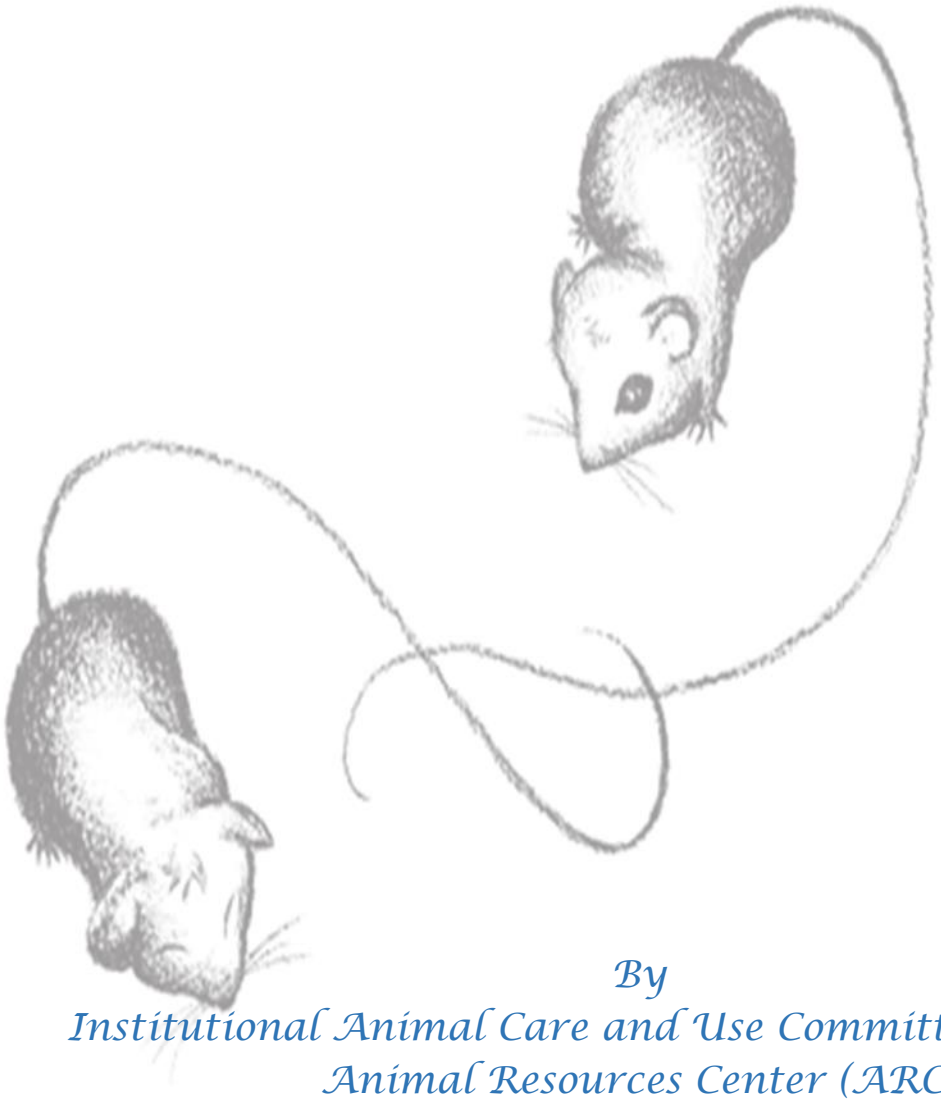
Handbook for the Use of Laboratory Animal



By
Institutional Animal Care and Use Committee (IACUC) &
Animal Resources Center (ARC)

Revised: July 2021

Contents

I- INTRODUCTION

A- Purpose

The purpose of this handbook is to familiarize the reader with:

1. the organization and function of the Animal Resources Center (ARC),
2. the policies governing the use of laboratory animals in research and teaching at the Universidad Central del Caribe (UCC), and
3. the services available at these facilities.

B- Description and Floor Plan

The UCC Animal Resources Center is located in the basement of the Basic Sciences Building and occupies 7,700 sq. ft. It provides housing for both aquatic animals and small rodents. In addition, that ARC have rooms equipped with specialized areas for the following services: as stock and treatment, necropsy, quarantine, clean cages, bedding diet, cage washing, food and storage. The facilities also include an area for sterilization. There is also a space designated for an administrative office and storage of dead animals.

II- ORGANIZATION- INSTITUTIONAL PROGRAM FOR ANIMAL CARE AND USE

A. The lines of authority and responsibility for administering the program and ensuring compliance with the Public Health Service (PHS) Policy are as follows:

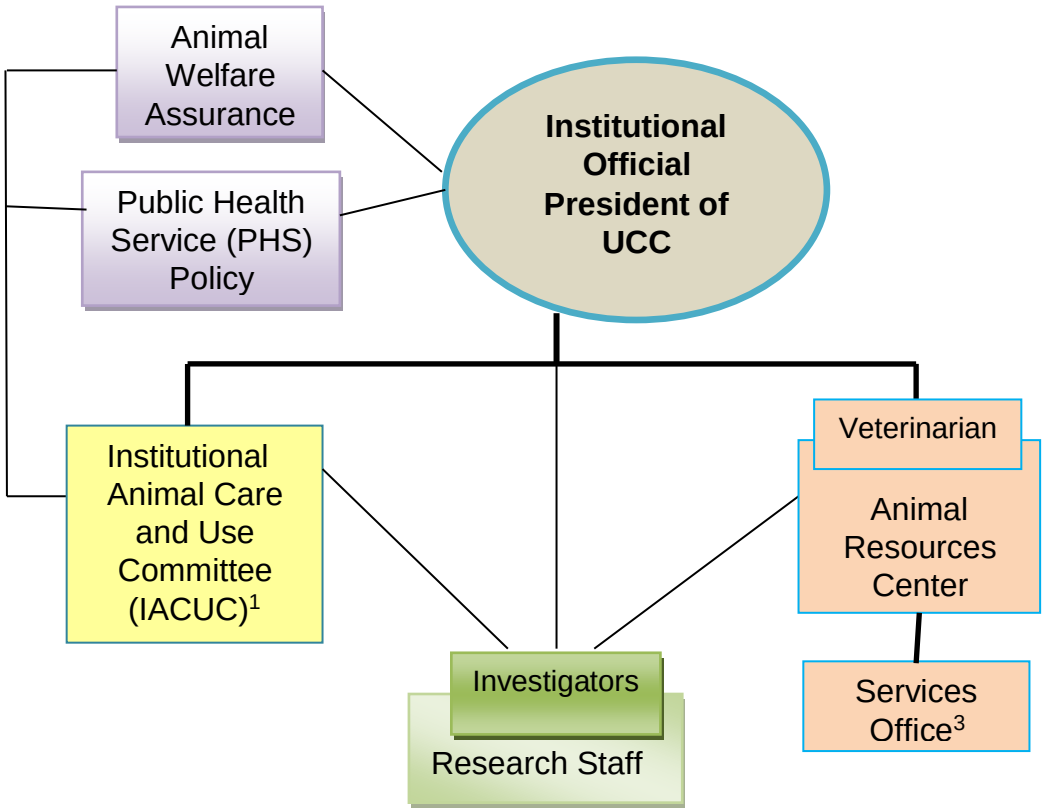


Figure 1: Organizational Scheme of Institutional Program for the Institutional Program for Animal Care and Use

As indicated above, there are direct and open lines of communication between the IACUC and the Institutional Official (IO) and between the Veterinarian and the IO.

[1]The Institutional Animal Care and Use Committee (IACUC) is the Universidad Central del Caribe review body for matters relating to the care, use and treatment of animals in these areas.

[2]The Office of Animal Resources Center (ARC) is responsible for oversight of all animal care and use and ensuring compliance with federal, state and local regulations. The Animal Resources Center (ARC) is under the administration of the President of the University. The unit's personnel consist of a supervisor, a part-time consulting veterinarian, and one full-time and one part-time animal caretaker. An Institutional Animal Care and Use Committee (IACUC) has been established to advise the President on animal usage and care matters. The IACUC is composed of a representative from the basic science departments, a representative of the medical school research committee, a consulting veterinarian, and a representative of the community. The Institutional Committee is responsible for reviewing all research and teaching protocols that use live animals for compliance with NIH policies and the appropriate federal and commonwealth laws. Users of the ARC may express their needs, recommendations or complaints to the Animal House Supervisor on routine affairs or the IACUC on more formal matters.

[3]Services offices include the research and administration offices of personnel that offer support to ARC.

B- Functions of ARC

The primary functions of the Animal Resources Center are to care for the animals and provide information concerning the purchase, basic husbandry, quarantine, veterinary medical care of laboratory animals used in research and teaching programs of the Universidad Central del Caribe, as well technical assistance, advice, and consultation dealing with animals utilized for research programs by making readily available animal materials and products and animal husbandry supplies.

III- POLICIES, REGULATIONS, AND STANDARDS RELATING TO THE CARE AND USE OF LABORATORY ANIMALS

A. National Regulations

1. Animal Welfare Act

The Animal Welfare Act (AWA), Public Law 89-544, and its amendments regulate the transportation, purchase, sale, housing, care, handling, and treatment of animals used for research, testing or teaching, for exhibitions, and sold as pets. The AWA specifically includes dogs, cats, nonhuman primates, guinea pigs, hamsters, rabbits, and any other warm-blooded animals that are

being used or are intended to be used for research, testing, teaching, experimentation, exhibition purposes, or as pets. Specifically exempted from the AWA are birds, rats of the genus *Rattus*, mice of the genus Mus, horses and other farm animals used for food or livestock and poultry used to improve animal nutrition, breeding, management, or production. 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 IACUC, adequate veterinary care and responsibilities of the attending veterinarian, training of all personnel using laboratory animals on humane methods of animal maintenance and experimentation, and record keeping.

The Institutional Animal Care and Use Committee must be composed of at least three members to include a veterinarian with special training in laboratory animal medicine/science, a person not affiliated with the institution other than by his/her committee membership, and a chairman. This committee is responsible for the review all protocols using animals to ensure that they meet the criteria listed in the AWA. In addition, the committee must conduct semiannual inspections of all animal study areas and animal facilities to ensure that the use of animals does not deviate from the approved protocol and the institution's program description. The importance of this requirement is underscored by the fact that the Chief Executive Officer of the institution must certify that the attending veterinarian and the Institutional Animal Care and Use Committee have the authority to enter any animal area at any reasonable time.

The AWA is administered by the United States Department of Agriculture (USDA), specifically the Regulatory Enforcement and Animal Care (REAC) component of the Animal and Plant Health Inspection Service (APHIS). Research facilities are subject to unannounced inspections by USDA veterinarians and are required to furnish annual reports that include, in addition to other information and assurances, the common names and numbers of animals being used. Procedures must be categorized by procedures, e.g., (a) no pain, distress or use of pain-relieving drugs; (b) pain or distress for which appropriate anesthetic, analgesic, or tranquilizing drugs were used appropriately during research and testing, and (c) that the principal investigator has considered alternatives to painful procedures.

Noncompliance with the USDA standards for the humane care, use, and transportation of animals may lead to substantial fines and/or suspension of animal research activities.

2. Public Health Service Policy on Humane Care and Use of Laboratory Animal (NIH Policy)

Reference: https://grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf

The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals incorporates the changes in the Public Health Service Act (PHS Act) mandated by the Health Research Extension Act of 1985, Public Law 99-158. The PHS Policy requires that each institution receiving PHS funds for activities involving animals submit detailed information regarding the institution's program for the care and use of ALL live vertebrate animals to the Office of Laboratory Animal Welfare (OLAW). This information is in the form of an Animal Welfare Assurance, and it must be updated annually and completely revised every five years. Significant changes in existing Assurance status or significant problems encountered in implementing this policy must be reported immediately to the OLAW.

Institutions are required to identify an institutional official who is ultimately responsible for the institution's program for the care and use of animals and a veterinarian qualified in laboratory animal medicine who will participate in the program. Each institution also is required to designate clear lines of authority and responsibility for those involved in animal care and use for PHS-supported activities.

The Policy clearly defines the role and responsibilities of IACUC in all aspects of PHS-supported research. The committee must be composed of at least five members to include an individual not affiliated with the institution, a veterinarian who has program responsibilities and training or experience in laboratory animal science and medicine, a practicing scientist experienced in research involving animals, and a member whose concerns are in a nonscientific area.

The Policy requires that the Institutional Animal Care and Use Committee review and approve those sections of PHS grant applications related to the care and use of animals before funds can be awarded. Institutions are required to conduct semiannual self-assessments of the program. Both major and minor deficiencies in the institution's program must be identified, and it must adhere to an approved plan and schedule for correcting significant deficiencies.

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

3. *Guide for the Care and Use of Laboratory Animals*

In 1963, NIH and the National Academy of Sciences Institute for Laboratory Resources (ILAR) published the first edition of the Guide for the Care and Use of Laboratory Animals (the Guide). The current Guide was revised in 2011. The Guide's purpose is to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. It is a long-

standing National Institutes of Health (NIH) policy that grantees and contractors using live vertebrate animals in projects or activities supported by NIH should be guided by the recommendations in this publication.
Reference: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf

4. *Policies of Various Granting Agencies*

Most granting agencies have established policies for the care and use of laboratory animals. Investigators should understand fully the requirements of each agency from which they seek funds. The Office of Grants Management or the office of the IACUC may be contacted for specific information.

5. *American Veterinary Medical Association Guidelines on Euthanasia*

Methods of euthanasia recommended by the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (revised 2020) are accepted by both the PHS Policy and the Animal Welfare Act as standard methods of euthanasia.
Reference: https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

6. *Use of Controlled Substances*

Potentially addictive or habituating drugs for human or animal use are classified under federal and state law. Examples of controlled substances include barbiturates and narcotics. The Department of Justice, Drug Enforcement Administration (DEA), enforces this law and requires appropriate security and record management of these substances. If you need more information, please refer to Pharmacology Department.

B- Local Regulations

1. Department of Environmental and Natural Resources

This governmental department of the Commonwealth of Puerto Rico oversees implementation and formulation of public environmental policy and for the protection and conservation of the natural, environmental, and energy resources of Puerto Rico.
Reference:
http://drna.pr.gov/historico/biblioteca/reglamentos_folder/6765.pdf/view

C. Institutional

1. Committee

The PHS Policy and the Animal Welfare Act require establishing a committee, referred to by the generic name of Institutional Animal Care and Use Committee (IACUC), whose function is to ensure that the care and use of animals are appropriately humane. The IACUC carry out the responsibilities of the IACUC at Universidad Central del Caribe.

Committee membership includes a Doctor of Veterinary Medicine with experience in laboratory animal science and medicine, an individual whose primary concerns are in a nonscientific area and who is not otherwise affiliated with the Institution (the "outside member"), and practicing scientists experienced in research involving animals. Members are appointed by the President of the Universidad Central del Caribe.

The committee meets at least twice a year (March and September), and it is responsible for monitoring the institution's animal care and use program, performing the semiannual inspection (March and September) of the institution's animal use areas, and ensuring that there are no deviations from approved animal use protocols that adversely affect animal welfare. This committee is authorized to suspend an activity involving animals if it determines that the activity didn't follow applicable provisions of the Animal Welfare Act, the Guide, and the PHS Policy.

This committee also reviews and approves, requires modifications in, or withholds approval of all protocols related to the care and use of animals at the Universidad Central del Caribe.

2. Training Programs

All personnel are required to acquire training before working with animals. A variety of training programs are available to personnel who care for or use laboratory animals. Lectures based on the information in this handbook are offered as needed for all personnel who use animals in research. In addition, specie specific "hands-on" training is also available for personnel.

The ARC Animal Care Technicians participate in continuing education sessions as well as on-the-job training programs. Research technicians whose jobs involve animal care should participate in an in-house continuing education program.

3. Reporting Deficiencies in Animal Care and Treatment

Any complaints or concerns by a Universidad Central del Caribe employee regarding the care and use of laboratory animals at this institution should be made to the Supervisor of the Animal Resources Center either verbally or in writing. If the complaint is directed against the ARC, the report should be made to the chairman of the IACUC.

Confidentiality will be maintained. The ARC Supervisor (or IACUC Chairman) will keep the individuals expressing concerns informed of the actions to be taken. The IACUC or the ARC Supervisor will conduct an initial review of the concerns. After notification of the issue and discussion with the Chairman of the IACUC, the problem may be taken before the IACUC for full review. The person who is the subject of the complaint will be notified in writing of the concerns expressed and allowed to respond. The IACUC will maintain a file documenting the complaint, the review, and the actions taken to rectify any problem(s) identified.

IV- OPERATING RULES AND POLICIES

A- Use of the Facility

In general, most laboratory space and animal rooms within the ARC are for common use. Exclusive use of space within the animal facilities must have prior approval from the ARC Supervisor and the IACUC. Use of the facilities by non-institutional researchers must be endorsed by the IACUC before consideration by the President of UCC.

Utilization of animals or facilities in any formal research and teaching protocol requires prior approval and authorization from the Institutional Animal Care and Use Committee (IACUC).

B- Hours of Operation

Personnel Work Schedules:

- Animal Caretakers
  7:30 am - 12:00 - 1:00 – 4:00 pm Monday thru Friday
  Morning- Sundays

- Supervisor
  8:00 am – 4:30 p.m. Monday – Friday

Lic. Betzaida Torres (Supervisor)	787-798-3001 (Office) ext. 2096
	787-638-1701 (Cellular)
Prof. Zilka Rios (Pres. IACUC)	787-798-3001 (Office) ext. 2082
	787-502-3871 (Cellular)

C- Emergencies

Any abnormal situation observed or related at the animal facility should be notified immediately to the security officer or the ARC Supervisor.

D- Security

Specific security measures have been implemented to protect faculty, staff, equipment, and the animals used in biomedical research at the Universidad Central del Caribe. Cooperation in enforcing these measures is essential.

During regular working hours (8:00 a.m. – 4:30 p.m.) access to the ARC will be through the doorway marked "ENTRANCE" in the basement of the Basic Sciences Building. All other doors will be always locked.

In order to provide access to the ARC to users during non-working hours, one key to the main entrance and the animal area will be given to each academic department. The security of this key will be the responsibility of the department chairman. Entrances into the animal housing facilities are always secured by an electronic code system. All persons who require the use of the Security Access Code (See Appendix #1) must be listed on approved and active protocols. Research personnel is responsible for relocking the Center. Loaning of keys requires ARC permission.

For access during non-working hours, weekends or holidays must be authorized by the Department of Dean of Administration through Form-S1. All student visitors (personal external to the UCC) must be previously approved by the Dean of Student and Dean of Administration (see Appendix #2 and #5).

Any person or activity in the animal facilities that appear inappropriate and/or suspicious should be reported immediately to the Animal Resources Center and/or a UCC security officer.

Any user who enters the Center during non-working hours must sign the register that will be located near the main entrance of UCC.

All personnel must follow the rules established by the ARC and must have an identification card. Also, the department's Chairman supervising the course is responsible for providing a list of the students that will need access to a particular area at least one week before the beginning of the course.

ARC Rules:

1-Security Cameras- The ARC has identified the installation of security cameras. These are running 24 hours, 7 days a week

2-Visitors

To protect research animals and minimize any possibility of disease transmission, visitors, curiosity seekers, including family members and especially children, are not allowed in Universidad Central del Caribe animal facilities without prior approval by the ARC Supervisor. Tours of Universidad Central del Caribe animal facilities are conducted by ARC personnel for interested groups.

3- Photographs or Videotapes of Animals

The use of animals in biomedical research is a very sensitive and emotional issue. Therefore, faculty members are urged to carefully consider all possible interpretations of pictures of research animals taken for documentation or publication. The ARC office is available to advise faculty in developing photographic materials and to help review materials for subject matter that the general public might misinterpret. Under no circumstances should photographic equipment be taken into the Universidad Central del Caribe animal facilities without the specific prior approval of the ARC Supervisor or/and Chairman of IACUC.

4-Inquiries Regarding Animal Use

Investigators and technicians should not attempt to answer questions from individuals outside the Universidad Central del Caribe regarding animal care and use at this institution. All questions should be referred to the ARC Supervisor or/and the Chairman of the IACUC. The Office of the Associate Dean for Research will handle all inquiries from members of the media and clear all

interviews in advance with Universidad Central del Caribe faculty and staff. The ARC Supervisor should be informed of all such requests for information and, when possible, provided with the name, address, telephone number, and affiliation of the individual(s) inquiring.

5-Break-ins

Anyone discovering a break-in of animal housing or use areas should inform the ARC Supervisor immediately. The area should not be cleaned or otherwise disturbed until permission is received from individuals responsible for the investigation.

6-Radios, cellular, phones, mp3 players, computers

Many species can hear sound frequencies inaudible to humans; hence equipment and materials that produce noise within the hearing range of nearby animals can have potential effects. For this reason, radios, cellular phones, mp3 players (including with the headphone), and any other generator of sound/frequencies should not be used unless they are part of an approved protocol or an enrichment program.

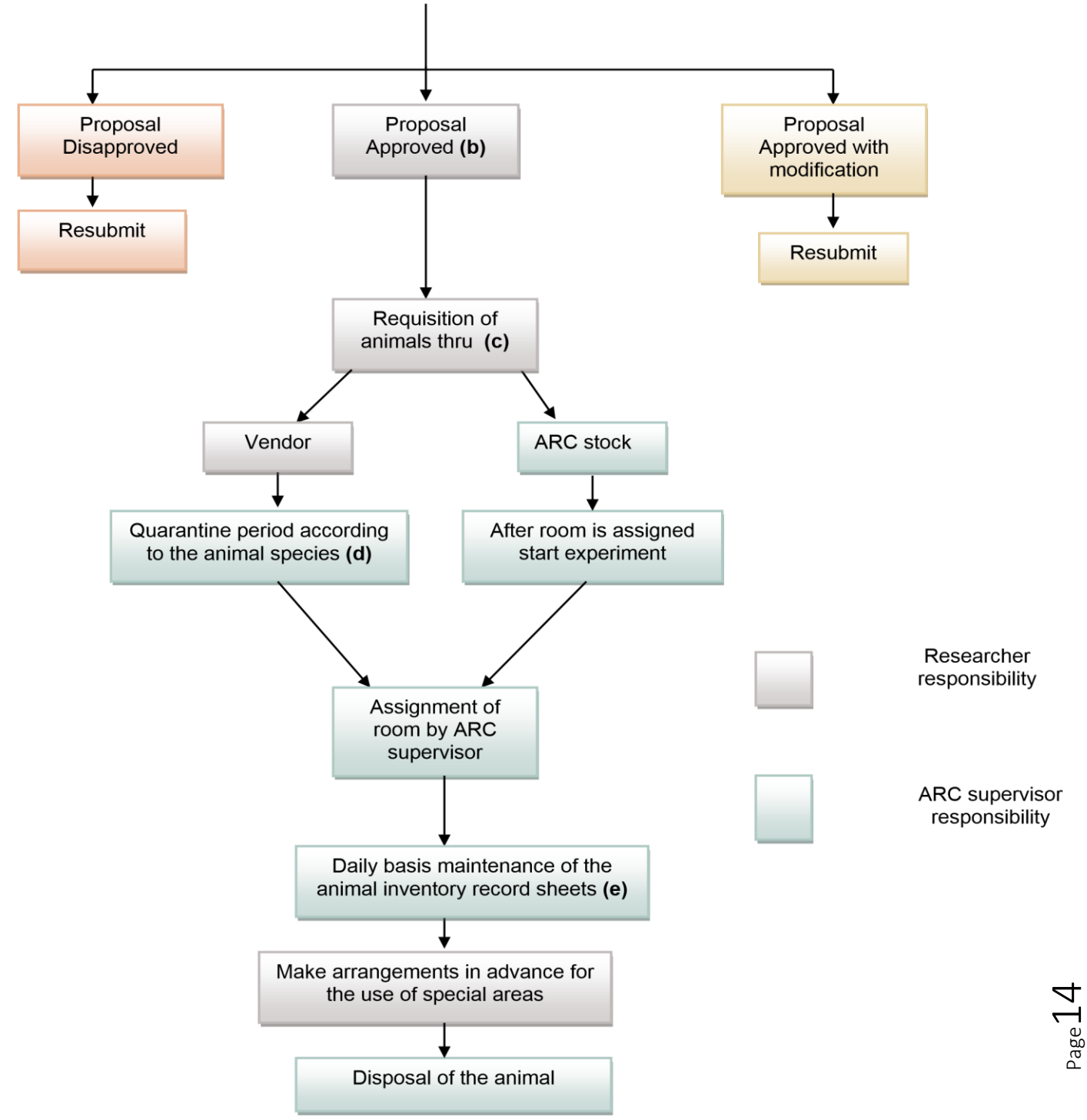
E- **Pets in Animal Facilities and Laboratories**

Pets are not allowed inside the laboratories and animal rooms.

F- **Procedure for requesting service from the ARC**

To receive services from the ARC, it is necessary to complete the following procedure:

Submission of the Application for Protocol Approval involving Animal Laboratory Use to the Institutional Animal Care and Use Committee (IAUCUC) **(a)**



G- Removal of Animals from the Institution

Animals must not be removed from the institution without prior, specific permission from the ARC Supervisor.

H- Transportation of Animals within the Institution

In planning the route by which animals will be transported between laboratories and the animal housing areas or other laboratories, care should be taken to minimize time spent in public spaces, including hallways or lobbies. The proper way to transport animals is in a plastic cage with a metal lid. Animals should be concealed from the public during transportation in public corridors by placing a drape loosely over the cage. Call the ARC office or ask the area ARC Supervisor for advice or assistance.

Only people authorized to handle animals will transport animals.

I- Animal to Breeding

(See Appendix #4).

J- Animal Identification

The Animal Welfare Act (AWA) and the Guide require specific information on all animal cages for identification purposes. Cage cards or tags supplied by the ARC have been designed to satisfy these requirements. Research or other data may be placed on a second card in the holder behind the identification card. However, the completed ARC card must be visible on all animal cages at all times.

K- Record Keeping

Each investigator is responsible for maintaining records that document efforts to avoid animal pain and distress during the research procedure. These records must be maintained for three years after completion of the project. Records for individual experimental groups of rodents and other small animals should include the Animal Project Number and source of the animal; date of experimental procedure; procedure performed including Animal Project Number and person(s) performing procedure; pre-surgical drugs, anesthetics, and post-surgical care; illnesses or injuries; medical treatment during the experiment; date of death or euthanasia; and disposition.

L- Experimental Procedures in Animal Housing Areas

Experimental procedures including euthanasia are not to be performed in occupied animal rooms unless justified for scientific or environmental control reasons and only if the investigator has obtained approval from ARC and/or the IACUC.

M- Animal Experimentation Involving Hazardous Agents

The UCC Radiation Officer must authorize the use of radioisotope material in animal studies.

The Institutional Chemical Committee must authorize the use of hazardous chemical agents in animal studies.

The introduction materials such as various infectious agents (bacteria, fungi, viruses, parasites and other), as well as potential sources of such agents (human blood or cells, human or animal cell lines) and recombinant or synthetic nucleic acid molecules to the animals must be reviewed and approved by the Institutional Biosafety Committee. (See Appendix #6)

N- Waste Disposal

The ARC will coordinate the disposal of animal wastes and carcasses. It will be disposed of in a safe and sanitary manner through a private company.

O- Blood Collection Techniques

Aseptic procedures should be used when collecting blood in all situations. Blood collection by cardiac puncture in any species or from the retro-orbital plexus in rats and mice should be performed only on anesthetized animals.

Consult the ARC veterinary staff for recommendations on the volume of blood and frequency of bleeding for each species. References:

1- http://www.ahc.umn.edu/rar/BLOOD.HTML

2- https://oacu.oir.nih.gov/sites/default/files/uploads/training-resources/blood_collection_tutorial.pdf

P- Animal Surgery

Recommendations for Aseptic Technique, Anesthesia, Analgesia and Post-Operative Care for Rodent Surgery. See appendix #7

Pre- and Post-Surgical Care of Animals

The health status of all animals used for a survival surgical procedures should be evaluated before surgery. The animal's cage should be tagged to withhold food and water overnight or longer as necessary, depending upon the species and the procedure. Pre-surgical tranquilizers can reduce animal anxiety, thus resulting in a much smoother, quieter induction and reduced requirement for an anesthetic agent.

The principal investigator is responsible for postoperative care of the animal with appropriate input from an ARC veterinarian. Immediate post-surgical care should include observing the animal to ensure uneventful recovery from anesthesia and surgery. The animal must be monitored and returned to the animal housing area until it regains sternal recumbence and hold up. Color and capillary refill time should be evaluated frequently.

Appropriate postoperative care for rodent species includes administering fluids, analgesics, and other drugs as indicated; clinical observations for signs of pain, abnormal behavior, appetite, and excretory functions; and providing care for surgical incisions.

1. Survival Surgery in Non-Rodent Mammals

Survival surgery is defined as any surgery from which the animal recovers consciousness for any period. Individuals performing survival surgical procedures must know aseptic surgical techniques and have adequate training and skills to conduct the procedure without causing undue post-surgical distress to the animal. Aseptic techniques must be used for all survival surgical procedures.

The IACUC will determine the classification of "major" or "minor" for each proposed surgical procedure. The guidelines used by the committee to make this determination are described in the current edition of the Guide.

Minor survival surgery does not expose a body cavity and causes little or no physical impairments to the animal. Wound suturing, peripheral vessel

cannulation, pump implantation in subcutaneous tissue, etc., are examples of minor survival surgery.

Minor surgical procedures may be performed in a suitably located and equipped laboratory setting using appropriate aseptic techniques. The laboratory should include a clean work area for the 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.

Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of the animal's physical or physiologic functions. Laparotomy, thoracotomy, craniotomy, joint replacement, limb amputation, etc. are examples of major survival surgery.

3. Surgery in Rodents

All procedures on rodent species may be conducted in a laboratory. For major survival surgical procedures, appropriate aseptic techniques including a clean work area; preparation of the surgical site including removal of the hair, disinfection of the skin and draping of the surgical site with sterile drapes; use of sterile supplies, instruments, and suture materials; and use of sterile gloves and a surgical mask by the surgeon and any assistants working in the surgical field should be used.

4. Non-Survival Surgery

If the animal does not regain consciousness postoperatively, major surgical procedures on non-rodent species may be conducted in a suitably located and equipped laboratory.

5. Multiple Survival Surgeries

Multiple-survival surgical procedures on a single animal are discouraged. Only under special circumstances, such as if the procedures are related to components of the research projects, more than one major surgical procedure on a single animal may be permitted with the approval of the IACUC. Occasionally, unanticipated additional surgeries to correct complications following the primary surgical procedures may be done as long as they are approved by an ARC veterinarian and do not cause an inordinate degree of pain or distress to the animal. Multiple-survival surgical procedures for teaching

protocols are not to be done. Cost is not an adequate justification for performing multiple-survival surgical procedures on an animal.

Q- Paralytic Agents

The use of paralytic agents is discouraged, particularly in surgical experimentation. It is recognized, however, that their use for specific applications has merit. If such agents must be used, written justification must be provided to the IACUC. Under no circumstances are paralytic agents to be used for surgery without appropriate anesthesia. The protocol must specifically note, in detail, how a proper level of anesthesia will be maintained throughout the time that the animal is under the influence of the paralytic agent.

R- Prolonged Physical Restraint

Prolonged physical restraint may be stressful to the animal and should be avoided unless justified as essential to the research objectives. All physical restraint for periods longer than one hour must be specifically justified in the protocol for consideration and approval by the IACUC. Convenience is not a justification for the use of prolonged physical restraint.

When prolonged physical restraint is required, animals should be conditioned to the equipment by gradually increasing times of restraint until the required restraint time is reached. The period of restraint must be limited to the minimum required to accomplish the research objectives restraint. For each situation, the IACUC will make a determination regarding the intensity of the attention required. Attention must be given 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, prompt veterinary care must be provided. This may require temporary or permanent removal of the animal from the restraint device. If the health problem is considered serious by the ARC clinical veterinarian tending the animal, the well-being of the animal must take priority over the experimental objectives.

S- Immunization of Research Animals

Because there are diverse opinions and techniques associated with animal immunization, protocols that propose to use procedures contrary to the following policies will be considered by the IACUC upon receipt of written justification and documentation. If appropriate documentation is lacking, it may be necessary to conduct a study designed to provide appropriate documentation.

1. Complete Freund's adjuvant

Many of the classical adjuvants, especially Freund's Complete Adjuvant (FCA), cause local inflammation and often chronic pain. When draining skin granulomas form and tissue is sloughed, the antigen-adjuvant emulsion may be lost.

Laboratory personnel using FCA should be cautioned about inadvertent self-injection on needle tips. This results in painful and long-lasting inflammation in humans.

Use of alternate adjuvants which produce less detrimental side effects is strongly encouraged.

2. Post-Injection Care

Animals given aqueous solutions of antigens after sensitization should be observed for signs of anaphylactic shock. Appropriate treatment should be administered if an acute reaction occurs.

Inflammatory reactions at injection sites should be reported to an ARC veterinarian for examination and treatment if indicated.

T- Unavoidable Pain or Distress

Every effort must be made to avoid or minimize discomfort, distress, or pain to experimental animals, consistent with sound research design. Procedures that may cause more than momentary or slight pain or distress must be justified for scientific reasons in writing by the investigator. To minimize distress to the animals, the earliest possible end point to the study must be defined and used. Whenever possible, this should be prior to death of the animal. The protocol must justify and clearly state the end point to be used.

U- Euthanasia

The euthanasia guidelines provided by the ARC are based on recommendations of the American Veterinary Medical Association Guidelines on Euthanasia. Any deviations from these guidelines must be justified in writing and approved by the IACUC. Reference: https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

V- Personnel Health Surveillance Program

All personnel working with animals must have evidence of a valid tetanus shot and any other specific requirements.

Any injuries should be promptly attended to and be reported to the Human Resources Office.

1- Animal Bites or Other Animal Related Injuries

In the event of an animal bite or other animal related injury, administer first aid and promptly report the injury to the ARC Supervisor and your employer. Go to a clinic/hospital if additional treatment is necessary. Complete the "Workers Compensation Worksheet for First Report of Injury" form and submit it to the designated person. Also, contact the Human Resources Office of the Universidad Central del Caribe.

2- Lab coat is required within these facilities.

3- Not allow the handling of contact lenses.

4- Are totally prohibited eating, drinking, chewing gum and application of makeup.

5- Everyone must discard the gloves, before leaving these facilities and try to wash your hands with soap and water, provided in the room.

VI- Humane Methods of Animal Maintenance and Experimentation

ARC at Universidad Central del Caribe is based on the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training, the recommendations of the Guide for the Care and Use of Laboratory Animals, and the requirements of the Animal Welfare Act and its amendments.

A. Animal Care

Animals are housed in cages designed to provide a physical and social environment that contributes to their well-being while minimizing variables that can modify an animal's response during experimentation. Environmental factors such as temperature and humidity ranges, lighting, and noise levels are considered in housing the various species. Palatable, uncontaminated, and nutritionally adequate food and fresh, potable, uncontaminated drinking water are provided to meet the requirements of each species. Bedding, if used, is

absorbent and free of toxic chemicals. Cage and equipment cleaning schedules and methods are designed to keep animals clean and dry and eliminate pathogenic organisms while providing minimal interference with normal physiological requirements of each species.

The veterinary care program consists of observing all animals to assess their health and welfare using appropriate methods to prevent, control, diagnose, and treat diseases and injuries; providing guidance to users regarding handling, immobilization, anesthesia, analgesia, and euthanasia; and monitoring surgery programs and post-surgical care.

Personnel caring for animals are trained by the ARC Supervisor in laboratory animal husbandry through formal courses and closely supervised on-the-job experience. They are taught to detect and report variations in normal function or behavior of the animals. Personnel learn to handle the animals in a calm, confident manner that minimizes stress and ensures the safety of both the handler and the animal.

B. Animal Experimentation

It is the responsibility of any investigator using animals in research to ensure that he/she and their employees, both professional and technical, know how to handle and care properly for the species being used. They also should be knowledgeable about the animal model and the techniques used. The veterinary staff should be consulted if there are any questions. In addition, information regarding the basic needs of each species is readily available from reference sources in the ARC library. Investigators should try whenever possible to reduce the number of animals used, refine techniques to minimize pain or distress suffered, and replace animals with alternative or adjunctive methods.

1. Reduction - The numbers of animals used in research can be reduced in a variety of ways.

    * Literature Review

    No experiment using animals should be performed without a thorough review of the literature to eliminate the possibility of needless repetition and to determine the most appropriate model to answer a particular research question. Through the inter-library loan system, the Universidad Central del Caribe library has access to literature concerning all aspects of animal experimentation or electronic methods. Specific information may be sought using a variety of databases including AGRICOLA which is maintained by the National Agricultural Library. (https://agricola.nal.usda.gov).

* Use Based on Requirements to Achieve Statistical Significance

All experiments should be planned to provide sufficient data points to determine statistical significance. Using insufficient numbers of animals may require a repetition of the experiment and, therefore, may be as undesirable as using too many animals.

* Disease-Free Animals

While the cost of disease-free animals, sometimes called SPF (Specific Pathogen Free), is high initially, the long-term benefits of using such animals usually far outweigh the initial cost.

* Sharing Animals or Tissues

In some cases, the organs, tissues, etc., may be commercially available. Several investigators sharing organs of a single animal reduces the number of animals necessary and the cost of the investigation.

2. Refinement

Whenever possible, investigators must design experiments so that death is not the end point. Minor modifications of the approach to the experimental problem may allow euthanasia of an animal before it suffers significant discomfort or anxiety. Along the same lines, when passing tumors or growing tumors *in vivo*, efforts should be made to collect tissues or evaluate effects prior to the time that the animal is incapacitated.

Anesthetic, analgesic, or tranquilizing agents should be administered for any procedure potentially causing more than minimal pain or distress to the animal; exceptions must be justified and approved by the IACUC. The principal investigator should be alert to, and recognize signs of pain or distress in the animal he/she is working. Changes in dietary or grooming habits or changes in body temperament may indicate that an animal is in pain or distress. If the investigator or research technician has any questions or needs assistance, an ARC Veterinarian should be consulted.

3. Replacement

* Teaching New Techniques

New techniques should be demonstrated or practiced on models or cadavers. Videotapes and slide-tape presentations should be developed and used as much as possible in training programs.

* Alternative or Adjunctive Methods

While an intact biological system may be required to answer some research questions, tissue culture, or other *in vitro* techniques, including computer or mathematical modeling may provide satisfactory alternative or adjunctive methods.

VII. ANIMAL RESOURCES CENTER (ARC)

The ARC is responsible for establishing and providing appropriate facilities for the care and use of animals; professional and technical expertise, consultation, and service in all phases of laboratory animal care and use; health care programs for laboratory animals, including diagnostic services; and continuing education in the care and use of animals by ARC staff.

A. Veterinarians and Staff

The Veterinarian provides expertise in the biology, diseases, and pathology of laboratory animals and techniques associated with using these animals in research.

B. Organization

The ARC is a research and academic service area responsible to the President of Universidad Central del Caribe through the Chairman of IACUC.

The Supervisor of the ARC coordinates and directs operations of animal health programs, animal care programs, business operations, and teaching programs. The Supervisor organizes and oversees the daily operation of the animal facilities. The Supervisor also assists in planning facility renovations, training technicians, and evaluating services coordinates the daily operation in the ARC, orders animals and supplies, and provides reports about animal usage, animal care, supplies, and requested assistance. Moreover, other issues relating to the finances of the ARC and communications between the ARC and other departments within the Universidad Central del Caribe are directed from the administrative offices.

C. Description of Facilities

The animal resources facilities consist of approximately 7,700 square feet of animal housing and support areas located on the same floor. Specialized space includes cage washing areas, autoclave areas, food storage, bedding storage, diet kitchens, stock and treatment, necropsy rooms, animal yard moat.

D. Animal Procurement

The researcher must process all animal orders through the purchasing department. Before ordering animals, the researcher must inform the ARC Supervisor to ensure there is adequate space to house the animals and select the vendor. After ordering, the researcher must coordinate with the ARC Supervisor for the arrival of the animals. Animals are generally not shipped after Wednesday of each week, so they will not arrive on the weekend.

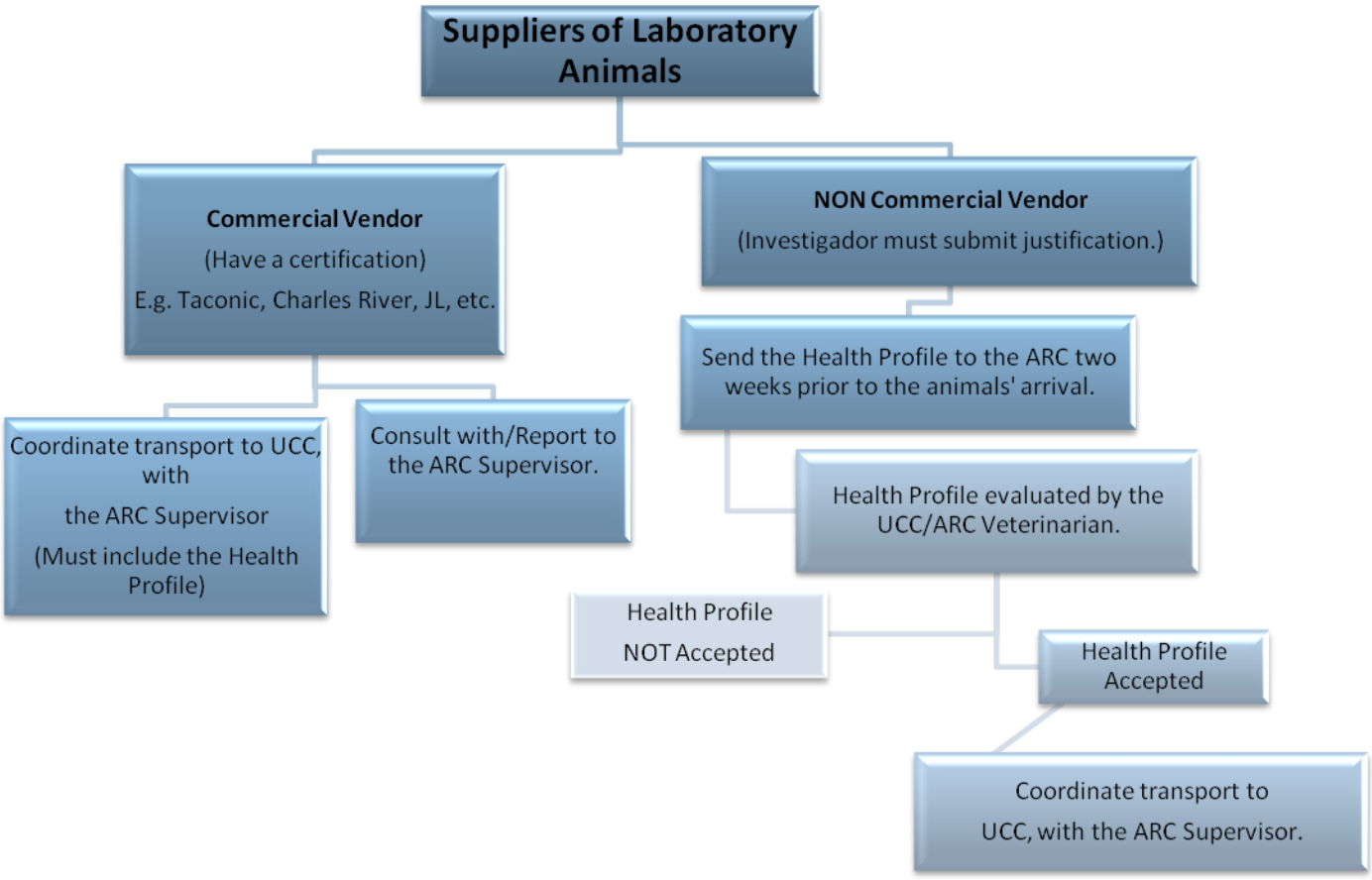
1. Ordering

All research and teaching protocols that use animals must be submitted to the IACUC for approval. After approval, the original should be given to the Animal Resources Center Supervisor, with a copy sent to the researcher requesting the permit.

IMPORTANT NOTE: During storms and hurricanes season (June 1 to November 30), if an imminent WARNING of Tropical Storm or Hurricane arises, issued by the National Weather Service (SNM), the principal investigator or representative will contact the Supervisor of the ARC, for the cancellation of the shipment of the animals to Puerto Rico.

Steps to follow when choosing a supplier for laboratory animals:

Note: The following are the main investigator's responsibilities when considering a supplier for laboratory animals.



2. Receiving

Animals are received at the ARC by the Supervisor or a delegate and checked for order specifications and signs of illness. The animals are then identified appropriately. The Supervisor ensures that the animals are housed in the appropriate animal room and notifies the investigator of the animal's arrival and location. If, by mistake, animals are delivered directly to an investigator and bypass receiving procedures, ARC should be notified immediately. If an investigator requires animals to be delivered directly to a laboratory for immediate use, the requirement must be specified on the purchase requisition.

To reduce the introduction of disease to research colonies, the animal facility maintain a quarantine system. All rodents (rats and mice) are isolated for 48 hours minimum. All animals are segregated by shipments and by origin (vendor). Daily observations are made for signs of disease, and extension of the isolation period may be necessary. If diseases are observed in these animals, the investigator is notified, and appropriate arrangements are made. Upon satisfactory completion of the quarantine period, the animals are moved to

colony rooms for long-term housing. Colony housing is accomplished by segregating animals by species and by origin (vendor).

3. Shipping

All shipments of animals to other institutions, regardless of whether or not they are to be returned, must be coordinated through the ARC to ensure that the necessary health certificates are obtained and in compliance with the other institution or agency. The researcher is responsible for the shipment.

4. Sources of Animals

The ARC staff is knowledgeable regarding the sources and availability of animals for use in research and should be consulted, especially if particularly strains are required.

The ARC receives periodic health assessment reports for laboratory animals available from various sources. Recommendations regarding the use of these animals are made based on their health status. An ARC clinical veterinarian must approve a request to obtain animals from an unapproved vendor.

- Commercial Vendors

  Commercial vendors, selected based on the consistent good health of their animals and dependable delivery and service, are used most frequently as a source of research animals. Animals generally not considered acceptable or with limited acceptability due to known disease problems may be purchased if required for a particular study. However, the investigator must realize that these animals will have to be housed and handled so that they do not jeopardize the health of any other animals or humans.

- Other Institutions

  The ARC must be notified if animals are to be acquired from other institutions. The ARC veterinarian will need information about the health status of these animals so that they may be shipped and housed in a manner that protects them from infection and prevents infection of resident animals.

E. Animal Care

1-Routine Animal Care

ARC personnel check all animals daily, including weekends and holidays, to ensure adequate food and water are available. Rooms are cleaned monthly. Cages are changed regularly depending upon the animal species.

Each day, the Animal Care Technicians tag cages containing sick animals and removes any animals that died overnight. The reports of sick and dead animals are submitted daily to the ARC Supervisor for further action.

2-Special Care Requirements

Animals receiving special care (i.e., special diet, fasting, etc.) are identified through tags or labels attached to the animal's cage. A supply of these tags is available from the ARC Supervisor. Care should be taken to select the most appropriate tag and complete it correctly before attaching it to the animal's cage.

Other special requirements such as altered lighting cycles or temperature, isolation, etc. or special care for large groups of animals may be arranged with the ARC Supervisor.

3-Standard and Special Diets

Standard dry diets are fed ad libitum in self-feeders to all rodents unless otherwise specified. The ARC does not provide special diets, but ARC personnel can assist investigators in locating sources for them. Dietary supplements are provided when necessitated by disease problems or dietary requirements.

4-Environmental Control

Each animal room has fresh air (i.e., non-recirculated air). Relative humidity and temperature are periodically monitored as appropriate. All rodent’s rooms are on individual time-controlled lighting systems set for 12 hours dark (6:00 PM-6:00 AM) and 12 hours light (6:00 AM-6:00 PM) unless the research protocol requires deviations.

In the room of the immunosuppressed animals (nude mice), time-controlled lighting systems set for 10 hours of light (8:00 AM-6:00 PM) and

14 hours dark (6:00 PM-8:00 AM) is maintained. Refer to Nude Mice Manual

To assure USDA and Guide standards are met, animals should be housed only in facilities approved by ARC. Housing animals in laboratories for periods longer than 12 hours must be approved by the IACUC.

5-Vermin Control

A private company comes to the ARC monthly to perform vermin control. Animal rooms, bedding room, and food room are not treated.

6-Disposition of Carcasses or Animal Wastes

After ensuring that the animal is dead, the carcass must be placed in a plastic bag and stored in the freezer. Final disposal is by a private company.

7-Other Support Services

ARC personnel is trained to provide various specialized animal care support functions, including establishing and maintaining animal breeding colonies. Contact the ARC office for information or assistance.

8-Per Diem Charges

The ARC must recover costs for the care of animals housed within its facilities through per diem rates charged to investigators to maintain their research animals. These rates are established through a cost analysis performed according to guidelines established by the NIH. Rates are implemented only after review and approval by the budget office, IACUC, and the Universidad Central del Caribe administration. Faculty members applying for grants may contact the ARC Director to obtain information concerning any anticipated increases.

The researcher should consult with the ARC Supervisor about estimated animal costs before submitting a grant application. (See: Appendix #3)

F. Animal Health

1-Routine Health Care

With the assistance of animal health technologists (AHT), the veterinarians perform preliminary physical examinations as needed. Animals are treated only after approval by the investigator, except during an emergency.

2-Emergency Health Care

If the individual(s) designated by the principal investigator as the emergency contact(s) cannot be reached within a reasonable length of time, an ARC veterinarian will provide supportive care according to his/her professional judgment. If the animal must be euthanatized, every effort will be made to save tissues needed for the research protocol. The investigator or his designee will be notified as soon as possible of any action taken.

3-Reporting Sick Animals

An animal observed to be ill or exhibiting abnormal behavior should be reported to the ARC as soon as possible so that a veterinarian can examine it.
Inconsistent laboratory results of experimental animals may suggest an underlying disease problem in the research animals used. If all other possibilities for the inconsistencies have been eliminated, please consult with the ARC veterinary staff for assistance.

4-Quarantine Procedures

As determined by a clinical veterinarian, animals will be quarantined upon arrival at Universidad Central del Caribe for a period dependent upon the species, source, and health status.

5-Zoonotic Disease

When people handle animals, the potential always exists for contracting zoonotic diseases such as leptospirosis, toxoplasmosis, or lymphocytic choriomeningitis (LCM); however, this potential can be almost eliminated by purchasing only disease-free animals from reliable vendors and by practicing good hygiene.

G. Diagnostic Laboratory and Necropsy

The ARC or veterinarians may recommend diagnostic laboratory procedures and necropsy analysis.

H. Billing Procedure

The ARC must recover its operating costs from charges for services rendered. Charges for services, assistance, supplies, and/or animal care are itemized on a monthly report to administration submitted to each investigator. (See annexed #3).

I- Emergency Plan

Storm or hurricane:
(See Emergency and Evacuation Manual of the Universidad Central del Caribe)

- During

All academic and administrative activities will be suspended.

- After

As conditions allow, ARC staff return to work to evaluate and reestablish the services of the ARC.

Depending on the environmental conditions (power, water, etc.) that may affect the ARC, the supervisor and/or the consultant veterinarian may establish a quarantine period for the use of laboratory animals.

The supervisor will maintain communication with the users of the ARC and the IACUC.

VII. Grant and Manuscript Preparation

A. Preparing NIH Grant Applications

The Public Health Service Policy requires that the use of all vertebrate animals in research be governed by the Principles for the Use of Animals and, in the case of warm-blooded vertebrates, the Guide for the Care and Use of Laboratory Animals. No PHS award involving the use of animals is made unless an Assurance (see Section I.A.2.) has been approved by the Office for Protection from Research Risks (OLAW).

Several sections of the application require information about the proposed animal use. Failure to supply the requested information may delay consideration or jeopardize funding. These sections are:

1. Page - 5. Vertebrate Animals: Indicate the IACUC (IACUC) approval date. Enter "pending" if the IACUC review is delayed beyond the submission date of the application. The UCC Animal Welfare Assurance Number is D16-00343.

2. Page 4 - Supplies: State the number of animals to be used, unit purchase cost (actual cost of animal + shipping + box charges), and unit care cost.

3. Page 5 - Supplies: Include the total initial cost of the animals and an inflationary increase of 4-10% per year for each succeeding year. Although 4% inflation is the maximum allowed on most grants, higher rates may be accepted if they can be documented.

5. Research Plan - F. Vertebrate Animals: Provide a detailed description of the proposed use of the animals in work outlined in the experimental design and methods section. Identify the species, strain, ages, sex, and numbers of animals to be used.

6. Justify the use of vertebrate animals, the choice of species, and the numbers used. If the animals are in short supply, costly, or to be used in large numbers, provide a specific rationale for their selection and numbers.

7. Describe the procedures for adequate maintenance and veterinary care of the animals involved. Reviewers will wish to know if the living conditions of animals will be appropriate for the species and contribute to their health and comfort and if medical care for animals will be available and provided as necessary by a qualified veterinarian. The following general statement may be used if the proposed work will necessitate no special care. "Housing and day-to-day care for the animals are consistent with the standards of the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. All animals are observed daily, and appropriate veterinary care is provided by the veterinary medical staff of the ARC. This staff consists of veterinarians with training and experience in laboratory animal medicine and science and technician."

8. Describe the procedures to avoid unnecessary discomfort, pain, or injury to the animals. Reviewers will specifically try to determine that procedures will avoid or minimize discomfort, distress and pain to the animals, consistent with sound research design; procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia unless the procedure is justified for scientific reasons by the investigator; personnel conducting procedures are qualified and trained in those procedures; and that animals that would otherwise experience severe or chronic pain or distress that cannot be

relieved will be painlessly sacrificed at the end of the procedure or, if appropriate, during the procedure.

9. Describe methods of euthanasia to be used and reasons for selection. Justification based on scientific reasons must be provided if methods of euthanasia used are not consistent with the recommendations of the American Veterinary Medical Association Panel on Euthanasia

B. UCC Application for the Use of Animal Subjects Form

Investigators are urged to submit the Application for the Use of Animal Subjects forms two months before the grant deadline to ensure no delays in approval and subsequent notification of the granting agency. Lack of timely receipt by NIH of the verification of approval can delay consideration and jeopardize funding. It is the investigator's responsibility to submit a completed Application for the Use of Animal Subjects Form to the IACUC, Prof. Zilka Ríos at, Basic Science Building, Microbiology Department, second floor for each new proposal, competitive renewal, modification of ongoing grant, grant supplement, or non-competitive continuation involving animals, regardless of funding source; any pilot project and modifications in approved animal use protocols; and any educational project in which vertebrate animals are used. Protocols using vertebrate animals must be approved before beginning the project.

Submit the original of the completed "*Application for Protocol Approval Involving Laboratory Animal Use"* (electronic protocol) (no handwriting) directly to the Chairman of IACUC. The investigator will be notified in writing of the decision of the IACUC. Upon approval, one copy of the protocol will be filed in the Chairman of the IACUC office and the other copy to the ARC office. Appropriate notification will be provided to the Office of Grants Management and the granting agency. The assigned Identification Number must be indicated on all orders for animals to be used on that protocol.

IACUC approval is valid for three years only. The protocol modifications will be notified using the "Approved Protocol Modification Application." Approximately two months before the expiration of project approval, the IACUC office will mail a courtesy notice to inform the protocol renewal. However, it is the investigator's responsibility to ensure all ongoing projects are submitted for annual review.

C. Manuscript Preparation

Publications of work based on animal studies should provide a complete and accurate description of the animals used, including common name; genus and species; strain, stock, or breed; source; age and/or weight; sex; method of identification; and microbiologic status. The animals' environment -the caging; room temperature, humidity, ventilation, and lighting; diet and water; and husbandry routines - should be defined to duplicate experimental results. Other information that should be provided

includes the time of sampling, where and how the samples were obtained, all drugs and dosages used, and the euthanasia method employed.

VIII- GLOSSARY

1. Necropsy- an examination of a dead body; postmortem.

2. Quarantine- the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined.

3. Euthanasia- the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress.

4. Carcass- the dead body of an animal, often specific to a slaughtered animal dressed as meat.

5. Recumbence- “lying down”

6. Laparotomy- a surgical incision into the abdomen.

7. Thoracotomy- surgical incision into the thorax.

8. Craniotomy- the surgical operation of opening the skull.

9. Contusion- a bruise; injury in which the skin is not broken.

10. Freund's adjuvant- a substance consisting of killed microorganisms, such as mycobacteria, in an oil and water emulsion that is administered to induce and enhance the formation of antibodies.

11. Animal Yard Moat- indoor yard

Appendix
#1

Security Access Code

Universidad Central del Caribe
Animal Resources Center
And
Institutional Animal Care & Use Committee
(IACUC)

Animal Resources Center Users

Ref: Installation of an Electronic Lock

In a visit to the Animal Resources Center carried out by different agencies, they recommended that access control be established for the facilities to increase security for animals, personnel, equipment, and the Center's structure.

The Institutional Animal Care & Use Committee (IACUC), in a meeting on January 19, 2001, recommended the purchase of an electronic lock.

This electronic lock was installed on door #241. This door provides access to the animal rooms, the laboratory, and the Center's warehouse. This particular model works through a code system.

Starting April 18, 2001, this system was implemented every day, 24 hours a day.

The established regulations for the security system are detailed below:

- A different code will only be given to each authorized personnel member. This code will be considered confidential.

- Authorized personnel will be identified as those specified and approved in the current Application for Protocol Approval Involving Animal Laboratory Use, and any changes should be notified and approved in the Update Approved Protocol Application; IACUC members; Animal Resources Center employees; personnel authorized by their director to take samples from the existing freezers in the area.

- The Center Supervisor must be informed of any visits beforehand.

- Maintenance personnel for air conditioners, elevators, etc. must use door #035. Should they need to use door #241, the Center Supervisor must be contacted.

- The security code may be changed following the current needs. Any changes will be notified beforehand.

- In case of a tropical storm or hurricane warning, as well as warnings for other adverse weather conditions, the system will become and remain inactive until conditions return to normal.

- If any personnel member transfers their code, admittance to the Center may be revoked at the discretion of IACUC members.

All recommendations should be submitted in writing and directed to Prof. Zilka Ríos (zilka.rios@uccaribe.edu) or Betzaida Torres (betzaida.torres@uccaribe.edu).

Appendix
#2

REQUISITOS PARA LA ACEPTACION DEL ESTUDIANTE VISITANTE EN LA UCC

REQUISITOS PARA LA ACEPTACION DEL ESTUDIANTE VISITANTE EN LA UCC

A manera de velar y cuidar por la seguridad institucional y de los estudiantes que nos visitan procedentes de otras instituciones universitarias, se ha preparado el siguiente documento. El mismo establece los requisitos a seguir con el fin de autorizar el acceso a las áreas a visitar en la UCC.

El estudiante deberá completar el formulario *"Registro de Estudiante Visitante",* el cual fue revisado el 17 de febrero de 2015. Una vez completado el formulario, deberá entregarlo al Decanato de Administración firmado por el investigador mentor y responsable de éste en la UCC con los siguientes documentos:

- ✓ Una foto 2X2
- ✓ Carta explicativa de la institución de procedencia con el propósito de su visita en la UCC
- ✓ Evidencia de matrícula de la institución de procedencia del curso que requiera experiencia en investigación o que haya un acuerdo colaborativo entre la institución de procedencia y la UCC
- ✓ Endoso del seguro de responsabilidad pública de la institución de procedencia con límite de un millón de dólares *(Si el estudiante nos visita en su carácter personal, deberá tramitar el mismo con una asegurada como individuo.)*
- ✓ Copia del plan médico
- ✓ Copia del Certificado de Vacunas administradas por el Departamento de Salud y las vacunas requisitos para trabajar en los laboratorios
- ✓ Pago de $40 por servicios de estacionamiento
  (Para registrar el vehículo, es necesario nos emita una copia de la licencia de conducir y la licencia del auto a registrar.)
- ✓ Pago de $15 para la identificación que utilizará durante el período que visite la UCC. Esta identificación deberá utilizarla en todo momento.
  (El estudiante realizará los pagos del servicio de estacionamiento e identificación en la Oficina de Recaudaciones una vez la Administración verifique que todos los documentos estén completados. Brindará copia del recibo de pago para recibir la identificación y el permiso de estacionamiento.)

El Decanato de Administración será custodio de los expedientes de estos estudiantes. Además, informará al investigador mentor y a la Casa de Animales por correo electrónico sobre la autorización de acceso a la UCC una vez haya cumplido con todos los requisitos. El estudiante está autorizado a acceder a las áreas relacionadas por el tiempo indicado en el formulario *Registro de Estudiante Visitante*. La Administración auditará los expedientes e informará al investigador cuando alguno de los

documentos haya cumplido su fecha de vencimiento, pues ningún estudiante está autorizado a permanecer en la UCC con un endoso de seguro vencido. Estos endosos no corren por año natural.

El estudiante deberá devolver su identificación y permiso de estacionamiento al Decanato de Administración una vez termine su trabajo en la UCC.

Appendix
#3

POLITICA EN LA FACTURACION DE ANIMALES DE LABORATORIO/ POLICY REGARDING THE BILLING OF LABORATORY ANIMALS

UNIVERSIDAD CENTRAL DEL CARIBE

POLITICA EN LA FACTURACION DE ANIMALES DE LABORATORIO
POLICY REGARDING THE BILLING OF LABORATORY ANIMALS

CENTRO DE RECURSOS ANIMALES
Animal Resources Center

Objetivos:
Goals:

1. Mejorar facilidades, equipo y mantenimiento del Centro de Recursos Animales. *Improve facilities, equipment and maintenance of the Animal Resources Center.*
2. Proveer información del costo de mantenimiento. *Provide information on the cost of maintenance.*
3. Incluir información del "per diem" en propuestas de investigación. *Include information from the per diem in research proposals.*

Empleados de la Universidad Central del Caribe
Employees of the Universidad Central del Caribe

Personal universitario es el término que incluye a todas las personas que rinden servicios a la Universidad Central del Caribe bajo un puesto, y comprende las siguientes clasificaciones que se definen más adelante: personal docente, personal no docente y personal administrativo. Se excluye toda persona que trabaje bajo un contrato de servicios profesionales (incluye: *Faculty Adjustment, Joint Appointment).*

University staff is the term that includes all the people who render services to the Universidad Central del Caribe who have a formal position, and includes the following classifications defined below: teaching staff, non-teaching staff and administrative staff. Any person working under a professional services contract is excluded (includes: Faculty Adjustment, Joint Appointment).

Centro de Recursos Animales (CRA)
Animal Resources Center (ARC)

El servicio principal del CRA es el cuidado diario de animales en proyectos de investigación, aprobado por el *Comité Institucional para el* Uso y Cuidado *de Animales (IACUC).* Esto incluye la alimentación, el suplido de agua y alimento, el cambio de la camada, la limpieza de las jaulas, la supervisión de la salud, el mantenimiento de la facilidad y la administración del programa del cuidado animal.

The main service of the ARC is the daily care of animals in research projects, approved by the Institutional Committee for the Use and Care of Animals (IACUC). This includes feeding, water and food supply, changing the litter, cleaning the cages, health monitoring, maintaining the facility and administering the animal care program.

"Per diem"
"Per diem"

Para recuperar el costo del cuidado animal, se carga un honorario diario llamado *el cuidado del animal diario* o *"per diem"*. Este honorario se establece por día, por animal y se registra a través de la hoja "*Daily Animal Inventory*" por el CRA y facturado a la cuenta del investigador, al final de cada mes. Las tarifas se basan en un análisis anual del costo diario asociado al mantenimiento de los animales. El "*per diem*" es basado en el alojamiento y mantenimiento convencional. De surgir un alojamiento y mantenimiento no convencional se realizará un análisis para el ajuste de éste. Los cargos diarios comienzan tan pronto los animales lleguen a las facilidades del CRA.

To recover the cost of animal care, a daily fee called daily animal care or "per diem" is charged. This fee is established per day, per animal and is recorded through the CRA's "Daily Animal Inventory" sheet and billed to the investigator's account at the end of each month. Rates are based on an annual analysis of the daily cost associated with keeping animals. The "per diem" is based on conventional housing and maintenance. If non-conventional accommodation and maintenance arise, an analysis will be carried out to adjust it. Daily charges begin as soon as the animals arrive at the CRA facilities.

"Cost Analysis and Rate Setting Manual for Animal Research Facilities"

Producido por el *"Cost Manual Revision Committee"* bajo el auspicio del área de Medicina Comparativa del *"National Center for Research Resources (NCRR),* como componente del *National Institutes of Health (NIH*).

Produced by the Cost Manual Revision Committee under the auspices of the Comparative Medicine area of the National Centers for Research Resources (NCRR), as a component of the National Institutes of Health (NIH).

Facturación
Billing

I. **Investigadores Internos con Fondos Restrictos (ingresos que se reservan para un determinado propósito o propósitos y no puede ser utilizado para cubrir otros fines o proyectos) en el Uso de Animales**

Internal Researchers with Restricted Funds for Animal Use (income that is reserved for a certain purpose or purposes and cannot be used to cover other purposes or projects) in the Use of Animals.

A- El CRA realizará un reporte para facturar el uso de animales, basado en el "per diem", a la cuenta de los fondos restrictos del proyecto del investigador, al final de cada mes.

The ARC will write a report to invoice the use of animals, based on the "per diem", charged to the account of the restricted funds of the researcher's project, at the end of each month.

B- La facturación del CRA al investigador estará acorde con el inventario diario de animales que se ubica en estas facilidades, con el propósito de tener un registro de la entrada y salida de los animales.

The invoicing of the ARC to the researcher will be in accordance with the daily inventory of animals located in these facilities, in order to have a record of the entry and exit of the animals.

C- El CRA considerará todos los proyectos con fondos restrictos. Este reporte será dirigido a la Oficina de Recursos Fiscales y ésta a su vez realizará una factura que deberá ser firmada por el investigador con el fondo restricto autorizado, para gestionar la transferencia de éstos.

The ARC will consider all projects with restricted funds. The written report will be addressed to the Office of Fiscal Resources and they in turn will make an invoice that must be signed by the investigator with the authorized restricted fund, to manage the transfer of the funds.

II. Investigadores Internos sin Fondos Restrictos para el Uso de Animales

Internal Researchers without Restricted Funds for Animal Use

A- De este investigador no tener una cuenta para su proyecto, ésta estará libre de cargo por un año, a partir de la aprobación de su proyecto por el IACUC.

If this researcher does not have an account for his/her project, it will be free of charge for one year, from the date of the approval of his/her project by the IACUC.

B- Se le realizara un reporte del "per diem" del uso de los animales en el CRA, al investigador y copia al director de su departamento adscrito. Dicho reporte tiene como propósito concienciar al investigador de sus gastos.

A report of the "per diem" of the use of animals in the ARC will be made, delivered to the researcher with a copy to the director of his/her assigned

department. The purpose of this report is to make the researcher aware of his expenses.

C- Luego del año, de este investigador todavía no tener cuenta en su proyecto, éste será facturado al departamento adscrito, a menos que el Presidente de la Universidad Central del Caribe autorice lo contrario.

If after the year has passed and the researcher still does not have an account for his/her project, it will then be billed to the department under which the researcher is assigned, unless the President of the Universidad Central del Caribe authorizes otherwise.

D- El investigador principal o el director del departamento podrá dirigirse a la oficina del Decano de Investigación para la disponibilidad de fondos.

The principal investigator or the department director may contact the Office of the Dean of Research for the availability of funds.

III. Investigadores Externos con Animales en el CRA.
External Researchers with Animals in the ARC.

A- Investigador externo es todo aquel investigador que labore en otras instituciones o agencias y que no sea empleado de la Universidad Central del Caribe.

An External researcher is any researcher who works at other institutions or agencies and who is not employed by Universidad Central del Caribe.

B- El investigador deberá proveer al CRA el número de cuenta del proyecto a facturarse y toda información requerida por la oficina de Recursos Fiscales y la oficina del Decano de Investigación.

The researcher must provide the ARC with the project account number to be billed and all information required by the Office of Fiscal Resources and the Office of the Dean of Research.

C- El CRA realizará un reporte para facturar a la oficina de Recursos Fiscales del investigador cada mes, en acorde con el inventario diario de animales que se presenta en estas facilidades.

The ARC will write a report in order to bill the investigator's Office of Fiscal Resources each month, in accordance with the daily inventory of animals present in these facilities.

D- Una vez realizada una primera factura de la misma deuda al no recibirse su pago en treinta (30) días, la oficina de Recursos Fiscales le enviará un segundo aviso al investigador con copia a la oficina del Decano de Investigación o administrador de los fondos. Al no ser recibida en treinta (30) días más, la oficina de Recursos Fiscales le notificará al Decano de Administración quien autorizará la cancelación de los servicios por el CRA.

Once the first invoice of the expenses incurred has been sent and payment is not received within thirty (30) days, the Fiscal Resources office will send a second notice to the investigator with a copy to the office of the Dean of Investigation or fund manager. After an additional thirty (30) days have passed without payment having been received after the issuance of the second notice the Office of Fiscal Resources will notify the Dean of Administration who will authorize the cancellation of services by the Animal Resource Center.

IV. Dirección de Fondos Adquiridos
Acquired Funds Management

A- Los fondos adquiridos a base de la facturación por el alojamiento y mantenimiento de los animales de laboratorio en el CRA, serán dirigidos a la cuenta de este Departamento.

The funds acquired on the basis of billing for the housing and maintenance of laboratory animals in the ARC, will be directed to an account of this Department.

B- Estos fondos serán usados por el CRA para las mejoras de las facilidades, equipo y mantenimiento.

These funds will be used by the ARC for improvements in facilities, equipment and maintenance.

V. Consideraciones
Considerations

A- Todo investigador deberá solicitar en su propuesta el costo de mantenimiento convencional de sus animales de laboratorio.

All researchers must request in their proposal the cost of conventional maintenance of their laboratory animals.

B- Deberá considerar la cantidad de animales experimentales y en reproducción (si aplica) en cada año fiscal.

You should consider in your budget the number of experimental animals as well as those used for breeding (if applicable) in each fiscal year.

C- Deberá contemplar cualquier solicitud para aumento de animales durante la vigencia de su proyecto. (Este no garantiza la aprobación del IACUC).

You must consider the possibility of any request to increase the number of animals during the term of your project. (This does not guarantee IACUC approval).

VI- Lo que incluye el costo de "per diem"
What the cost of the "per diem" includes

A- Rutina y mantenimiento convencional. *Routine and standard husbandry.*
B- Rutina de cambios de jaulas. *Routine cage changes.*
C- Atención veterinaria de rutina (diagnóstico y tratamiento). *Routine health care (diagnostics and treatment).*
D- Cuidado de emergencia (no causado por el protocolo de investigación). *Emergency Care-(Not caused by research protocols).*
E- Consultoría veterinaria. *Veterinary Consultations.*
F- Monitoreo de salud ocupacional o exposiciones incluyendo mordeduras y rasguños de animales. *Occupational health monitoring or exposures including animal bites and scratches.*
G- Disposición de tejidos y animales como resultados de eutanasia *Disposal of tissues and animals as a result of euthanasia.*
H- Manejo en la salud de la colonia (calidad y la aprobación de los vendedores). *Colony health management (vendor quality and vendor approvals).*
I- Desarrollo de protocolo y adiestramiento. *Training and protocol development.*
J- Rutina de inspecciones de facilidades, mantenimiento y reparaciones. *Routine facility inspections, maintenance and repairs.*
K- Facturación rutinaria. *Routine billing.*

VII- El "per diem" no incluye. Posible costo adicional
The "per diem" does not include. Possible additional cost

A- Quarantine Assays.

B- Gestión de cuarentena de proveedores no aprobados. *Quarantine management from unapproved vendors.*
C- Procedimientos de rederivación. *Rederivation procedures.*
D- Actividades de investigación (Injecciones, muestras de tejidos, anestesia, apoyo quirúrgico y cuidado post- op y servicios de apareos). *Research activities (Injections, tissue samples, anesthesia, surgical support, post-op care, and breeding services).*
E- Servicios técnicos especiales incluyendo requisiciones de drogas, materiales solicitados por el PI. *Special technical services including requisition of drugs, supplies or materials requested by PIs.*
F- Solicitud especial de PPE (*Personal Protective Equipment*). *Special PPE (Personal Protective Equipment).*
G- Transferencias, importaciones / exportaciones o costos de envío no aprobados. *Unapproved transfers, imports/exports or shipping costs.*
H- Atención de emergencia o tratamientos causados por actividades de investigación. *Emergency care or treatments caused by research activities.*
I- Necropsias relacionadas con la investigación. *Research related necropsies*
J- Gastos de envío y manejo. *Shipping and handling fees.*
K- Cambios adicionales en la jaula más allá de las prácticas estándar del CRA, debido al modelo de investigación o los requisitos del protocolo. *Extra cage changes beyond ARC standard practices due to research model or protocol requirements.*
L- Requirements (i.e. PU/PD models or reduced bedding requests)
M- Equipo especializado/instalaciones en lso cuartos, almacenamiento y costo de remoción. *Special equipment/room installation, storage, or removal costs*
N- Solicitudes de alojamiento o sala de procedimiento especializado. *Dedicated housing or procedure room requests.*
O- Solicitudes especiales de facturación. *Special invoicing requests.*
P- Mantenimiento especial (dieta, agua, camada) *Special husbandry (diets, water, bedding).*

Aprobado por: Waleska Crespo

Approved by:

Fecha: 30-abril-2020

Date: 30 / abril / 2020

Appendix
#4

Standard Operating Practice (SOP)
Apareos
(Breeding)

UNIVERSIDAD CENTRAL DEL CARIBE
Standard Operating Practice (SOP)
Apareos
(Breeding)
Centro de Recursos Animales

Funciones del Centro de Recursos Animales (CRA):

Functions of the Animal Resource Center (ARC):

Las funciones principales del CRA son: cuidar a los animales que se albergan en estas facilidades y proporcionar información sobre la compra, manejo básico, cuarentena, atención médica veterinaria de animales de laboratorio, utilizados en los programas de investigación y enseñanza de la Universidad Central del Caribe, así como asistencia técnica, asesoramiento y consultas sobre animales utilizados en programas de investigación, poniendo a disposición los suministros para los animales.

The main functions of the Animal Resource Center are to care for the animals that are lodged in these facilities and provide information on the purchase, basic management, quarantine, and veterinary medical attention of laboratory animals used in the research and teaching programs of the Universidad Central Caribe, as well as providing technical assistance, advice and consultations on animals used in research programs, and making available supplies for animals.

Adquisición de Animales

Acquisition of Animals

El investigador al adquirir animales de laboratorios, tiene dos opciones:

The researcher, when purchasing laboratory animals, has two options:

1- **Recurso: Casa Comercial-** estas casas suplidoras de animales, son aquellas que son proveedores en animales de laboratorio. Se especializan en la reproducción, productos y servicios en animales. Estos servicios incluyen: información sobre modelos de animales, educación, entre otros. Estas deben estar certificadas o tener licencia que garanticen su integridad en todos sus procesos. En estas existen revisión de informes de salud, también de una comprensión de las prácticas de manejo, así como de la metodología y la frecuencia de las pruebas.

   Una vez aprobado este recurso para obtener los animales de laboratorio, por medio del *Application for Protocol Approval Involving Laboratory Animal Use* (protocolo), el investigador deberá coordinar con el Supervisor del CRA la compra y el recibo de éstos.

   1- Commercial establishment resource - establishments supplying animals are suppliers of laboratory animals, specializing in reproduction, products, and services. These services include information about animal models and education, among others. These establishments must be certified or have a license that can guarantee integrity in all their processes. In these establishments, there are reviews of health reports and an understanding of management practices, as well as a methodology and a specific frequency of tests.

Once this resource has been approved for obtaining laboratory animals, utilizing the Application for Protocol Approval Involving Laboratory Animal Use (protocol), the investigator must coordinate with the Supervisor of the ARC the purchase and receipt of the animals.

2- **Recurso: No Comercial**- este recurso puede ser un hospital, universidad o alguna otra agencia. Estos no se dedican al comercio de la reproducción de animales. Esta alternativa puede ser seleccionado por el investigador principal, cuando el animal no se puede obtener comercialmente.

Una vez sea justificado y solicitado este recurso por el investigador principal, por medio del *Application for Protocol Approval Involving Laboratory Animal Use* (protocolo), y aprobado por el IACUC, este investigador deberá coordinar con el Supervisor del CRA la compra y el recibo de los mismos. En este proceso se le estará indicando el tipo de *"health profile",* para ser enviado por el recurso seleccionado. Este *"health profile"* no deberá tener más tres meses de realizado.

2- Non-commercial resource - this resource can be a hospital, university, etc. These resources are not engaged in the trade of animal reproduction. This alternative can be selected by the principal investigator when the animal cannot be obtained commercially.

Once this resource is justified and requested by the principal investigator, the Application for Protocol Approval Involving Laboratory Animal Use (protocol) is implemented, and, once approved by the IACUC, the investigator must then coordinate with the Supervisor of the ARC the purchase and receipt of the animal. In this process, the health profile of the animal in question will be indicated to the non-commercial resource and that is the one meant to be sent by the selected resource. This health profile should not have more than three months of completion.

3- **Recurso: Apareo en UCC**- este es sólo autorizado cuando los animales no pueden ser adquirido comercialmente. Una vez sea justificado y solicitado este recurso por el investigador principal, por medio del *Application for Protocol Approval Involving Laboratory Animal Use* (protocolo) y aprobado por el IACUC, éste deberá cumplir con el "*Mice Breeding Protocol"* y su adiestramiento, coordinado con el Supervisor del Centro.

El CRA puede tener una variedad de cepas en ratones con unas especificaciones diferentes, por tanto, es importante que el investigador provea su personal para el trabajo con los mismos. El CRA no será responsable de los factores que puedan impedir que estos animales se reproduzcan efectivamente, no obstante, el CRA estará velando por el cumplimiento en el manejo realizado con la reproducción de los animales. El CRA está basado en el mantenimiento de una colonia de animales experimentales y no en reproducción.

3- Resource-Breeding at the UCC- this is only authorized when the animals cannot be acquired commercially. Once this resource is justified and requested by the principal investigator, the Application for Protocol Approval Involving Laboratory Animal Use (protocol) is implemented, and once approved by the IACUC, it must then comply with the Mice Breeding Protocol, including any training involved, and coordinated with the Supervisor of the Center.

The ARC can have various strains of mice with different specifications; therefore, the researcher must provide their personnel to work with these animals. The ARC will not be responsible for factors that may prevent these animals from reproducing effectively. The ARC is founded on maintaining a colony of experimental animals and not on the reproduction or breeding of these animals.

Método de Apareo Opcional

Optional Mating Method

Actualmente este Centro sólo tiene una cepa de ratas (Sprague Dawley), donde se beneficia varios proyectos de investigación. Por tanto, para poder controlar el inventario y los costos que afectan el presupuesto de estas facilidades, el Centro realizará los apareos en esta cepa de ratas. No obstante, el investigador principal deberá siempre someter en su protocolo el recurso de la casa comercial.

Currently, the Center only has one strain of rats (Sprague Dawley) for the use and benefit of several research projects. Therefore, to control the inventory and the costs that affect the budget of these facilities, the Center will be breeding this strain of rats. However, the principal investigator must always submit a commercial resource in his protocol for animal acquisition.

Para tener disponible estos animales, estos deberán ser solicitados al CRA, por medio de la forma *"Internal Form".* En esta forma deberá observar lo siguiente:

To have these animals available, they must be requested to the ARC through an Internal Form provided. This form should be completed in the following manner:

- Llenar la descripción de la información solicitada. Fill in the requested information.
- El tiempo de solicitud de los animales, dependerá de los requisitos de los animales (edad, sexo, etc.). Enviar solicitud de (6) a (7) semanas con anticipación, como mínimo. Puede consultar con el Supervisor del Centro.

  The point in time to request the animals will depend on the requirements of the animals (age, sex, etc.). Send the request at least (6) to (7) weeks in advance. The advice of the Center Supervisor can be sought on this matter.

- Cantidad de animales: el CRA tiene un espacio límite para albergar los animales.

  The number of animals: the ARC has limited space for housing animals.

- La solicitud **NO LE GARANTIZA LA DISPONIBILIDAD DE LOS ANIMALES.**

  The request **DOES NOT GUARANTEE THE AVAILABILITY OF ANIMALS.**

El investigador con su personal será responsable de cancelar cualquier solicitud de animales, al menos con (2) semanas de anticipación e informarlo por escrito al Supervisor del CRA. Esto con el propósito, para que el CRA pueda redirigir los

animales a otro proyecto de investigación y minimizar los costos de mantenimiento de los mismos.

Each investigator with their staff will be responsible for canceling any animal requests at least two (2) weeks in advance and informing the Supervisor of the ARC of this in writing. This has the objective of having the ARC redirect the animals to another research project and thus minimize the animal maintenance costs.

La disponibilidad de los animales en el CRA, les será informado por medio de un reporte. De no tener animales disponibles, el investigador podrá comprar los animales mediante los procesos establecidos por el CRA y la oficina de Compra.

Information on the availability of the animals will be made utilizing a report. If there are no animals available, the researcher will purchase the animals through the processes established by the ARC and the Purchasing Office.

Se llevará un expediente de todos los animales disponibles para el investigador. De igual manera, de todo aquel investigador que una vez solicite y luego no use los animales. De este investigador presentar un exceso de animales solicitados y no usados, sin previa cancelación, el CRA realizará lo siguiente:

- Un reporte, enviado al investigador principal, con copia al presidente del IACUC y a la oficina del Decanato de Investigación.
- el número de animales solicitados en la solicitud:
  - ✓ puede ser cancelada
  - ✓ haber una reducción de animales
  - ✓ ser cancelada su solicitud y/o ser redirigida a otro proyecto.
  - ✓ Esto hasta que haya un comunicado por escrito de compromiso de uso de estos animales.

A file of all the animals available for the researcher and every researcher who has requested and does not use the animals will be kept. If this researcher shows an excess of animals requested and not used, without prior cancellation, the ARC may do the following:

- A report will be made and sent to the principal investigator, with a copy to the President of the IACUC and the Deanship of Research.
- the number of animals requested:
  - ✓ can be canceled outright
  - ✓ a reduction can be made in the requested number of animals
  - ✓ the request can be canceled and/or be redirected to another project.
  - ✓ These events can continue to occur until there exists in writing a commitment from the researcher to the use of the animals requested.

Todo animal en proceso de reproducción: entrará en inventario. Por lo tanto, estos animales serán considerados en los gastos diarios (perdiem) del investigador por el CRA y en toda propuesta sometida por el investigador.

Every animal in the reproduction process will enter inventory. These animals are to be considered and

reflected in the researcher's daily expenses (per diem) by the ARC and any proposal submitted by the researcher.

Toda reproducción de animal de laboratorio estará sujeto a lo siguiente:
All reproduction of laboratory animals shall be subject to the following:

- espacio disponible available space
- presupuesto del investigador (proyecto) a sufragar el costo del perdiem.
  budget of the researcher (project) that covers the cost of the per diem.
- presupuesto del CRA ARC's budget
- cumplimiento por el investigador y su personal de las normas establecidas por el CRA y el IACUC.
  compliance by the researcher and his staff with the rules and regulations established by the ARC and IACUC.

2019.10.04

Appendix
#5

VISITING STUDENT SPECIAL PROCEDURES DUE TO COVID-19

Visiting Student Special Procedures Due to COVID-19

School of Medicine

COVID-19 is a rapidly evolving situation, which requires the review and updating of security protocols to ensure UCC's safety of all staff, students and external visitors. We have determined that, for the duration of the pandemic, additional procedures to the regular requirements for Visiting Students, will be in place. The regular ones can be retrieved from UCC webpage http://www.uccaribe.edu/research/?page_id=5226 We count of your understanding and cooperation.

New Covid-19 Procedures:

1) **Student itinerary**: The mentor must list the places the student will be visiting at UCC (departments, laboratories, instrumentation areas and facilities to be used.

2) **Student schedule**: The mentor must state the days and working hours. At daytime schedule, or on weekdays, from Monday through Friday. In the research labs, his /her mentor will physically supervise the student at all times.

3) **Safety orientation:** Before start working in the lab, the mentor shall provide an orientation to the student, in order to follow UCC's and the lab's protocols; to keep social distancing and the safety/cleanliness precautions required to be in the workplace required during the pandemic. The student will sign a ratification of the orientation provided. A copy of this document will sent to Mrs. Joelis Burgos, at Deanship of Admissions and Student Affairs, to be included in the student file.

4) **Negative COVID-19 test result:** During this pandemic situation, following the Puerto Rico Health Department requirements for the safe entry to a workplace, the student must hand-in a negative result for COVID-19 molecular test from 72 hours prior to his/her arrival to work. A copy of this document will also be sent to Mrs. Joelis Burgos, at Deanship of Admissions and Student Affairs, to be included in the student file.

Once all documents are collected and revised:

1) The Deanships of Administration will certify the completeness of documents, and send a written notification to the Interim Dean of Medicine for access approval or disapproval.

2) After approval, the Deanship of Administration will grant access to the visitor student by adding them to the LIST of personnel with access to UCC (the list that the security guard holds in the entrance of UCC).

Appendix
#6

Policy of Use of Cell Lines in Live Animals

Policy of Use of Cell Lines in Live Animals

The confounding effects of viral infections (often without signs of clinical disease) of experimental animals, e.g. mouse hepatitis and ectromelia are well documented in the literature. [2] These infections can have devastating effects on experimental results as well as on production of animals from breeding colonies.[3] of equal concern is the potential for infections among laboratory workers. Infections with zoonotic agents such as Hantavirus [4] and lymphocytic choriomeningitis[5] have been traced to contaminated biological material transplanted into animals.

Biological material and animal products such as cell lines, tissues, and tumors have been repeatedly incriminated as vehicles for the introduction of animal pathogens into animal colonies. The prevention of accidental introduction of viral infections into research animals is essential to maintenance of the animal colonies at the university. To maintain the integrity of the animal colonies the Institutional Animal Care and Use Committee has established the following guidelines regarding the introduction of cells, tumors, and other biologic products into experimental animals:

1. Investigators are responsible for ensuring that the biologic materials used in their study will not endanger the health of study animals or other animals housed in the facility. Investigators planning to introduce tumors, tissues, or cells into experimental animals must complete the relevant section of the Animal Exposure.

2. Cell lines, tumors, or other biologic materials originating from rodents or passage through rodents must be certified free of murine pathogens prior to their introduction into UCC animal facilities. This includes cell lines purchased from ATCC or other commercial sources.

3. Investigators must provide the IACUC with documentation that the material has been tested and verified free of pathogens before introducing these materials into animals. Cell lines, tumors or other materials for which no documentation is provided must be tested. The preferred method for testing material is by polymerase chain reaction (PCR) procedures.

4. Any material found to be positive for murine pathogens must not be used. Once the material is shown to be cleared of the agent(s) and the proper documentation is on file in the ARC office, the material may be used for experiments.

5. Human cell lines that have not been passage through rodents do not require testing for murine viruses.

6. Cell lines passage through rodents and frozen for storage and later use must be retested when thawed. More frequent testing of cells lines may be required if: 1)

rodent pathogens are detected in the room where the cell lines are being used; 2) the material will be transferred to animals in a campus facility other than that in which it was originally used; 3) cell lines have been passage through animals housed in an off campus facility and returned to USC; or 4) as otherwise determined by the Attending Veterinarian and/or IACUC.

References:

1. Adapted from: University of South Carolina, Graduate Science Research Center/IACUC, South Carolina,USA.
2. Committee on Infectious Diseases of Mice and Rats, Institute of Laboratory Animal Resources. Infectious Diseases of Mice and Rats Pub 397 (National Academy Press, Washington, DC, 1991.
3. Baker, D.G. Natural pathogens of laboratory mice, rats, and rabbits and their effects of research. Clin. Microbiol. Rev. 11, 231-266 (1998).
4. Lloyd, G. & Jones, N. Infection of laboratory workers with Hantavirus aquired from immunocytomas propagated in laboratory rats. J. Infect. 12, 117-125 (1986).
5. Hinman, A.R. et al. Outbreak of lymphocytic choriomeningitis infections in medical center personnel. Am. J. Epidemiol. 101, 103-110 (1975).
6.Herman, P., & Pauwels, K. (2014). Biosafety Recommendations on the Handling of Animal Cell Cultures. Animal Cell Culture, 9, 689–716. https://doi.org/10.1007/978-3-319-10320-4_22
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7122109/
7. Biosafety levels https://www.phe.gov/s3/BioriskManagement/biosafety/Pages/Biosafety-Levels.aspx

Appendix
#7

Recommendations for Aseptic Technique, Anesthesia, Analgesia and Post-Operative Care for Rodent Surgery

Recommendations for Aseptic Technique, Anesthesia, Analgesia and Post-Operative Care for Rodent Surgery

Goal: To minimize pain and distress in rodents during and after experimental procedures. This document are properly recommendations in the correct technique and that anesthesia, postoperative pain medication and care are provided to the animals.

Investigator Responsibility:

- Ensure adequate aseptic technique
- Monitoring animals for pain and distress
- Intervening to reduce pain and distress
- Judicious use of anesthetics and analgesics
- Implementation of humane endpoints

Rationale: Federal animal welfare laws and policies mandate the scientist's responsibility for the humane care and use of laboratory animals. Minimizing pain and distress also reduces the impact of these extraneous factors (e.g. as non-experimental variables) on research.

Who is involved: Investigators, Research Assistant, Animal Caretakers, Veterinary staff.

A dynamic collaboration between scientists, animal caretakers, and veterinary staff involving continuing observations of the animals will be most productive for developing humane interventions that benefit the scientific outcome of a study.

Recommendations for Aseptic Technique

The following procedures are recommended to ensure adequate aseptic technique:

A. Animal Preparation

1. The hair over the surgical site should be clipped using #40 or #50 clipper blade, taking care not to cut the skin. This should be performed in an area separate from where the surgery is to be conducted.
2. Rodents should be anesthetized according to the Application for Protocol Approval Involving Laboratory Animal Use Form.
3. Once the toe pinch response is lost, anesthetic depth is sufficient for surgery; the animal's ears and feet, and mucous membranes of the eyes and nose should be pink indicating adequate oxygenation.
4. If the animal's eyes are open, artificial tears ointment should be applied for protection and lubrication if the animal is anesthetized for more than 5 minutes.
5. The surgical site should be scrubbed (two or more times) with Betadine, Chlorhexidine, or another approved antiseptic scrub. In between scrubs, rinse the

site with 70% ethyl or isopropyl alcohol or sterile water. Clean (preferably sterile) gauze must be used and you must start at the incision site and spiral outwards (do not go back to the incision site with the same gauze). Follow this by wiping with the comparable solution. Although not the preferred recommendation, if a final 70% ethyl or isopropyl alcohol wipe is used, you must ensure that drying time is permitted prior to making an incision as ethanol residues can cause tissue damage in the incision. Please see Table 1.

6. The rodent should be carefully placed onto a warm surface and positioned for surgery.

B. Surgeon Preparation

1. Surgery should be conducted in a disinfected, uncluttered area that promotes asepsis during surgery. Please see Table 2.
2. Scrubs and personal protective equipment (as dictated by the facility requirements and including a mask) should be donned by the surgeon.
3. Hands should be scrubbed thoroughly with antibacterial soap and new gloves (disposable or sterile) should be worn.

C. Surgical Instruments

1. Between animals, the instruments should be cleaned of particulate matter and placed in a scientifically acceptable disinfectant solution or a glass bead instrument sterilizer. The instruments should be wiped dry prior to use. If a hot bead sterilizer is used, allow adequate time for the instruments to cool before use. Please see Table 3.
2. After all surgeries are completed, the instruments should be thoroughly cleaned prior to packing for the autoclave.

D. Surgical Procedure

1. The animal must be maintained in a surgical plane of anesthesia throughout the procedure (i.e., absence of toe pinch reflex).
2. Surgical drapes may be helpful for some procedures.
3. Begin surgery with sterile instruments and handle instruments aseptically.
4. When using “tips only” technique, the sterility of the instrument tips must be maintained throughout the procedure.
5. Instruments and gloves may be used for a series of similar surgeries provided they are maintained clean and uncontaminated between animals. Please see Table 4.
6. Monitor and/or maintain the animal’s vital signs.
7. Absorbable suture material or electrocautery should be used to control bleeding.
8. When the ventral abdominal cavity is opened, the abdominal lining, (peritoneum), and muscle layer must be closed with an appropriate number (for the length of the wound) of absorbable sutures. The skin should be closed separately.

9. When the peritoneal cavity is opened from a dorsal approach (incision on the back), it is recommended that absorbable sutures be used to close the peritoneum prior to skin closure.
10. Please see Table 5 for wound closure selections.

Mouse & Rats Anesthesia and Analgesia

Federal regulations mandate that animals undergoing potentially painful procedures be provided with adequate anesthesia and analgesia. The Universidad Central del Caribe, Institutional Animal Care and Use Committee (IACUC) has developed the following recommendation to help research investigators with updated practice standards for rodent (mice & rats) anesthesia and analgesia, related to changes in literature and updated information in the field of laboratory animal medicine. Exceptions to these principles are permitted only if scientific justification is provided in the Form Application for Protocol Approval Involving Laboratory Animal Use (protocol) and approved by the IACUC.

When writing the IACUC protocol, it is encouraged to include dose ranges to allow for appropriate flexibility. Specific doses drawn from the suggested ranges may be procedure-, strain-, gender-, and age-specific; please consult with Animal Resources Center (ARC), veterinary staff if needed.
This recommendation document concentrates on the following topics regarding the use anesthesia and analgesia in mice:

- Definitions
- Anesthesia
    - Non-painful procedures
    - Minor surgical procedures
    - Major surgical procedures
- Analgesia

A. Definitions

Non-painful procedures (e.g., imaging, restraint)
Sedation—the animal has suppressed spontaneous movement and decreased agitation, curiosity and aggression. The animal can respond to external stimuli (including pain) if the stimulus is of adequate intensity. The state is not associated with any analgesic effect.
Surgical plane of anesthesia—the animal is unconscious and does not move in response to a noxious stimulus. The animal should not respond to external stimuli (including pain).
Minor surgery—Minor survival surgery does not expose a body cavity and causes little or no physical impairment (e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy, and device implantation in the subcutaneous space). These procedures are routinely done on an “outpatient” basis in veterinary clinical practice.
Major surgery—Major survival surgery (e.g. laparotomy, thoracotomy, joint replacement, and limb amputation) penetrates and exposes a body cavity, produces substantial impairment of

physical or physiologic functions, or involves extensive tissue dissection or transection. (e.g., thoracotomies, laparotomies, craniotomies, head caps)
Analgesia—Relief of pain to a normally painful stimulus.
Pre-emptive analgesia—Analgesia delivered before the painful stimulus. Provision of pre-emptive analgesia is consistent with standard veterinary practice

B. Anesthesia

The use of ISOFLURANE inhalant anesthesia for rodent procedures is recommended, due to its wide safety margin, reliability, ease of administration, and rapid return to consciousness for animals after exposure has ended. Use of anesthetic regimens other than isoflurane may be chosen if required for the specific research model. Scientific justification for other anesthetic protocols may be required by the IACUC.

Inhalant anesthetics (i.e., isoflurane) – Delivery of inhaled anesthetics by mask or endotracheal tube via a precision vaporizer is recommended for all non-aquatic species. Adjusting the inhaled percentage of anesthetic gas to deepen anesthesia is far safer than repeated re dosing of injected drugs. Volatile anesthetics are easier to decrease as well, even compared to drugs for which there is an injectable antagonist or reversal agent. A disadvantage of the inhalant anesthetic agents is the lack of residual analgesia once the vaporizer has been turned off; pre-emptive analgesia is necessary.
Injectable anesthetics (i.e., ketamine combinations, pentobarbital) – Injectable anesthetics are appropriate for many procedures. There is, however, a great deal of variation in depth and duration of anesthesia among rodent strains and individual animals.
Local anesthetics (i.e., lidocaine, bupivacaine) – Local anesthetics are usually injected at the site of the incision and may be appropriate to consider as supplements to either inhalant or injectable anesthetics.
Please see Table 6

C. Stages of Anesthesia

During induction of general anesthesia, animals pass through various stages indicative of the level of anesthesia.

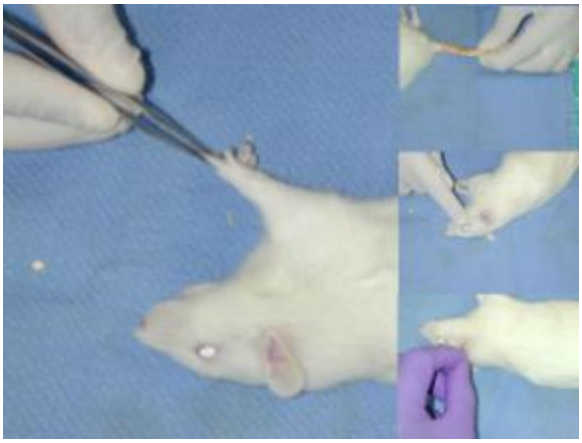
- Stage 1 — excitatory, disorientation, vocalization, urination, defecation.
- Stage 2 — loss of consciousness with or without struggling and whining, many reflexes are intact but righting reflex is lost, rapid irregular breathing and rigidity.
- Stage 3 — surgical stage of anesthesia, with loss of reflexes, muscle relaxation, deep and rhythmic breathing, planes 1-4 (light to deep).
- Stage 4 — medullary paralysis with respiratory arrest, hypotension and imminent death. Cardio-pulmonary resuscitation and drugs to reverse anesthesia must be given or animal will die.

D. Signs of inadequate anesthesia

Adequate general anesthesia is accompanied by loss of muscle tone reflected in loss of purposeful movements, however, hamsters and gerbils may retain "swimming" or purposeless movements even in deep surgical anesthesia. There is loss of reflexes for example corneal, pinnae and pedal. There should be no response to aversive stimuli e.g. tail pinch, pinching abdominal skin with forceps and a lack of vocalization. Twitching of whiskers is lost with progression from light to medium anesthesia. There are changes in the depth and frequency of respiration and cardiovascular parameters.

E. Monitoring the depth of anesthesia

- Assess movement, stimulus perception and reflexes - [cornea, toe, tail or ear]
- Observe chest wall movement
- Pulse, heart rate, direct or indirect blood pressure (cuff or Doppler)
- Mucus membrane color at muzzle, feet, ears, tongue
- Temperature
- Ancillary equipment e.g. pulse oximetry, end tidal carbon dioxide (capnometry).



F. Analgesia

As with anesthesia, the recommendations for analgesics will depend upon the procedure being performed. Pre-emptive analgesia refers to providing the analgesics prior to the painful event and is recommended unless scientifically justified. The first dose of the analgesic should be administered PRIOR TO the surgical procedure, i.e. before the “first cut” is made. Use of analgesic protocols may be chosen if required for the specific research model—all surgical protocols require anesthesia and analgesia, unless specifically justified by the PI and approved by the IACUC.

Opioids (i.e., buprenorphine, morphine) – Opioids are very effective analgesics for surgical pain but may have effects on cardiovascular function and can be sedating.

Non-steroidal anti-inflammatory agents (i.e., meloxicam, carprofen, ketoprofen) – Newer, longer-lasting non-steroidal anti-inflammatory analgesics (NSAIDs) may have longer durations of action than available opioids. These drugs are frequently co-administered with an opioid to combine potency of effect with duration of action.
Please see Table 7

G. Best Practices

Multi-modal drug administration – Using a combination of agents (multi-modal anesthesia and analgesia) is recommended. This practice can help maximize the desired effects while minimizing the side effects that occur with over-reliance on a single agent.

Pre-emptive analgesia – Pre-emptive analgesia or administration of pain relief before the painful stimulus is recommended:

- To ensure that pain is being treated as the general anesthetic is wearing off;
- To lower the overall amount of general anesthetic required; and,
- To prevent sensitization of pain mechanisms ("ramp up")

Frequency of analgesic administration – Analgesic doses and frequencies should be carefully considered. Careful planning is required for overnight pain management. Many analgesics administered at 5 pm will wear off before 8 am the next morning. Multimodal analgesia is recommended to combine potency of effect with duration of action.

Additional supportive care – See: *Post Procedure Care of Mice and rats in Research: Reducing Pain and Distress.* Non-pharmaceutical methods to enhance the administration of anesthetic and analgesic agents should be used and include:

- Keeping the animal warm during and after anesthetic procedures
- Fluid administration
- Keeping recovering animals isolated in a quiet area
- Providing supplemental foods. Contact with ARC the veterinary staff for additional information on supportive care.

Monitoring – Plans for intra- and post-operative monitoring must be included in the IACUC. Monitoring anesthesia includes responsiveness to painful stimuli, character of respiration, and skin or mucous membrane color as seen by observing the ears, tail, and oral mucosa or foot pads. Pedal withdrawal reflex (footpad-pinch) is recommended for assuring adequate depth of anesthesia prior to first incision and as a repeated check throughout the procedure. Depending on the procedure, other monitoring may be indicated such as heart rate, blood pressure, body temperature, and tissue oxygenation. Monitoring should be recorded through the post-operative period to complete recovery.
Please see Table 8

Dose ranges and titration – All drugs, dose ranges and routes of administration must be listed in the IACUC. Dose ranges are starting points which must be titrated up or down for the individual animal, or for the particular application (procedures conducted, animal age and strain differences). When laboratory experience finds that recommended dose ranges are consistently too high or too low for the particular application, the veterinarian should be informed, and a protocol amendment submitted to the IACUC. Anesthetics are always titrated

to effect. It is not acceptable to conduct surgical procedures unless the animal is fully anesthetized.

Recordkeeping – Administration of anesthesia and analgesia and peri-operative monitoring should be recorded. Depending on the species, records may be kept in the animal's individual medical record or in laboratory records and on postoperative card cards. Records should extend through the period of complete recovery, and should document post-operative care and analgesia that is provided. Minimum required documentation includes:

- Procedure date
- Individual animal or cage ID
- Procedure performed
- All drugs administered o All observations made
- Any Surgical or anesthetic problems

Training – The very best anesthetic plans are only as good as the skill and care with which they are applied. Required procedure-specific training will be detailed on the IACUC.

H. Controlled Substances

Several commonly used anesthetics and analgesics (i.e., opioids, ketamine) are controlled substances and require special procedures to be completed prior to use in animal research.

More information can be found at the Pharmacology Department of UCC.

Post Procedure Care of Mice and Rats in Research: Reducing Pain and Distress

A. Sources of pain or distress: Pain or distress may be caused by spontaneous or experimentally induced disease or injury. Other factors such as extreme homeostatic challenges may contribute to an animal's distress or discomfort.

B. Systematically monitoring for Pain and Distress: Animals must be monitored by the investigator when distress or illness is expected. The frequency of monitoring depends upon the severity of the animals' condition, the expected rate of change in the animals' status, and the impact of the procedure on the animals.

- ✓ At a minimum, all post procedural animals should be monitored once a day (until stable).
- ✓ Major survival surgery would require at least twice a day monitoring for the first 24-48 hours which corresponds to the need for the administration of pain-relieving drugs.
- ✓ Studies of chronic or progressive diseases may require increasingly more frequent monitoring as the disease gradually develops.

- ✓ Some situations may require hourly or even continuous monitoring during critical periods in which rapid change in the animal's condition would be anticipated.

A monitoring plan should be described in detail for each procedure that would be expected to cause pain or distress.

C. Detecting Clinical Signs of Pain and Distress

Signs of pain and distress in rodents are not easy to detect because of their small body size, their tendency to conceal outward signs of pain, and their habit of hiding or freezing when disturbed. Pain and distress can be detected by carefully observing subtle changes in behavior. Healthy mice and rats have clean, sleek, well-groomed fur, and good skin and mucosal color. They are alert, socially active, inquisitive, and tend to explore the cage perimeter. Normal posture is somewhat stretched out and the animals move quickly and smoothly around the cage.

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D. Appearance and Behavior: Observations

1. From the cage exterior:

Routinely inspect the rodents through the top and sides of the cage. Get in the habit of removing the cage from the shelf and looking through all sides of the cage. Signs of distress may be missed in animals on lower or upper shelves because of low lighting or difficult access. Newborns may be inconspicuous within piles of bedding or nestles.

2. Remove Wire lid:

Lift the wire lid to elicit a response to your presence. This disturbance may prompt the animals to move about the cage. Examine the animals' behavior, gait, and hair coat. Abnormal mice or rats may huddle in their cage or may fail to move around and explore. Rats may vocalize when approached. Inspect an animal's mode and speed of movement. Observe the tail position when the animal moves

- ➢ Is the gait awkward?
- ➢ Does the animal teeter or stumble?
- ➢ Is the back hunched and abdomen tucked under while walking?
- ➢ Is the tail stiff and upright?

E. Appearance and Behavior: Assessment for Abnormalities

<u>Use of monitoring sheets is suggested to track as many of the following parameters as needed:</u>

- ✓ Activity Level
  Inactivity: hunched, huddled, lethargic
  Hyperactivity: restlessness, lack of inquisitiveness

- ✓ Attitude
  Arousal, depression, awareness of surroundings
- ✓ Behavior, spontaneous (observed without disturbing the animal)
  Vocalization, self-trauma, isolation from cage mates
- ✓ Behavior, provoked (observed when the animal is disturbed or prodded)
  Vocalization, hiding, aggressiveness, minimal response
- ✓ Body condition and Weight
  Thin, emaciated, abdominal distention, missing anatomy
- ✓ Food and Fluid intake
  Inappetance or anorexia; dehydration; pica behavior (eating foreign objects)
- ✓ Fecal and urinary output
  Fecal color, size, quantity and consistency; urine color and quantity
- ✓ Fur and skin
  Unkempt, greasy or dull fur; porphyrin (red) staining around the eyes and nostrils; cyanotic, pale or congested mucous membranes or skin (ears, feet, tail), skin lesions; soiled perineum
- ✓ Eyes
  Clarity/condition of lens and cornea; position of globe (sunken or protruding more than normal), condition of eyelids, discharge or encrustation, porphyrin (red) staining
- ✓ Posture
  Hunched back, tucked abdomen; prostrate; head tucked down
- ✓ Locomotion
  Gait, ataxia, lameness, action of each limb, position of tail when walking
- ✓ Neurological
  Tremor, convulsion, circling, paralysis, head tilt, head pressing, coma
- ✓ Vital signs
  Respiratory distress (open mouthed breathing, pronounced chest movement)
- ✓ Other clinical parameters relevant to your study
  Presence and status of tumors, infection, or surgical wounds,
  device placement and integrity

F. Physical Exam: Assessment

1. Body Weight (BW): A change in BW is a sensitive indicator of rodent health; Baseline measurements are important for long term studies. Reduction in body weight may reflect starvation, dehydration or a combination of both. Failure of young animals to gain weight is equivalent to a loss of body weight. It is helpful to compare body weights of treated animals to normal controls.

2. Body Condition: Evaluate rodents for emaciation or cachexia (body wasting) by palpating the lumbar spine and iliosacral areas. A scoring system can be applied to the progressive loss of fat and muscle mass to gauge the severity of emaciation. Generalized loss of muscle mass makes the spine appear prominent.

3. Weight Loss: Rodents typically have a reduced food and water intake 1-2 days post-surgery. Low food intake may be more severe and prolonged if animals are experiencing pain and distress (e.g. if pain control is inadequate). Nutritional support and fluid therapy are important for enhancing post-op recovery and for non-surgical studies that result in morbidity and inappetance.

Nutritional Support: Rodents have high energy requirements due to their small size and high metabolic rates. They also often have minimal fat reservoirs that can be mobilized to supply needed energy. Nutritional support is critical on recovery to avoid hypoglycemia (especially if the animal was fasted prior to anesthesia induction). Stimulating appetite to increase food intake is helpful to promote a more rapid recovery in rodents. Something that tastes different and better than the daily ration may be appealing to rats and mice and so may stimulate their appetites. All nutritional supplements must be approved by veterinary.

Methods to provide nutritional support include:

- Providing a high-quality pelleted rodent diet as soon as the animal has recovered sufficient to ambulate and eat.
- Mice should not be held of feed for longer than 4 hours; rats for not longer than 6 hours.
- Administering supplemental fluid and nutritional support by feeding gelatin or agar-based diets, ground moistened feed, or small amounts of peanut butter or other high caloric paste-type diets.
- Injecting small volumes (3-12 ml, depending on the size and species) of a warmed 5% w/v dextrose solution subcutaneously.

Normal Daily Food Consumption:
Mouse…….12-18 gm/100 gm body weight
Rat……...5-6 gm/100 gm body weight

4. Fluid and Electrolyte Balance:

Volume deficits can be estimated by comparing pre-surgical body weight and post-surgical/post-anesthesia recovery body weight of an individual animal. Regular, frequent weighing of animals can be used to assess both nutritional and fluid intake deficits during the longer-term postoperative recovery period. Decrease in skin turgor/skin elasticity (which is best assessed by “tenting” the skin over the dorsal lumbar area and evaluating how quickly it returns to its normal position) corresponds to mild to moderate (10%-20%) dehydration. Volume deficits can be corrected by the subcutaneous or intraperitoneal injection of warmed saline, warmed lactated Ringer’s solution, or other warmed balanced replacement fluids. The selected route replacement fluids are administered needs to consider the rate of absorption from the specific site. If fluids cannot be administered intravenous, the intraperitoneal route provides the most rapid absorption into the vascular system. If significant blood loss has occurred, blood transfusions can be administered (usually via the lateral tail vein or jugular vein). With transfusions into inbred and F1 hybrid

rodent strains, as well as naïve animals, blood typing is usually not needed and transfusion reactions seldom occur. Animals that do not have normal daily water consumption within 24 hours of recovery from anesthesia must have the estimated water intake deficit administered to them parenterally or orally (i.e. such as via oral gavage), on a daily basis, until normal intake has resumed. Animals that do not exhibit normal intake of water will not have corresponding normal intake of solid food.

Total Blood Volume:
Mouse……..5.85 ml/100 gm body weight
Rat……...57.5-69.9 ml/100 gm body weight

Normal Daily Water Consumption:
Mouse……. 15 ml/100 gm body weight
Rat……...10-12 ml/100 gm body weight

5. Body Temperature: Due to their large ratio of body surface area to mass and high metabolic rate, rodents lose body heat at a faster rate than large animals. A large decrease in body temperature can be a reliable predictor of death in some studies and may guide the decision of when to euthanatize an animal. Temperature can be measured by implantable microchips (subcutaneous), telemetry, or various ear/skin/rectal thermometers.

Maintaining body temperature and treatment of hypothermia: Rodents lose heat rapidly when under general anesthesia (1 degree per 5 min). It is important to conserve body heat during anesthesia by providing a heat source, thermal insulation or a combination. Keep animals warm until their activity has returned to normal.

Normal Body Temperatures:
Mouse……..98.8-99.3 F (37-37.2 C)
Rat……99.4 F (37.5 C)

Caution!! Provide gentle heat only (max of 40° C or 104°F) to prevent overheating which can cause injury or death. Burns can occur when an animal is positioned too close to a heat lamp (minimum distance is 18 inches from the animal). If recovering animals are warmed within a cage, offer an area for escape from the heating device so as the animals recover, they can leave the heated area for a cooler part of the cage.

Practical ways to provide heat:

- Insulated pouch or wrap
- Chemical warming pads --often too hot and must be wrapped in a towel
- Circulating water warming pads
- Warming racks
- Heat lamps -keep a thermometer near the animal to measure ambient temp

- Electrical heating pads and warming trays must be used under the cage and not in direct contact with the animal

G. Monitoring Tumor Growth

The growth of solid tumors and tumors that cause ascites produces pain and distress in rodents. Some examples:

- ✓ Pain is associated with distension of overlying tissues and ulceration of involved skin
- ✓ Tumors that impinge on joints can impair body movement and locomotion and can restrict the animal's access to food and water
- ✓ Growth of a tumor may cause inappetance and loss of body condition
- ✓ Cytokines released in response to tumor growth may cause tumor cachexia

The specific tumor model will determine the clinical signs to be monitored protocols must describe the type of tumor to be studied, the expected clinical signs, and a proposal that is appropriate for monitoring the expected clinical outcome. During critical periods when the tumor's size or its effect has the potential of causing pain or distress, the animals must be inspected at least daily by the principal investigator or his staff. Animals must be euthanatized before they become moribund or die from tumor load. The animals must also be euthanatized when the tumor mass becomes excessive, ulcerates impairs the animal's bodily functions or behavior.

H. Alleviation of Pain and Distress: General Approach

The effective recognition of pain and distress should not rely on a single clinical observation, but rather on a composite of signs and measurements that together reflect animal well-being. A strategy to manage the adverse effects of the experimental procedures is addressed in the protocol and must include a consistent plan for at least daily monitoring for the potential adverse effects.

A number of analgesic options are available such as local anesthetics (lidocaine, bupivacaine, and xylocaine), opioids (buprenorphine), and non-steroidal anti-inflammatory drugs (carprofen, ketoprofen, banamine, tylenol derivatives). Be familiar with the effective dosing schedule and reassess the animal for pain as the analgesic effect wanes. A green cage tag should indicate the date of surgery and be attached to the cage of post op animals that need to be assessed and treated for pain. An emergency contact number for the PI or other responsible person should be available in the event that an animal is found in pain or distress. Other information may be required.

I. Monitoring Systems

An example of a post-op monitoring system is demonstrated, but can and should be modified to fit your individual research needs.

Post-Operative Monitoring Forms (Parts A-C):

A. Immediate Post-operative period (end of surgery until fully conscious)

Date:_____ Time:_____ Initial Body Weight_____
Animal/Cage Number _____Procedure_____

□Analgesics *given perioperatively*
(Only drugs listed in the protocol should be administered)
- □Buprenorphine (0.05mg/kg given SQ)
- □Carprofen (5 mg/kg given SQ)
- □Bupivacaine (1-2 mg/kg applied topically)

□Fluids (Warm saline given IP or SQ *(during peri-operative period*)
3 ml per 25 g mouse and 15 ml per 250 g rat per day (once or split in two)

□Heat (warm water blanket, chemical packs, hot water bottles)
provided throughout surgery until animal is conscious

□Special diet (Transgenic dough diet, ClearH_20 gel diet, Nutrigel Transgel, crumbled pellets)

Complete Part B for 72 hours from the time of recovery (including weekends) or state the reason below:

- □Spontaneous death
- □Euthanasia
- □IACUC exemption from post procedure monitoring
- □Other: _____

B. Recovery Period

Animal/Cage Number_____ Date:_____ Procedure:_____

Analgesic (Given every 8-12 hours as needed)
Drug:_____
Dose and route:_____

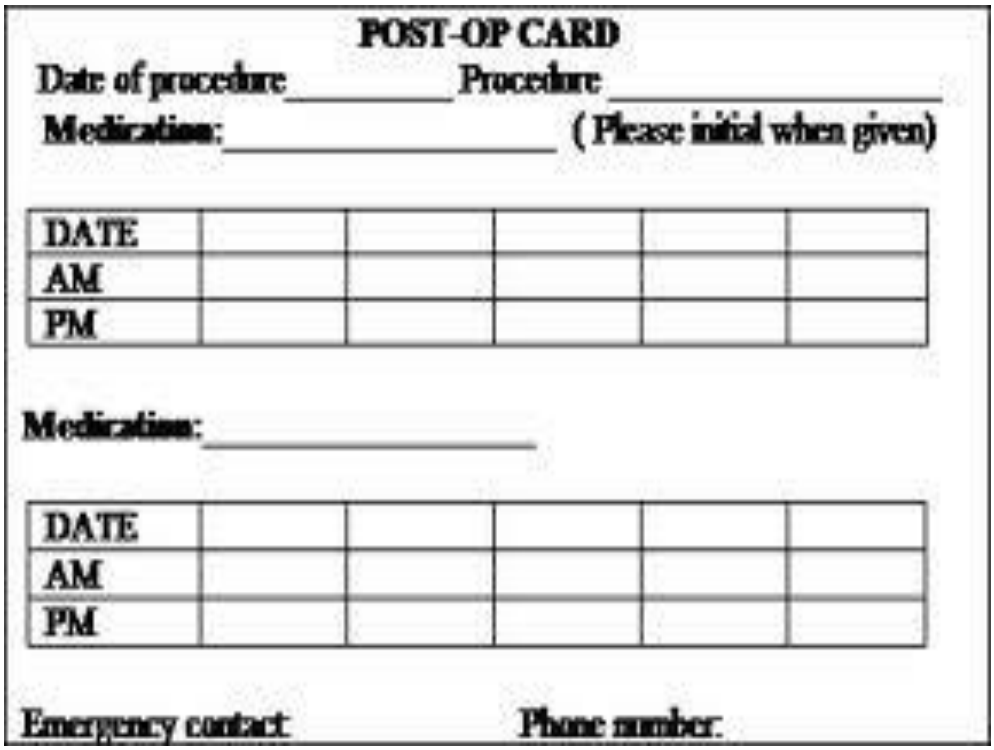
	12 hour	24 hour	36 hour	48 hour	additional
Date					
Time					

Review all boxes; N=normal; If abnormal, write in comment.

	Up to 24 hours	24-48 hours	48-72 hours
Date and Time			
Activity Level			
Attitude			
Behavior (Spontaneous)			
Behavior (Provoked)			
Food and Fluid Intake			
Fecal output			
Urine output			
Fur and Skin			
Eyes			
Posture			
Locomotion			
Neurological			
Respiratory			
Implant Evaluation			
Wound Evaluation			
Other experimentally related signs			

C. Long term monitoring (as stated in your protocol)

Date	Weight	Activity	Appearance	Appetite	Fecal/urine	Other comments	Initials



POST-OP CARD

Date of procedure_____ Procedure _____

Medication:_____ (Please initial when given)

DATE					
AM					
PM					

Medication:_____

DATE					
AM					
PM					

Emergency contact Phone number:

Tables

Table 1 – Skin Disinfectants

Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol or sterile water, followed by final wipe with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals. Please also refer to item A5 for additional guidance.

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

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

Table 2 – Recommended Hard Surface Disinfectants

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

Agent	Examples *	Comments
Quaternary Ammonium	Roccal® Quatricide®	Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within three minutes of contact.
Glutaraldehydes	Cidex®, Cetylcide®, Cide Wipes®	Rapidly disinfects surfaces.

Phenolics	Lysol®, TBQ®	Less affected by organic material than other disinfectants.
Chlorhexidine	Nolvasan® Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.
Hydrogen Peroxide (Clorox Healthcare®)	Clorox Healthcare® Hydrogen Peroxide Cleaner Disinfectant	Rapidly disinfects surfaces without harsh chemical fumes or orders.

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

Table 3 – Recommended Instrument Sterilants

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

Agent	Examples *	Comments
Steam Sterilization (moist heat)	Autoclave	Effectiveness dependent upon temperature, pressure and time (i.e., 121°C for 15 minutes vs. 131°C for 3 minutes)
Dry Heat	Hot Bead Sterilizer Dry Chamber	Fast. Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.
Gas Sterilization	Ethylene Oxide	Requires 30% or greater relative humidity for effectiveness against spores. Gas is irritating to tissue and requires specialized equipment for use. All materials require safe airing time.
Chlorine	Chlorine Dioxide	Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Glutaraldehydes	Cidex®	Several hours required

	Cetylcide® Metricide®	for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.
Hydrogen Peroxide-Acetic Acid	Actril® Spor-Klenz®	Several hours required for sterilization. Corrosive and irritating. Instruments must be rinsed with sterile saline or sterile water before use.

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

Table 4 – Recommended Instrument Disinfectant

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

Agent	Examples *	Comments
Chlorine	Sodium hypochlorite (Clorox® 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)	Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within three minutes. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.
Chlorhexidine	Nolvasan® Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be rinsed with sterile saline or sterile water before use.

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

Table 5 – Wound Closure Selection

Material *	Characteristics and Frequent Uses
Polyglactin 910 (Vicryl®) Polyglycolic Acid (Dexon®)	Absorbable. 60-90 days. Ligate or suture tissues where an absorbable suture is desirable.
Polydiaxanon (PDS®) Polyglyconate (Maxon®)	Absorbable. Six months. Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.
Polypropylene (Prolene®)	Nonabsorbable. Inert.
Nylon (Ethilon®)	Nonabsorbable. Inert. General closure.
Silk	Nonabsorbable. Excellent handling. Preferred for cardiovascular procedures. Caution: Tissue reactive and may wick microorganisms into the wound.
Chromic Gut	Absorbable. Versatile material.
Stainless Steel Wound Clips or Staples	Nonabsorbable. Requires instrument for removal.
Cyanoacrylate (Vetbond®, Nexaband®)	Skin glue. For non-tension bearing wounds. The glue requires adequate moisture and pressure to properly bond wound. Please note that if too much glue is applied, an exothermic (burn) reaction can occur.

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

Table 6: Recommendations for types of anesthetics

Drug	Mouse	Rat
Isoflurane	3-4% for induction and 1-3% for maintenance	
Pentobarbital [1]	40-85 mg/kg	IP 40-50 mg/kg
Ketamine/ Xylazine[2]	90-120 mg/kg* (or 70-100 mg/kg 5-10 mg/kg* (or 5-12 mg/kg SC or IP (not IM) *May not provide surgical anesthesia in mice. Consult with an ARC veterinarian before using this mixture in mice.	90 mg/kg 10 mg/kg SC or IP (not IM)

[1] Not recommended for survival surgery.

[2] Xylazine is a potent respiratory depressant. Re-dosing, if necessary, should be done with 1/2 the original dose of Ketamine alone.

Tribromoethanol [3]	125 mg/kg IP	300 mg/kg IP
A note regarding urethane: Urethane is a known carcinogen and may only be used for anesthesia in non-survival surgeries. Scientific justification in the approved IACUC is required for use		

Table 7: Recommendations for types of analgesics

Drug	Mouse	Rat
Buprenorphine	0.05-0.1 mg/kg SC Every 8-12 hrs	0.05 mg/kg SC Every 8-12 hrs
Meloxicam	5-10 mg/kg SC or PO Once daily	1-2 mg/kg SC or PO Once daily
Carprofen	5-10 mg/kg SC Every 12-24 hrs	4-5 mg/kg SC Every 12-24 hrs
Ketoprofen	2-5 mg/kg SC Every 12-24 hrs	2-5 mg/kg SC Every 12-24 hrs.

Table 8. Recommendations for types of analgesics for different procedures and expected pain levels.

Type of pain Severity	Type of pain Severity	Examples of procedure	Duration#	Recommended analgesics
Surgical	mild	Punch biopsy, vascular cut down	once	Local +/- NSAID
Surgical	moderate	Head cap, craniotomy, subcutaneous procedure	1 full day	Local with either NSAID or Narcotic
Surgical	severe	Thoracotomy, laparotomy	3 full days	Local with both NSAID and Narcotic
Chronic	Mil-moderate	Arthritis	long term	NSAID

[3] Not available as a pharmaceutical, scientific justification in the approved IACUC is required for use. Solution must be labeled with date prepared, stored at 4°C, and discarded if any signs of decomposition including discoloration, precipitate or toxicity are observed.

References

- Hawk, C.T., Leary, S., & Morris, T. (2005). Formulary for Laboratory Animals. (3rd Ed.). Ames, Iowa: Blackwell Publishing (http://www3.research.usf.edu/cm/docs/Formulary_for_Lab_Animals_3rd_ed.pdf)

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