, the mice can be euthanized and the spleen removed for subsequent spleen cell collection and fusion to myeloma cells. A final I.V. or S.Q. booster is given 72 hours before the removal of the spleen.

2. Monoclonal Antibody Production (Ascites)

   a. The abdominal area is wiped with a clean cotton ball saturated with 70% alcohol.

   b. Ascites is prepared by injecting 10^6 hybridoma cells in 0.5 ml saline solution IP into the mice.

   c. After visible tumor growth (approximately a period of 1-3 weeks), but before visible discomfort to the mice, the ascites fluid is harvested by inserting a 20 gauge needle intraperitoneally in an awake animal.

   d. This procedure can be performed 1-3 times.

   e. Prior to the final time, the mouse is euthanized with CO_2 and the remaining ascites fluid is harvested.

F. Guidelines for Maintenance of Tumor Cell Lines and Hybridomas in Rodents

1. Cell lines should be tested for murines viruses. Untested mice, or those contaminated with murine viruses, must be isolated.

2. Mice inoculated with cells producing solid tumors must be observed regularly (minimally 3 x per week) to ensure that they are euthanized before tumors ulcerate, or achieve a size so as to interfere with normal activity.

3. After inoculation with ascites producing tumor lines, mice must be observed at least 3 x during the first week and daily thereafter (including weekends and holidays) to monitor the degree of abdominal distention and for signs of illness. Ascites fluid must be removed before abdominal distention is such as to cause discomfort or interference with normal activity. Animals must be euthanized if they exhibit signs of poor condition (ruffled coat, huddling, etc.).

G. Guidelines for Estimating the Number of Animals

Federal regulations and the Guide for the Care and Use of Animals (Guide) require that Principal Investigators (PIs) provide justification for the total number of animals proposed for use in research and teaching. The IACUC is charged with evaluating the number of animals and the justification for those numbers. This assessment is essential to determine whether the numbers are sufficient to answer the scientific questions and goals proposed. The rationale provided for the number of animals needed in a study should be based on the nature of the study. Therefore, although statistical analysis is the preferred method for justification, this approach may not be appropriate for certain types of studies.

General Points to include in the Justification (as appropriate)
Budgetary constraints, time constraints, or the number of experiments that the laboratory personnel can perform in a week, month, etc. are not acceptable for justifying animal numbers.

The number of animals requested should cover the three-year life of the protocol. Studies should be designed to provide a statistically significant result with a minimum number of animals.

The justification should include the:

- Number of animals per experimental group;
- Number and types of experimental groups; and,
- Number of replicates for each study

• If eggs, fetuses or embryos are used before they reach specific stages based of neural development, they do not require statistical justification. However, justification should include the number of pregnant females required to produce the proposed number of offspring.

Expected morbidity or mortality that may impact the number of animals needed should be included in the justification.

For studies where statistical justification is not possible, sequential sampling is often the preferred method of addressing the number of animals that will be required

It is important to use the appropriate number of animals in a study. Using more animals than are needed to support your objectives is a waste of the extra animals; using too few animals gives you results that are unreliable and wastes all the animals used. Therefore, it is necessary to use systematic methods to estimate the best number of animals to use and to be able to justify this choice in the Animal Use Form. The methods used depend on the type of study and described below.

1. Statistical Design

What best describes your study?

a. Comparative study - use power analysis to determine how many animals are needed to give you a high enough probability to come to a correct conclusion. Methods for computing power for basic are given below.

b. Descriptive study - use confidence intervals to give a measure of the precision of your estimate.

c. Tissue Harvest - indicate how you determined how much tissue was needed and why.

d. Teaching and Training - for one or two animals this should be self-

evident. If several animals are used further explanation will be required.

2. Statistical Procedures

   a. What are your main research questions. If this is a comparative study, please state in terms of hypotheses to be tested.

   b. Please describe each group of animals; include any special characteristics, planned treatment for the group and the number of animals per group. State if groups are matched in any way.

   c. What are your key outcome variables. State when during the course of the study they will be measured.

   d. How do you plan to analyze the data. State statistical tests by name.

3. Sample Size

   a. What percent of animals do you expect may have missing data due to mortality, attrition or other causes?

   b. State how you determined your proposed sample size. Include preliminary values and/or estimates used in power analysis or computing confidence intervals.

H. Guidelines on Euthanasia as an Alternative to Death as an End-point in Rodents

Legal, regulatory, and moral guidelines require that animal pain, distress, and suffering be minimized in any experiment. For these reasons, investigators are encouraged to administer euthanasia in death-end-point experiments prior to the actual death of the animal if experimental validity will not be compromised. These objectives assume that investigators can differentiate between animals which are found morbid (i.e., affected with disease or illness), and those which are found moribund (i.e., in the state of dying).

Investigators must be able to judge and perform euthanasia on moribund rodents based on objective signs of dying, depending on experience with the animal model, professional judgment, and the experimental protocol. Some of the known signs of illness or dying which may be applied are listed below to assist investigators in decision making. The use of this information is encouraged with the understanding that the combination of signs indicating euthanasia may vary with experimental end points.

Animals that are found in moribund state should receive euthanasia. If death itself is the required end point of the experiment, the investigator must receive approval

to conduct such studies by providing appropriate justification in the IACUC protocol. Inconvenience or increased costs are not justifiable reasons. Investigators are expected to make a good faith effort to justify their end points, or assure that they can judge animals found moribund and agree to perform euthanasia.

In summary, all investigators are expected to monitor experimental animals at least daily (including weekends and holidays), to perform euthanasia on any animals which they judge should receive euthanasia, to use alternative end points to death when possible, and to minimize animal numbers within statistical constraints in general, but especially in death-end-point protocols.

1. Signs for Judging Morbidity (disease/illness) in Rodents

- rapid breathing rate
- breathing rate very slow, shallow, and labored
- rapid weight loss
- ruffled fur (rough hair coat)
- hunched posture
- hypothermia or hyperthermia
- ulcerative dermatitis or infected tumors
- inappetence
- diarrhea or constipation

2. Signs for Judging the Moribund Condition (state of dying) in Rodents

- signs for morbidity plus
- impaired ambulation (unable to reach food or water easily)
- evidence of muscle atrophy or other signs of emaciation (body weight is not always proportionate)
- any obvious prolonged illness including such signs as lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), prolonged inappetence, bleeding, difficulty breathing, central nervous system disturbances, or chronic diarrhea or constipation
- inability to remain upright

3. Signs of Pain in Animals

- animal not alert
- abnormal movement of abnormal postures
- inappetence or dehydration
- guarding reaction when likely areas of pain are palpated
- vocalization when palpated or moved
- self mutilation
- restlessness or lethargy
- shock

I. Cold Blooded Vertebrate Animals

Cold blooded vertebrates are sentient beings. They must be provided with proper care and husbandry with considerations unique to each species.

Importation and maintenance of the African clawed toad, *Xenopus laevis*, must be under permit from and in full compliance with regulation of the California Department of Fish and Game.

Provisions for other cold-blooded vertebrates must meet or exceed Department of Fish and Game regulation and meet standards as specified in Amphibians (1974) by the Institute of Laboratory Animal Research Husbandry of Laboratory Animals. This document is available from the office of the Director of BRC.

J. Guidelines on Using Neuromuscular Blocking Agents

The use of neuromuscular blocking agents (such as, tubocurarine chloride, metocurine iodide, pancuronium bromide, vencuronium bromide, atracurium besylate, gallamine triethiodide) must be approved for research procedures where the animal needs to be completely immobilized. These drugs may not be used alone for restraint, but only in conjunction with drugs which produce surgical analgesia.

Since these drugs readily cross the placental barrier the use of pregnant bitches will not be considered.

Due to the inherent difficulties in assessing the level of surgical anesthesia in paralyzed animals, the use of these drugs will be approved only if it is clearly established that they are essential for the proposed research, and that the investigator is able to monitor the animals appropriately.

The IACUC has adopted the following guidelines for the review of protocols which include the use of paralyzing drugs:

Surgical anesthesia must be induced, and the animal intubated, prior to administration of the neuromuscular blocking drug.

The use of an analgesic (e.g., xylazine) is recommended, in addition to the general anesthetic.

Controlled ventilation should be established prior to injection of the neuromuscular blocking drug.

During the period of paralysis, the heart rate and electroencephalogram must be monitored continuously for signs of reaction to pain and stress due to lightening of the anesthesia.

Core temperature, blood gases and fluid, and electrolyte balance must be maintained within normal levels during the period of paralysis. Provision must be

made for periodic voiding of the urinary bladder.

In cases of uncertainty concerning the experience of the investigator to monitor the animals the IACUC may require the veterinarian to observe the procedure using the proposed methods of anesthesia and analgesia, but without administration of the neuromuscular blocking drug, to assure that the anesthetic technique is sufficient to relieve any pain and stress associated with the procedure.

K. Visitors to Animal Facilities

The following policy is intended to ensure a minimally disruptive environment for resident animals, to protect the health of research animals, to protect the confidentiality and integrity of research, and to help in the accurate representation of policies and procedures.

All visitors are informed of the risks involved when entering an animal facility. All visitors must understand that there are inherent risks to entering an animal facility including animal bites, scratches, allergic responses, and risk of injury from equipment and understand that the possibility exists of exposure to pathogens which are infectious to humans (zoonoses exposure), specially to pregnant women or immunosuppressed visitors. It is recommended that visitors are healthy prior to entry, including no signs of upper respiratory tract infections, influenza, or strep.

Visitors to the animal facilities are generally prohibited, unless prior written approval by the Director of the BRC or his/her designee is given.

Conditions under which access to animal facilities may be granted, both for visitors and for the use of electronic, film, or tape recording of facilities and animals, are as follows:

Visitors are defined as researchers, contractors, media, research sponsors as well as accrediting, auditing, and regulatory agencies.

- All visitors must sign-in with the BRC Director or their designee upon arrival. This requirement is waived for government inspectors, other regulators, or auditing agencies (e.g., AAALAC site visitors, IACUC members, research animal veterinarians).

- All visitors must abide by the BRC Standard Operating Procedures (SOPs) pertaining to protective clothing (PPE).

- All visitors must be accompanied by The Lundquist Institute PIs or designees, or by a BRC employee (e.g. facility manager, research animal veterinarian).

- The use of any recording device (e.g., digital camera, camera phones, video recorder or sound recorder) is prohibited unless the Director of the

BRC or his/her designee grants permission to record, photograph or film within the BRC.

Student/staff/faculty who may be photographed in the course of their work should be informed when such activity is imminent; any individual who declines to be photographed, filmed, or recorded is not required to be subject to recording.

Role of the Investigator

The PI must get approval from the Director of the BRC or his/her designee, prior to any visitor(s) entering the BRC. PI is also responsible for any training and confidentiality agreements prior to visitor entering BRC.

Role of the BRC Staff

The BRC Director, Attending Veterinarian and the BRC animal care staff, must ensure the safety of all animals. It is imperative that all staff watch for guests behaving in an inappropriate manner. If any suspicious behavior is observed, the BRC Director must be notified promptly. In the event that s/he is not available, the IACUC Chair, Director of Compliance, Director of Human Resources or campus police must be notified.

Failure to follow the constraints of this policy will result in the visitor being immediately escorted out of the BRC vivarium and may result in the visitor being removed from the Lundquist's premises. Administrative disciplinary action may also be taken regarding the Lundquist's employee who escorted an unauthorized visitor or a visitor who acted inappropriately into the BRC vivarium, including revocation of facility access. The Director of BRC has the authority to make the short-term decision on revoking access to the vivarium for the Lundquist employee violating this policy. Long-term access decisions will be made through the IACUC. Human Resources and the individual's supervisor will be involved in any disciplinary action of the employee.

In addition to institute sanctions, individuals may also be subject to criminal sanctions or civil liability under federal or state law.

V. APPENDICES

Appendix A

The Lundquist Institute
Institutional Animal Care and Use Committee
Fiscal Year 2020-2021

Position	Name	Department/Title
Officers		
Chair	Raimund Hirschberg, MD	Medicine/Nephrology and Hypertension
Vice Chair	David Naylor, MD, PhD	Neurology
Members		
Radiation Safety	David Applebaum, MS	Environmental Health & Safety Director
Veterinarian	Catalina Guerra, DVM	BRC Director
Nonscientist	Rebecca Yuan, MPIA*	Sr. Research & Sponsored Programs Officer
Nonscientist	Sheila Jefferson, AA *Alternate for Rebecca Yuan	Sr. Research and Sponsored Programs Officer
Scientist	Marc Swidergall, PhD	Infectious Disease
Veterinarian	Jeffrey L. Lee, DVM	BRC Consultant
Scientist	Basil Ibe, PhD	Neonatology
Scientist	Yan-He Lue, MD	Medicine/Endocrinology
Scientist	Youngju Pak, PhD	Biostatistician, Medicine
Nonaffiliated	John W. Roberts, PhD	Offsite
Scientist	Yan-Qiong Xiong, MD, PhD	Medicine/Infectious Diseases
NA	Rosa Harmon, MS, RVT, CPIA	Compliance Manager, Compliance Office

Appendix B

1. Procedures for Institutional Animal Care and Use Review Committee (IACUC)

After administrative review and processing by Research Administration, copies of animal use protocols are submitted to the Compliance and Regulatory Affairs Office.

The Compliance and Regulatory Affairs Office coordinates the review of the protocols as follows: One copy of the protocol and supporting documents (Animal Use Review Form) is sent to the institutional veterinarian for review. One copy of the protocol—supporting documents is sent to another faculty member of the IACUC for scientific review. The member is chosen for experience with the animal species to be used. One copy of the protocol and supporting documents is reviewed by the Associate Vice President, Compliance and Regulatory Affairs for discrepancies, omissions and lack of detail in the supporting documents.

Following receipt of the separate reviews, a memorandum detailing changes to be made and/or information to be supplied is drafted and sent to the investigator for comment. If the investigator responds prior to 72 hours of the IACUC meeting, his/her response is distributed to the IACUC members at the meeting.

All members of the IACUC receive a copy of the agenda, animal use forms, continuing reviews, and amendments seven to ten days prior to the meeting.

At the IACUC meeting, protocols are presented for review by the convened committee; questions raised by the committee members are noted, and there is a vote on each protocol. The IACUC may approve, require modifications (to secure approval), or withhold approval of each protocol.

Decisions regarding protocols require the vote of a simple majority at a convened meeting at which a quorum exists in order to approve, require modifications (to secure approval), or withhold approval of each protocol. No IACUC member may participate in the review or the deliberations concerning a study in which the member has a conflict of interest except to provide information to the IACUC.

No member who has a conflict of interest may contribute to the constitution of a quorum when considering a protocol.

Designated Member Review (DMR):

a. For protocols which the IACUC determines require modification (to secure approval) the IACUC chair may designate reviewers from within the IACUC membership to review the investigator's response to the committee's memo (see below).

b. The investigator may petition for DMR if there are exigent circumstances, such as the need to provide approval to a funding agency before the time of the next

scheduled convened IACUC meeting. Such a request may be accepted at the discretion of the IACUC Chair or designee(s). Alternatively, the IACUC Chair or designee may choose to require review at a convened IACUC meeting.

To utilize DMR, the Compliance Office will prepare and distribute to each IACUC member a packet which includes a cover memo, explaining the reasons for the request for DMR, a copy of the protocol and the animal use form (and any other supporting documentation). Committee members are given a five (5)-calendar day consideration period to review the protocol document and may choose to communicate that they agree either to:

(1) allow designated reviewers to review the protocol, or
(2) hold the protocol for the next scheduled convened IACUC meeting for review

Member responses are sent to the Compliance Office either via email, online survey or in hard copy. The email or reply to the online survey will be construed as a signature; the hard copy response should be signed. At the end of the consideration period, the Compliance Office will tally the member votes to determine that more than half of the voting members respond; if this occurs, and if no member who responds requests convened IACUC meeting review, Compliance Office staff will ask the designated members for their review comments and/or approval. Members who do not respond before the expiration of the deadline are assumed to have no objections to DMR.

The designated reviewers are designated by the IACUC Chair (or his or her designee). These designated members have authority to approve, require modifications in (to secure approval), or request full committee review. The designated reviewers may not withhold approval; this action may only be taken if the review is conducted at a convened meeting at which a majority of the members are present. If the designated reviewers approve the protocol, the regular process is followed for sending an approval letter, and the protocol is placed on the agenda of the next convened meeting as an information item.

Review and approve, require modifications in (to secure approval), or withhold approval of proposed significant changes regarding the use of animals in ongoing activities according to PHS Policy IV.C. The IACUC procedures for reviewing proposed significant changes in ongoing research projects are as follows:

All significant changes in ongoing research protocols are reviewed by the IACUC at a properly convened meeting. Investigators are required to fill out the Protocol Amendment Request Form detailing the proposed change and, if necessary, a revised Animal Use Review Form, incorporating the proposed change. Proposed changes could include introduction of new procedures, change of species, increasing the number of animals, change in personnel, etc. No change in a protocol can be implemented without first having been reviewed and approved by the IACUC.

Notify investigators and the Institution in writing of its decision to approve or withhold approval of those activities related to the care and use of animals, or of modifications required to secure IACUC approval according to PHS Policy IV.C.4. The IACUC procedures to notify investigators and the Institution of its decisions regarding protocol review are as follows:

Protocols approved without modifications are immediately processed by the Compliance and Regulatory Affairs Office; a letter of approval is sent to the investigator and Grants and Contracts is instructed to prepare and send a letter, indicating approval to appropriate external agencies.

For protocols requiring modifications (to secure approval), a memorandum is prepared by the Compliance and Regulatory Affairs Office detailing the concerns of the committee and sent to the investigator for response. The chairperson will designate members to review the investigator's written response, (typically the Associate Vice President, Compliance, the Director of BRC and the consulting veterinarian); based upon the investigator's response, the reviewers may approve the protocol, require further modifications, or request review at a convened meeting.

For protocols in which approval is withheld, there are usually more substantive issues to be addressed by the investigator, relating either to the study design or to lack of detail, thus preventing adequate review and approval. Investigators are informed by memorandum of the committee's concerns and are requested to provide either a revised protocol or more detail in their experimental design. If the investigator responds, this response is presented at the next convened meeting at which time the IACUC can approve, require modifications (to secure approval) or withhold approval. The investigator may, at the discretion of the IACUC, be invited to attend the meeting to respond orally to substantive issues. The IACUC may also, at its discretion, seek review of a protocol by a qualified external reviewer if it feels that its membership does not possess the necessary expertise to review the protocol adequately. Such review by an external reviewer is, however, only advisory in nature and the IACUC may choose to accept or reject the reviewer's comments.

At the beginning of every IACUC meeting, the minutes from the previous meeting are reviewed and approved by the convened committee. Once approved, the chairperson signs the minutes, and a copy of the approved minutes is sent to the Institutional Official as notification of IACUC decisions to approve or withhold approval of activities related to care and use of animals. The minutes include a list of approved protocols, protocols for which approval was withheld and any discussion that might have taken place regarding those procedures.

Conduct continuing review of each previously approved, ongoing activity covered by PHS Policy at appropriate intervals as determined by the IACUC, including a complete review at least once every 3 years according to PHS Policy IV.C.1.-5. The IACUC procedures for conducting continuing reviews are as follows:

Investigators are required to complete a continuing review form annually. The form requests information regarding the number of animals used in the preceding year, the number of animals proposed to be used in the coming year, whether there have been any changes in procedures that substantially alter the use of animals, whether the investigator has become aware of any alternatives to the use of live animals since the last review, whether there have been any complications in the handling, care or use of the animals, whether there has been any change in the personnel handling the animals.

These forms are placed on the IACUC agenda, and reviewed by the committee at a convened meeting.

Additionally, the procedures for the Post-approval Monitoring Program (PAM) created by the IACUC are as follows:

Selection of Protocols
Selection of protocols for post-approval monitoring includes a cross section of species and pain categories with an emphasis on the following:

a. Studies classified as Pain Category E.
b. Protocols involving USDA-covered species, especially socially sensitive species, such as dogs, rabbits, pigs and sheep.
c. Protocols associated with past compliance issues.
d. Protocols the IACUC or veterinarians designate for review.
e. Protocols that require the animals brought to individual labs.
f. Protocols with a study, group, individual, or activity where a suspected or actual problem has been reported to the IACUC, Biological Resources Center (BRC) or Compliance Office.
* It should be noted that it is the intent of the IACUC that all active protocols will be monitored at some point.

Principal Investigator Notification
The Principal Investigator (PI) will receive notification in advance that the protocol will be monitored. In addition to the notice, the PI will receive a copy of the PAM checklist. This allows sufficient time to gather relevant information as outlined in the checklist, review the protocol, and prepare for the monitoring meeting. There will be a follow-up phone call or email after the initial notification to schedule the PAM and answer any questions the PI may have. Whenever possible, Post-Approval Monitoring should start off with a meeting between the individual conducting the PAM, the PI, and others participating with the experiments covered by the protocol. This meeting may take place in the PI's office, laboratory, a nearby conference room, or other appropriate location.

Pre-review of the Protocol
Before the meeting, the Post Approval Monitor will review the animal use protocol, using the PAM checklist. During the pre-review process, the Monitor can formulate specific questions for use during Post Approval Monitoring.

During each monitoring session
The Monitor will compare procedures conducted in the laboratory with those listed in the approved protocol. Discrepancies noted between the procedures performed in the lab and those listed in the protocol will be brought to the attention of the PI. Such discrepancies may include:
a. Personnel performing procedures are not listed in the approved protocol.
b. Procedures performed in the lab are not listed in the approved protocol.
c. Anesthetics, analgesics, tranquilizers, antibiotics, or other medications used in the lab are not noted in the protocol, are different from those listed in the protocol, or are not used in accordance with the protocol.
d. Procedures listed in the protocol to promote animal welfare (e.g. post-op monitoring procedures) are not being performed, or documented, as approved in the protocol.
e. Survival surgery is not performed aseptically.
f. Euthanasia procedures that differ from those listed in the protocol and/or a method for ensuring death (e.g. after CO2 exposure) are not employed.
g. Lab personnel appear to lack the necessary training to appropriately perform procedures listed in the protocol.
h. Supporting documentation for animal care, post-op care, or other study procedures is incomplete or unavailable.
i. Conditions are not safe for humans and/or animals.
j. Outdated materials (drugs, experimental agents, suture, sterile supplies, etc.) are used.

Note: Animal misuse, mistreatment, neglect or willful disregard for appropriate animal care will be immediately reported to the IACUC Chair, the Attending Veterinarian and the Compliance Office.

Process of Sharing Information Concerning the Review
a. When possible, the Monitor will discuss findings with the Principal Investigator and other lab personnel before leaving the laboratory.
b. Any animal welfare concerns raised may be referred to the IACUC Chair, the Attending Veterinarian, and the Compliance Office for resolution. A draft report will be sent to the IACUC Chair.
c. A final written report will be sent to the Principal Investigator, Lab Manager, and IACUC File. A report of all protocol monitoring will be presented to the IACUC by the Attending Veterinarian on a monthly basis.

Process of Follow-up
a. The Attending Veterinarian and Compliance Office will follow up on any issues raised during a protocol audit that requires protocol modifications, orientation of new personnel, or additional training.
b. If Protocol modification is required, the Compliance Office will follow up to ensure that a Protocol Amendment is submitted in time for the next IACUC meeting and will be available to respond if the PI has questions on the Amendment submission process.

c. On occasion, additional monitoring sessions may be part of the follow-up to assist with proper corrective actions. These will be scheduled as appropriate for the situation.
d. In most cases, issues raised can be addressed by either amending an existing protocol, or reverting to the procedures which are already listed in the approved protocol.

Process for PI Appeal
Investigators who disagree with the Monitor's findings and/or recommendations may appeal to the IACUC. This can be done by sending an e-mail to the Compliance Office. This will be passed on to the IACUC Chair. This may result in a response from the IACUC either upholding the report or recommending modification of the original findings, a request for the PI to appear at a meeting with the IACUC to present their case.

Every three years, the investigator must complete a new Animal Use Form, incorporating any changes that have occurred over the prior period of approval and reflecting the current status of the project, with regard to procedures employed. These forms are also placed on the agenda and reviewed at a convened meeting of the committee.

Be authorized to suspend an activity involving animals according to PHS Policy IV.C.6. The IACUC procedures for suspending an ongoing activity are as follows:

Ongoing procedures can be stopped at the discretion of the attending veterinarian if, in his/her professional opinion, this is necessary to protect the animals from inhumane treatment or from non-approved procedures. Subsequently, the actions of the veterinarian would be reviewed at a convened quorum of the committee and, if appropriate, a suspension vote would be taken. If the IACUC suspends an activity involving animals, the Institutional Official, in consultation with the IACUC, shall review the reasons of the suspension, take appropriate corrective action, and report that action with a full explanation to OLAW, USDA, AAALAC and the funding agency. Investigators are informed of the committee's decision in writing with an explanation of its actions.

Appendix C.

1. Sample IACUC Approval Letter

RESEARCH COMPLIANCE
https://lundquist.org
p: (310) 222-3624 | f: (310) 782-0486
e: complianceac@lundquist.org

IACUC APPROVAL PERIOD:
[approve_date]-[iacuc_expiration]
Note to Staff: IF RC APPROVAL REQUIRED: Enter Date of RC Approval here

ANNUAL REVIEW REQUIRED PRIOR TO:
[iacuc_expiration]

OR

3-YEAR RENEWAL REQUIRED PRIOR TO:
[iacuc_full_renewal]

INITIAL APPROVAL DATE:
[IACUC_INIT_APPR]
Note to Staff: REMOVE IF YEAR -01

Date

PI Name
PI email

SUBJECT: IACUC Project #; (Ref#)
TITLE: " "

Dear Dr.,

Your research project was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center on _____ (note to staff: "the above date" if RC not needed or date of IACUC approval if RC approval is needed) and by the Research Committee on _____. The following conditions will apply to this project:

1. Prior to initiation of the Study, approval of all other institutional committees involved is necessary. If this research is not externally funded and/or scientifically reviewed, review and approval by The Lundquist Institute Research Committee approval is also required.

2. The project will be subject to annual reviews of each previously approved animal research application, including a complete *de novo* review no less than every 3 years.

   The date of the **annual** review is noted above. THE ANNUAL REVIEW FORM IS AVAILABLE IN iRIS AND A COURTESY REMINDER WILL BE SENT TO YOU AT LEAST 3 MONTHS BEFORE THE END OF THE APPROVAL PERIOD NOTED ABOVE. Nevertheless, it is YOUR responsibility to complete this form and submit to the Compliance Office in a timely manner, if you wish to continue the study beyond this approval period.

   The date of the **3-Year Renewal** is noted above. THE 3-YEAR RENEWAL FORM IS AVAILABLE IN iRIS AND A COURTESY REMINDER WILL BE SENT TO YOU AT LEAST 3 MONTHS BEFORE THE END OF THE APPROVAL PERIOD NOTED ABOVE. Nevertheless, it is YOUR responsibility to complete this form and submit to the Compliance Office in a timely manner, if you wish to continue the study beyond this approval period.

3. You agree that any issues related to the humane care of the animals under your supervision will be brought to the attention of the institutional veterinarian and the investigation will be immediately reported to the IACUC and no such change will be initiated without Committee approval.

Note: The institutional veterinarian has oversight responsibility for the animal husbandry program and for carrying out all of the policies and procedures established by the IACUC.

4. The approval of this protocol is limited to the animal model(s) and procedures described in the application. If you require any modifications or additions to this protocol, you must obtain approval from the IACUC before initiating such changes. The approval of your project includes:

ANIMAL SUBJECTS

The total number of animals, by species, approved for this protocol is:

[number_of_animal_approved] (iacuc_species_list)
Pain Category: [iacuc_usda_class]

*This number does not take into account the number of animals you have used.
REMOVE IF NEW STUDY

FUNDING

According to the information provided in your application, the funding source for this research project may include the following:

Funding Type:
Funding Agency: [study_sponsor_no_abbrev]
Pass-Through Institution (if applicable):
Lundquist Institute PI: [pi_name]
Award Project Title:
Funding Agency Award Number: [study_sponsor_award_number]

Sincerely,
[electronic_signature]
Rosa Harmon
IACUC Compliance Manager

Cc: [pc_name],
Catalina Guerra, DVM, Director, C.W. Steers Biological Resources Center;
Office of Grants and Contracts or Theresa Caban, Industry Contracts

RH/ng

THE LUNDQUIST INSTITUTE FOR BIOMEDICAL INNOVATION AT HARBOR-UCLA MEDICAL CENTER
1124 West Carson Street, Martin Bldg., (RB-1) 2nd Floor, Torrance, CA 90502 | lundquist.org

Appendix D

1. BRC Conference Room Training Videos

BIOMETHODOLOGY

- Humane Handling and Lab Techniques for the Cat
- Humane Handling and Lab Techniques for the Rat
- Humane Handling and Lab Techniques for the Guinea Pig
- Humane Handling and Lab Techniques for the Dog
- Humane Handling and Lab Techniques for the Rabbit
- Humane Handling and Lab Techniques for the Mouse
- Humane Handling and Lab Techniques for the Hamster
- Charles River Laboratory — Surgical Procedures on Swine

These tapes have accompanying handouts and manuals

Appendix E

1. Animal Purchase Requisition Deadlines

THE LUNDQUIST INSTITUTE

Animal Order Deadlines

Below are the deadlines the animal vendors have given us to be able to place orders. Please turn in your animal purchase requisitions before these deadlines.

Remember, the BRC Office also needs time to check your animal project for number of animals you have left and expiration dates, we need to check your billing number also for available funds and expiration dates before we can then assign your order a PO# through the purchasing system.

Thank you for your cooperation.

Specie	Delivery Day(s)	Vendor Deadline
Rabbits	Normally Mon or Tues	One week before
Rodents	Tuesdays Thursdays	Thursday before 12 noon Thursday before 12 noon
Rodents from NCI	Only Tuesdays	A week prior on Tuesdays before 12 noon
Rodents from NIA	Only Tuesdays	A week prior on Mondays before 12 noon
Sheep	Mondays	3 weeks-notice to allow for Q-Fever test

For all other species, please contact
the C. W. Steers Biological Resources Center at 310-222-1262
Delivery dates depend on availability.
For special orders, please turn in requests as early as possible.

Remember...
Only the C. W. Steers Biological Resources Center Office can place animal orders.
You are welcome to call vendors for availability or information before turning in orders.

Appendix F

1. Per Diem Rates

C.W. Steers Biological Resources Center

Animal Per Diem Rates

Species		Rates Per Day
Cat		$24.90
Chicken		$6.20
Cow		$25.42
Dog		$24.90
Goat		$25.42
Guinea Pig		$2.42
Hamster		$3.42
Mouse	Regular	$1.69
Immuno-Deficient Mouse	Immuno-Deficient (nude, clean rm)	$3.39
Rabbit		$6.20
Rat	Regular	$2.83
Rat	Large rodent cage (up to 4 animals)	$2.83
Rat	Large rodent cage (5 to max 7 animals)	$5.66
Immuno-Deficient Rat	Immuno-Deficient (nude, clean rm)	$5.66
Sheep (Indoors)	Cage (inside housing, pregnant)	$32.27
Sheep (Outdoors)	Pen	$25.42
Swine (Indoors)	inside, metabolic cages, special housing	$24.90
Swine (Outdoors)	pen (group, regular housing)	$20.22

Note: These rates are current as of 7/1/2019. Rates have not increased since 7/1/2013.

Appendix G

1. Special Services Charges (Not sure which you wanted to use here)

CWS Biological Resources Center
Building E-2 North/Box 486
Tel: 310.222.1282 Fax: 310.222.1282

Special Services Billing Form

Date _____

Investigator _____

Project _____

Description of services provided:

Supplies and Drugs Used	**Qty**	**Price**
Subtotal Supplies		$ -
Overhead on supplies (29.5%)		$ -
Subtotal supplies plus overhead		$ -

C.W. Steers BRC Personnel Involved		**Time(hrs)**	**Fee Charged**
DVM ($90/hr)			$ -
DVM overtime ($135/hr)			$ -
RVT ($60/hr)			$ -
RVT overtime ($90/hr)			$ -
LAT ($30/hr)			$ -
LAT overtime ($45/hr)			$ -
Subtotal Personnel			$ -

Medical Equipment Usage			
Medical Equipment			
Medical Equipment			
Subtotal Equipment			$ -

Subtotal Personnel and Equipment	$ -

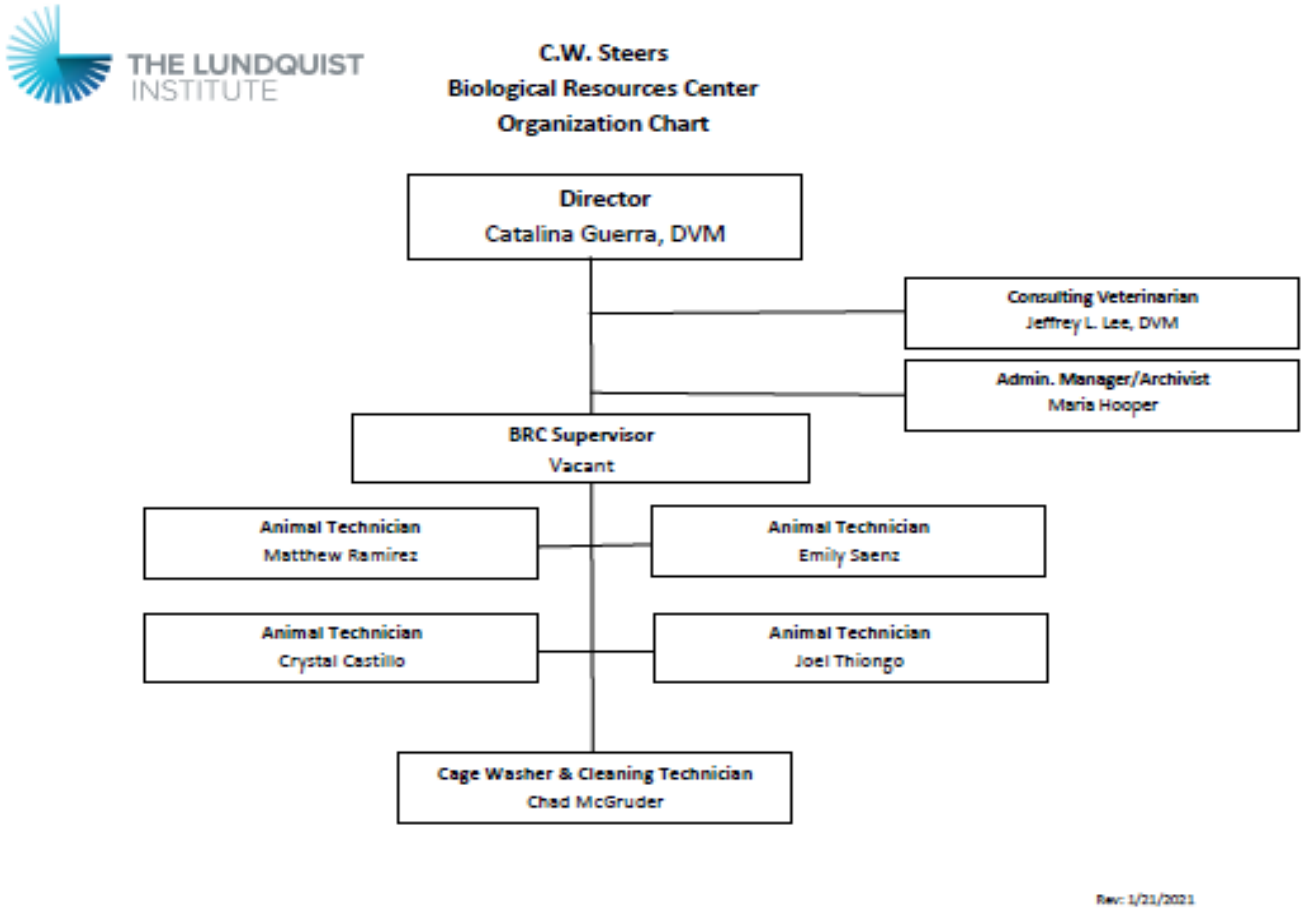
TOTAL AMOUNT TO BE RECHARGED	$ -

Signature _____ Date _____

Rev. 1/2021

Appendix H

1. C. W. Steers Biological Resources Center



Appendix I

1. Space Recommendations for Animal Caging

Minimum Space Requirements for Laboratory Animals						
Animal	**Weight**	**Housing**	**Floor Area/Animal**		**Height**	
	grams		sq. in.	sq. cm.	in.	cm.
Mice	<10	Cage	6.0	38.71	5	12.70
	10-15		8.0	51.62		
	15-25		12.0	77.42		
	>25		>15.0	96.78		
	grams		sq. in.	sq. cm.	in.	cm.
Rats	<100	Cage	17.0	109.96	7	17.78
	100-200		23.0	148.40		
	200-300		29.0	187.11		
	300-400		40.0	258.08		
	400-500		60.0	387.12		
	>500		>70.0	451.64		
	grams		sq. in.	sq. cm.	in.	cm.
Hamsters	<60	Cage	10.0	64.52	6	15.24
	60-80		13.0	83.88		
	80-100		16.0	103.23		
	>100		>19.0	122.59		
	grams		sq. in.	sq. cm.	in.	cm.
Guinea Pigs	<=350	Cage	60.0	387.12	7	17.78
	>350		>101.0	651.65		
	kilograms		sq. ft.	sq. m.	in.	cm.
Rabbits	<2	Cage	1.5	0.14	14	35.56
	2-4		3.0	0.28		
	4-5.4		4.0	0.37		

	>5.4		>5.0	0.46		
Cats	kilograms		sq. ft.	sq. m.	in.	cm.
	<=4	Cage	3.0	0.28	24	60.96
	>4		>4.0	0.37		
Dogs	kilograms		sq. ft.	sq. m.	in.	cm.
	<15	Pen/Run	8.0	0.74	-	-
	15-30		12.1	1.12		
	>30		>24.0	2.23		
	<15	Cage	8.0	0.74	32	81.28
	15-30		12.1	1.12	36	91.44
Non-human Primates	kilograms		sq. ft.	sq. m.	in.	cm.
Group 1	<1	Cage	1.6	0.15	20	50.80
Group 2	1-3		3.0	0.28	30	78.20
Group 3	3-10		4.3	0.40	30	78.20
Group 4	10-15		6.0	0.56	32	81.28
Group 5	15-25		8.0	0.74	36	91.44
Group 6	25-30		10.0	0.93	46	116.84
Group 7	>30		15.0	1.40	46	116.84
Pigeons			sq. ft.	sq. m.		
	-	Cage	0.8	0.074	see note below *	
Quail			sq. ft.	sq. m.		
	-	Cage	0.25	0.023	see note below *	
Chickens	kilograms		sq. ft.	sq. m.		
	<0.25	Cage	0.25	0.023	*Sufficient headroom for birds to stand erect.	
	0.25-0.5		0.50	0.046		
	0.5-1.5		1.00	0.093		
	1.5-3		2.00	0.186		
	>3		>3.00	0.285		

Appendix J

1. Housing Temperatures for Laboratory Animals

Animal	Temperature (°F)
Mouse	68—79
Rat	68—79
Hamster	68—79
Guinea Pig	68—79
Rabbit	61—72
Ferret	40—65
Dog	64—84
Mini Pig	61—81
Farm Pig	61—81
Sheep	61—81
Chicken	61—81

Ventilation: Minimum 10 room air changes per hour

Appendix K

1. Automatic Watering System

The following areas in the Biological Resources Center are supplied with automatic watering systems:

RB-2 Annex Rooms 2, 4 and 6	Dog Areas
RB-2 Annex Rooms 14 and 16	Rabbit Rooms
RB-2 Annex Rooms 17 through 20	Rodent Rooms

Animals housed in these areas will be put on the automatic watering system unless specially requested by the investigator through the Special Husbandry Request Form, which can be obtained in the BRC office.

When returning cages to racks, which are supplied with automatic watering, special care must be taken in order to ensure that the cages are placed in the rack in the slots without pushing on the sipper tube or obstructing the sipper tube. This will prevent the accidental flooding of the cage or the inability of the animal to reach its water.

Also, care should be taken when moving the racks themselves, as the manifolds for the watering system can be damaged if they hit walls or doors.

Appendix L

1. Disposal of Animal Carcasses

Policy

All uncontaminated animal carcasses are to be brought by investigator's staff to the PRC cooler located in Building PRC, Room 121, or F-0 and/or the cooler located in RB-2 room 134. The cooler is to be used for the storage of dead animals (non-radioactive only).

Procedure

All animals must be wrapped in a black plastic bag before being placed into the cooler. The stainless steel rack in the cooler is used only for animals that are to be saved for postmortem procedures. These animals must be tagged with the investigator's name and the date. When the animal carcasses are no longer needed, please place them in the disposal barrels. Carcasses on the shelf for more than one week will be discarded.

Contaminated Carcasses

After euthanizing sheep, please put their carcasses into strong plastic bags and seal them. The reasons for this request are twofold. It is often not possible to tie off the bag once the carcass is in the bin, and it is important to contain sheep and sheep parts, and thus minimizing human exposure to *Coxiella Burnetii*.

Carcasses contaminated with radioactive materials and infectious agents should be disposed of in accordance with safety policies.

Please contact the Safety Office, 310-222-2456, to make special arrangements for these carcasses.

Appendix M

1. Medical Records

Individual records are maintained for larger animal species, i.e., rabbits, dogs, sheep, and pigs. The investigator is required to maintain these records.

An Operative Record Form is use to record medication and anesthetics given to the animal during surgery. Also note post-operative recovery on operative record. This form is to be kept in the animal's records.

Once consciousness is regained, return, animals to their cage. Monitor all animals as deemed necessary to assure uneventful recovery from surgery. Duration of post-surgical observation period will vary with type of surgery performed and condition of the animals; however, all animals should be closely monitored for a minimum of 24 hours after surgery.

Post-Surgical Treatment must be instituted whenever required. Use the Treatment Form for record keeping.

Post-surgical observation and treatment are the investigator's responsibility and must be performed in accordance with recommendations made by BRC Veterinary Staff.

Appendix N

1. Training Manuals Available at C.W. Steers Biological Resources Center

Amphibians
Cat
Cattle
Dog
Ferret
Guinea Pig
Hamster
Mouse
Pig
Rabbit
Rat
Sheep

Appendix O

1. Standard Operating Procedure – Surgical Preps

I. Preparation of Surgical Materials

The sterile preparation of instruments, gowns, towels, and drapes is essential for all Major Survival Surgeries.

That is, the contents of the surgical pack and the manner in which it is assembled is important for asepsis and efficiency of operation. Sterilization indicators or sterility monitors must be included in every pack.

Instruments must be clean before they are sterilized; items such as drapes, lab sponges, sponges, towels, and gowns must be laundered, dried, and properly folded; packs must be properly wrapped. Monitors must be included in every pack.

Use steam under pressure (i.e., autoclave) for the sterilization of gowns, towels, laparotomy sheets, gauze sponges, standard instruments, and drapes.

A pressure of 15 pounds at 240 degrees Fahrenheit for 15 minutes is sufficient to kill both spore forming and non—spore forming bacteria, but penetration of steam into larger surgical packs will be slower so that at least 30 minutes should be allowed for sterilization of all packs.

Use gas sterilization (i.e., ethylene oxide) or dry heat for the preparation of special instruments and other delicate articles, such as implants and catheters.

*NOTE: Disposable caps •and masks, and prepackaged Betadine soaked scrub brushes, sterile suture materials, and sterile surgeon gloves are preferred over other items which must be sterilized.

II. Preparation of Animal

Prep should take place outside the operating room. Clip an area that is much larger than the surgical site. Wet a 2 inch stack of surgical sponges and pour Betadine or Nolvasan scrub over them.

Take the sponge from the top of the stack by its corners, and starting at the center of the clipped area, scrub the skin using circular motions and work toward the periphery of the clipped area. Once the clipped area is reached, discard the sponge and repeat the process with a clean Betadine soaked sponge. Repeat this process for 5 minutes. Follow with an alcohol spray to rinse the area.

III. Preparation of Surgeons

After the animal is positioned on the O.R. table, spray Betadine solution onto' the area as a final prep; do not rinse or wipe off. Cover the animal with sterile drapes exposing only the surgical field. Place the drapes around the surgical site using aseptic technique.

Before prep, change into surgical scrubs, remove rings and watches, and put on a cap and mask.

With arms held in the upright position, perform a 5 minutes scrub using Betadine scrub and Chlorhexidine and tap water.

The scrub must contact the surgeon's hands and arms from the fingertips to the elbows for 5 minutes. There should be a good lather, and all areas should be well, scrubbed.

With arms still in the upright position, the surgeon rinses the hands and forearms individually allowing water to run from finger- tips to elbow to sink. Arms remain in the upright position, and hands and arms are thoroughly dried with a sterile towel using aseptic technique; also, gowning and gloving must be performed in an aseptic manner.

Detailed information on preparation of instruments, animals, and personnel is available in the BRC.

THE LUNDQUIST INSTITUTE

DEA Controlled Drug Inventory Form- Class II-V

Drug_____ Concentration (mg/mL) _____

Size(mLs)_____ Balance Amount (mLs)_____

Date	Amount Plus/Minus	Amount Remaining	Investigator	Animal(s)	Comments/ Lot No	Initials

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SURGICAL RECORD

DATE	PROJECT # / PROTOCOL #	SPECIES	SEX	I.D. NUMBER	LOCATION

INVESTIGATOR	SURGICAL PROCEDURE	☐ SURVIVAL ☐ NON-SURVIVAL

PHYSICAL EXAM: Fasted: yes / no Blood drawn: yes / no **PERSONNEL:**

Weight: _____kg. Scale Number: _____ Anesthetist _____

Examination: ☐ NSF Temperature: _____°F Surgeon _____

Comments: _____ Asst. Surgeon _____

Anesthesia: Start:_____ / Heartrate:_____bpm / SaO2____ / Cuffed Endotracheal Tube Size: _____

Anesthetic Agent	Dose	Route / Time

Fluids:	Amount:

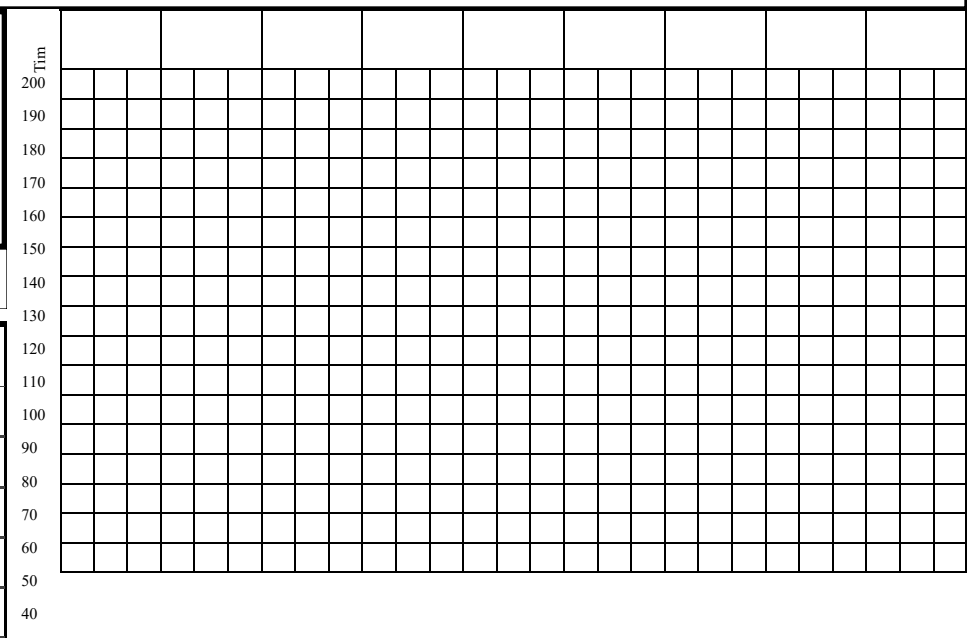
Inhalant Anesthetic Agent:	Flow Rate:
Isoflurane	

SURGERY: Start:_____ Finish:_____ Extubation Time:_____

MONITORS:
____ Gas Analyzer
____ Blood Pressure
____ ECG
____ Pulse Oximeter
____ Invivo Datascope
____ Other: _____

Anesthesia Machine / Vaporizer / and Respirator Number:_____

Remarks/Other Agents
(Agent / Dose) (Route/Time)



X=heartrate, O=SaO2 , Respirator Set at_____bpm

Comments:

Anesthetist:	Date:
Recorder : ☐ check if same as above	Date:

___See Attatched Supplement , Page___of___

POST-OPERATIVE RECOVERY FORM

THE LUNDQUIST INSTITUTE

ANIMAL #:_______ PROJECT #:____________ DATE of SURGERY:_________

POST-OPERATIVE PROCEDURES:

Time: _____ A.M.☐ P.M.☐ **Temp: ________ oF** **Pulse: _______ /min.** **Resp: ______ / min.**
Initials: _______
MM: Color: Pink ☐ Pale ☐ White ☐ Cyanotic ☐ Other ☐ __________
Hydration: Moist ☐ Tacky ☐ Dry ☐
CRT: < 1 sec. ☐ < 2 sec. ☐ > 2 sec. ☐ Other ☐ ________________
Incision Site: WNL ☐ Abnormal ☐ _________________________
Check Heating Pad: ☐ **Comments:** _______________________________

Time: _____ A.M.☐ P.M.☐ **Temp: ________ oF** **Pulse: _______ /min.** **Resp: ______ / min.**
Initials: _______
MM: Color: Pink ☐ Pale ☐ White ☐ Cyanotic ☐ Other ☐ __________
Hydration: Moist ☐ Tacky ☐ Dry ☐
CRT: < 1 sec. ☐ < 2 sec. ☐ > 2 sec. ☐ Other ☐ ________________
Incision Site: WNL ☐ Abnormal ☐ _________________________
Check Heating Pad: ☐ **Comments:** _______________________________

Time: _____ A.M.☐ P.M.☐ **Temp: ________ oF** **Pulse: _______ /min.** **Resp: ______ / min.**
Initials: _______
MM: Color: Pink ☐ Pale ☐ White ☐ Cyanotic ☐ Other ☐ __________
Hydration: Moist ☐ Tacky ☐ Dry ☐
CRT: < 1 sec. ☐ < 2 sec. ☐ > 2 sec. ☐ Other ☐ ________________
Incision Site: WNL ☐ Abnormal ☐ _________________________
Check Heating Pad: ☐ **Comments:** _______________________________

Time: _____ A.M.☐ P.M. ☐ **Temp: ________ oF** **Pulse: _______ /min.** **Resp: ______ / min.**
Initials: _______
MM: Color: Pink ☐ Pale ☐ White ☐ Cyanotic ☐ Other ☐ __________
Hydration: Moist ☐ Tacky ☐ Dry ☐
CRT: < 1 sec. ☐ < 2 sec. ☐ > 2 sec. ☐ Other ☐ ________________
Incision Site: WNL ☐ Abnormal ☐ _________________________
Check Heating Pad: ☐ **Comments:** _______________________________

Time: _____ A.M.☐ P.M. ☐ **Temp: ________ oF** **Pulse: _______ /min.** **Resp: ______ / min.**
Initials: _______
MM: Color: Pink ☐ Pale ☐ White ☐ Cyanotic ☐ Other ☐ __________
Hydration: Moist ☐ Tacky ☐ Dry ☐
CRT: < 1 sec. ☐ < 2 sec. ☐ > 2 sec. ☐ Other ☐ ________________
Incision Site: WNL ☐ Abnormal ☐ _________________________
Check Heating Pad: ☐ **Comments:** _______________________________

IV Fluids: Type: Norm-R ☐ LRS ☐ 0.9% NaCl ☐ Other ☐ ____________________________
Amount given: From end of surgery until IV catheter is removed: ________________________ **ml.**

Addt'l Drugs: Drug: ______________________________ **Dosage:** ______________**mg.** **Route:** ____________
Drug: ______________________________ **Dosage:** ______________**mg.** **Route:** ____________

Comments: ____
____

Signature: ____ ***Date:*** ______________

Post Operative Treatment Form

Investigator:	**Project # :**		
Species:	**Animal # :**		
Date of Surgery:	**Suture Removal**	**Date:**	**Init:**

ANALGESICS

Day	Time	Drug	Dosage	Route	Initial	Date

☐ It was determined that no analgesics were needed

TREATMENT

Day	Time	Drug	Dosage	Route	Initial	Date

POST OPERATIVE CARE

Day	Temp °F	Comments	Initial	Date

Post-Procedure Monitoring Form for Rodents

IACUC Protocol Number	
Principal Investigator	
Responsible Research Staff Name	
Lab Phone	
After House Contact Number	
LABioMed Email	

Cage ID	
Procedure Date	
Specie	☐**Mouse** ☐**Rat**
Animal ID	
Procedure	

Date	Time	Animal ID	Body Condition: B.Wt. (g)	Hydration: Urine/Feces	Incision, Lesion, Tumor Appearance	Pain Assessment	Rx Treatment	Dose (mg/kg)	Volume	Route (IV, IP, IM, SQ)	Initial

Appearance: N=Normal, A=Abnormal Pain Assessment: 1 (Normal)- 5 (Moribund, Very Painful)

This form is an example, and PI's are able to use either this form or their own, to monitor their animals post-procedure, as long as the documentation of monitoring is available in the animal housing room for the BRC staff. This form can be used to monitor more than one animal. After all the rodents listed on this sheet are euthanized or have died, this sheet should be kept on file in the lab or given to BRC. Frequency of monitoring must be done according to the IACUC policy dealing with the experimental condition under study.

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Rabbit Surgery Record

Date: _____ **Protocol:** _____

Investigator: _____ **Procedure:** _____

Animal #	Wt. In Kg.	Anagelsic agent, dose and route (time given) _____ _____ _____	Ketamine in mg (IM)	Xylazine in mg (IM)	Time Given	Animal Recover OK (time)	Initials/ Date	Post Pocedural monitor (time)	Initials/ Date	Post Pocedural monitor (time)	Initials/ Date	Additional Anagelsic needed (Y or N)	Anagelsic agent, dose and route (time given)

Rodent Surgery Record

Date: _____ **Investigator:** _____ **Surgeon:** _____

Protocol: _____ **Procedure:** _____ **Species:** _____

Animal ID or Cage #	Wt. In grams	Anesthetic Agents (dose and route)	Time Given	Anagelsic agent (dose and route)	Time Given	Animal Recover OK (time)	Date and Initial	Post Pocedural monitor (time/date)	Additional Anagelsic needed (Y or N)	Anagelsic agent (dose and route)	Date and Initial