INSTITUTE OF BIOSCIENCES AND TECHNOLOGY
TEXAS A&M HEALTH SCIENCE CENTER

GENERAL INFORMATION FOR INVESTIGATORS

Texas A&M University Health Science Center

Program for Animal Resources

Revised February 2015

TABLE OF CONTENTS

I. Introduction

This guide provides information to investigators and their staff regarding the humane care and use of animals involved in research at the Texas A&M University Health Science Center Institute of Biosciences and Technology (IBT). The vivarium is managed by the Program for Animal Resources (PAR). This guide briefly describes the use of the equipment and supplies maintained at the facility, procedures that must be followed, and the responsibilities of personnel when working in these facilities. The PAR has a large investment in the rodent caging systems within the facility. These caging systems are designed to provide maximum protection against contamination by viruses, pathogenic bacteria, ectoparasites, and endoparasites. It is essential that all personnel follow the appropriate procedures outlined in this guide.

Personnel who do not follow these procedures may be denied access to the facility and may also lose their animal research privileges.

Keeping the facilities clean, secure and the animals healthy is critical for the success of everyone's research. If you see an investigator or a PAR employee who is not following the rules please take steps to correct the situation. You can contact the PAR manager, PAR supervisor, or veterinary staff. If possible, obtain the name of the person involved so that they can be contacted and retrained as necessary.

II. Contacting the PAR and Emergency Numbers

A. Contact List: *PROGRAM FOR ANIMAL RESOURCES PHONE NUMBERS*

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	OFFICE	***Cell***
Mary Cole *(Office Associate)*	**713-677-7745**	
Philip Shirley LATg (*LAC Manager*)	**713-677-7471**	**281-435-2788**
Jonathian Few *(Veterinary Technician I)*	**713-677-7720**	
David Brammer (*Attending Veterinarian*)	**281-410-8164**	
Dr. Allison Rice-Ficht (*VP for Research*, *Institutional Official*)	**979-436-0592**	
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Contact numbers for the Manager, Supervisor, Technicians, and Clinical Veterinarians are prominently posted throughout the facility.

B. For Problems During Working Hours

For Facilities-related issues contact the PAR Coordinator at 713-677-7452, the PAR Manager at 713-677-7471, or any of the PAR staff.

For Animal Husbandry issues, contact the PAR Coordinator, Supervisor, Veterinary Technician, or any of the PAR staff.

For Animal Health issues, contact the veterinary technician or any member of the veterinary staff.

C. After Hours and on Weekends

For Facilities related issues and Animal Husbandry issues contact security at 713-677-7464 or call the PAR Manager at 281-435-2788.

For Animal Health issues, call the UTHSC Veterinarian on call at 713-500-7542.

EMERGENCY VETERINARY SERVICE

U.T. MEDICAL SCHOOL
(CENTER FOR LABORATORY ANIMAL MEDICINE AND CARE)

713-500-4453

D. Obtaining Information on the Status of the Vivarium and Animal Care Issues

Information affecting the vivarium or animal care will be posted outside the entrance of the vivarium as needed. The PAR Manager will also distribute information by email to the principal investigators and their technicians. The information should be passed on to the members of the research team.

E. Emergencies and Updated Information on the Status of the Vivarium and Animal Care Issues

IBT Emergency contact information can be accessed by dialing security at 713-677-7464.

III. Regulations Governing Animal Care

1. The PAR is required by federal regulation to follow specific standards and procedures outlined by

the following agencies:

National Institutes of Health – **Office Laboratory Animal Welfare (OLAW)**
Animal Welfare Act, United States Department of Agriculture (USDA) standards
Public Health Service, PHS Policy and U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training
Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), National Research Council Guide for the Care and Use of laboratory Animals

2. If you would like more information about any of these agencies, visit the PAR Manager

IV. Protocols for Animal Research

A. The IACUC

1. The federally mandated Institutional Animal Care and Use Committee (IACUC) at the IBT is responsible for reviewing and approving protocols.

2. Do not conduct experiments, even pilot studies, which are not described in your approved animal protocol. Amend protocols to reflect changes in procedure, animal numbers, etc. If you have any questions about whether a change needs an amendment, discuss it with the IACUC chair or with a PAR veterinarian.

3. It is the responsibility of the PI to insure that everyone working on an animal project is familiar with the approved and active version of the animal protocol that covers the work being done. Any IBT employee should check with the PAR Manager to see the animal protocols on which they are listed.

B. Adding Personnel

1. **The fastest way to add personnel to a protocol is to submit an amendment with no other changes other than the addition of the person and a description of their experience.** Personnel changes can be approved administratively by the IACUC, typically within one day.

2. If you have other modifications to add to the protocols, wait until the personnel change is approved and then submit a second amendment with the new procedures, drugs, animal numbers, etc. These other types of amendments must be reviewed and will take more time.

C. How to Submit a Protocol

1. Protocols requesting approval to use animals in research or amendments to existing protocols are submitted through iRIS (Integrated Research Information Systems) https://imedris.tamu.edu/
2. If you need assistance with IACUC submissions please contact TAMHSC Research Compliance

D. Protocol Review Process

1. The IACUC meets once a month on the first Monday of the month. Protocol must be submitted no fewer than nine (9) days prior to the next meeting in order to be reviewed.

2. The time required for the review process depends on the nature of the submission. Personnel additions can typically be approved within 1-2 days. Amendments typically take 1-2 weeks, unless they require full committee review (see below). Amendments do not extend the approval interval for a protocol.

3. 3 year renewals and new protocols are not approved until after the IACUC meets. If revisions are required, the protocol is not approved until those are completed to the committee's satisfaction. Most revisions or iterations are reviewed by a designated reviewer and do not need to wait for the next IACUC meeting.

3. Approvals for new protocols and renewals are issued as soon as possible after the completion of the IACUC meeting.

4. Renewals are due annually and should be submitted no fewer than 30 days before the expiration date. (The expiration date is one year from the date of the AUP's original approval).

5. In each annual renewal, the investigator should update the protocol to include all amendments.

6. The indications for full committee review are listed below. Protocols meeting these criteria undergo full committee review and discussion when they are first submitted and every three years thereafter (so-called major renewals). In the interim years, the renewals (so-called annual renewals) are also reviewed by the IACUC.

7. Annual renewals or amendments that introduce new manipulations that meet the criteria for full committee review will be reviewed and discussed at a meeting of the IACUC, i.e. full committee review.

E. Policies and Ethics Involved for Specific Procedures

1. Euthanasia as an Alternative to Death as an End Point

1. Legal, regulatory, and moral guidelines require that animal pain, distress, and suffering be minimized in any experiment. For these reasons animals should be euthanized in death-end-point experiments prior to their actual death if experimental validity will not be compromised. If death is a required end point, the investigator may receive approval to conduct such studies by providing appropriate scientific justification in writing.

2. The scientific rationale should answer the following questions: What alternatives were considered and why can you not use morbidity instead of death as an endpoint? Can pain-relieving measures be used, and if not, why not? How many animals will be used this way and how is the minimal number required? What additional information will be gained in the interval between morbidity

and death? How will animals be monitored and how will this be recorded? What arrangements are available for prompt removal of dead animals?

3. In all cases investigators are expected to continue to monitor experimental animals at least twice daily (including weekends and holidays), to euthanize any animals which they judge to be moribund, to use alternative end points to death when possible, and to minimize animal numbers within statistical constraints in general, but especially in death end-point protocols.

4. The following are signs and symptoms for judging morbidity (disease/illness) and moribund condition (state of dying) in rodents:

Morbidity

1. Rapid breathing rate
2. Breathing rate very slow, shallow, and labored (preceded by rapid breathing)
3. Rapid weight loss
4. Ruffled fur (rough hair coat)
5. Hunched posture
6. Body temperature less than 30 C (hypothermia)
7. Hyperthermia
8. Ulcerative dermatitis or infected tumors
9. Anorexia (loss of appetite)
10. Diarrhea or constipation

Moribund Condition

Signs and symptoms for morbidity plus:

1. Impaired ambulation (unable to reach food or water easily)
2. Evidence of muscle atrophy or other signs of emaciation (body weight is not always appropriate)
3. Any obvious illness including such signs as lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), anorexia (loss of appetite, especially when prolonged), bleeding, difficulty breathing, CNS disturbance, or chronic diarrhea
4. Inability to remain upright

2. Multiple Survival Surgical Procedures

1. Multiple survival procedures may be permitted only with prior approval of the IACUC. Cost alone is not an adequate justification for performing more than one survival surgery on an animal. Post operative analgesia is highly recommended in cases of multiple major survival surgery.

2. Any proposed multiple survival surgery must be described in detail on the animal care and use form. The following are examples of multiple survival procedures that may be approved:
    a. Serial surgeries essential to the design of a given study.
    b. Entry into a body cavity which is done by a probe or needle, e.g. a *laparoscopic examination or needle electrode placement, which does not result in any injury likely to produce pain.
    c. Clinical procedures, which are not related to the research project, but which may be necessary for the health and well being of the animal.

d. Procedures which are commonly performed multiple times in a clinical setting, such as multiple hysterotomies for delivery of the contents of a pregnant uterus.
e. Tumor reduction can be performed only on subcutaneous tumors and the procedures can only be done twice.

3. In all cases, the usages must be scientifically justified in the context of the overall purpose of the research project.

3. Major Procedures

1. Major procedures are those that enter a body cavity, or those that have the potential of inducing a handicap. For example, placement of a stent in the carotid through a percutaneous route does not enter a body cavity, but it does have the potential of inducing a carotid occlusion and causing a stroke. Thus it would be considered a major procedure.

2. Major procedures, in non-rodent species, must be done in a dedicated sterile operating room with a separate facility for preparation of the animal.

3. Rodent surgery can be performed under aseptic conditions, meaning that: (1) the surgical area is clean (wiped down with a disinfectant (i.e., Nolvasan, Virkon, etc..), (2) fur is clipped from surgical site and skin is cleaned with Nolvasan, (3) instruments are sterilized either by autoclaving instrument pack or by soaking in a chemical sterilant (Instracal, Amerse) for at least 15 min., and (4) the surgeon wears a mask and sterile gloves or latex gloves thoroughly cleaned with Nolvasan, Virkon, or another germicidal agent.

4. Polyclonal Antibodies

If a commercial company makes antibodies that can be ordered from a catalogue you do not need an animal protocol. However, if you use a commercial company antigen for the production of antibodies you will need an active animal use protocol. The company can ensure the purification of the antibodies and supply their immunization, serum collection and euthanasia protocol, their USDA registration number, and their Public Health Service (Office of Laboratory Animal Welfare) assurance number.

5. Production of Monoclonal Antibodies

1. Tissue-culture methods should be used to produce monoclonal antibodies unless there is a clear reason to do otherwise. The investigator should clearly explain in the protocol why in vitro methods are not feasible or not appropriate in their circumstances. The following scenarios, modified from the information distributed by NIH, are examples of circumstances under which ascites might be required.

   a. Some hybridoma cell lines do not adapt well to tissue-culture conditions. The concentration of antibody generated may be too low and purification or concentration from in vitro systems can lead to protein denaturation and decreased antibody activity. As general guidelines, when a supernatant of a dense hybridoma culture grown for 7–10 days (stationary batch method) yields an mAb concentration of less than 5 micrograms/ml this would be considered to be low yield. If hollow-fiber reactors or semipermeable-membrane systems are used, 500 mg/ml

and 300 mg/ml, respectively, are considered low mAb concentrations.

b. Tissue-culture methods can yield mAb that do not reflect the normal modification of proteins with sugars, and this abnormality might influence binding capacity and other critical biologic functions of mAb

c. Contamination of valuable cell lines with fungi or bacteria may requires prompt passage through a mouse to save the cell line

d. When more than 5 mg of mAb produced by each of five or more different hybridoma cell lines is needed simultaneously. It is technically difficult to produce this amount of mAb since it requires more monitoring and processing capability than the average laboratory can achieve.

e. When analysis of mAb produced in tissue culture reveals that a desired antibody function is diminished or lost it may be necessary to passage the hybridoma through mice to recover the line.

f. When more than 50 mg of functional mAb is needed, and previous poor performance of the cell line indicates that hollow-fiber reactors, small-volume membrane-based fermentors, or other techniques cannot meet this need during optimal growth and production.

2. The maximum dose of pristane to be used for priming is 0.2 ml to minimize animal distress.

3. There does not appear to be convincing evidence that significant pain or distress is associated with the injection into the mouse of pristane, but during the accumulation of ascites there is likely to be pain or distress, particularly when some cell lines that are tissue-invasive are used and in situations of significant ascites development. Therefore, after injection of hybridoma cells, mice should be evaluated at least daily, including weekends and holidays.

4. Animals must be weighed often to ensure that ascites fluid does not exceed 20% of the animal's body weight. Once ascites production begins, animals must be checked daily and moribund animals must be euthanized. Taps can be done at a rate of one per 48 hour period, not to exceed a total of 3 taps (as more frequent taps result in higher levels of endotoxin.)

6. Retro-orbital Bleeding

1. Retro-orbital bleeding must be done with the animal under anesthesia. .
2. The frequency and number of times this procedure can be done will depend upon the merits of the proposal and the skill of the investigator and must be approved by the IACUC.

7. Tumor Burdens in Animals

These standards refer specifically to rats and mice and are as follows:

1. The maximum tumor burden on any one animal should not exceed an estimated 10% of the animal's body weight.

2. The maximum measurable total tumor burden on any one animal should not exceed one solitary mass of 1.5 centimeters in diameter on a mouse or 2 centimeters on a rat.

3. Multiple masses with a combined total diameter of 1.5 cm for mice and 2 cm for rats are permitted.

4. If an investigator requires tumor burdens larger than those specified above, or requires generation of partially necrotic or ulcerated tumors, a written scientific justification must be submitted to and approved by the IACUC.

5. Fluid neoplasia (e.g. hybridomas) and non-neoplastic ascites are addressed under hybridoma production standards.

8. Gloves and Instruments in Rodent Procedures

1. Investigators must wear sterile gloves or latex gloves thoroughly cleaned with Nolvasan (Chlorhexedine), Virkon, or another germicidal agent, while performing survival surgery on rodents. They may use either pre-packaged sterile gloves or bulk gloves that are sterilized immediately prior to use.

2. When performing procedures on multiple animals at one sitting, it is necessary to change gloves or re-sterilize the gloves between animals. The instruments must also be re-sterilized between animals.

9. Tagging Animals

Ear notches and ear tags as a routine means of identification are all accepted procedures. If investigators wish to use another method, they should contact the PAR manager or veterinary staff to discuss alternatives.

V. Description of PAR Resources

A. Barrier Levels

1. The housing system and procedures for entering any rodent facility are based on a graduated scale of four Barrier Level categories (BI, BII, BIII, and BIV), with the highest level requiring the most stringent procedures. Lower barrier levels have less rigorous operational requirements. As you progress from BI to BIV, procedures to enter a facility become more rigid. The B level of a facility also dictates the institutions from which mice can be imported, and the type of quarantine that is required. **If you work in multiple animal facilities, it is vital that you never work with animals from another facility *before* working with animals at the PAR facilities.** Virtually all mice in the PAR are barrier-contained by the use of ventilated racks. Below is a summary of the operational requirements for the PAR:

Mice are housed in ventilated cages (barrier at cage level)
Cages and supplies are sanitized in washers.
Acidified water is used
Feed is irradiated
Protective clothing consists of scrubs or disposable gown, mask, and gloves.
Cages and animals are manipulated inside a work station
Animals from other institutions are quarantined before entry.

2. There is an active sentinel program in place in the facility. The sentinel program consists of test animals that are exposed to soiled bedding from other cages. These animals are sacrificed at regularly scheduled intervals and tested for the presence of pathogens. The health reports for all PAR rodents are located in the Manager's office.

B. The IBT - PAR Animal Facilities

The Program for Animal Resources animal facility is a two story, 26,279 square foot structure. The

17,542 square foot first floor contains 4 animal housing rooms able to accommodate 34 racks holding 100 cages, 4 adjacent procedure rooms that are assigned to specific investigators. Support areas outside the actual animal holding area include a surgery room, transgenic lab, central materials supply room, procedure rooms, break room with adjacent locker/shower facilities for the animal care staff, 2 administrative offices, laundry room (complete with washer and dryer) and 2 small storage rooms with shelving.

The first floor support areas inside the animal holding area are (1) a biohazard suite that includes two animal holding rooms one accommodating 2 racks holding 100 cages and the other accommodating 3 racks holding 100 cages, a lab, 3 biological hoods, and an autoclave; (2) the quarantine suite that contains 5 cubicles and a small anteroom; (3) a sentinel housing room that contains 2 cubicles; (4) a cage wash area that contains tunnel and rack washers, a water bottle filler, an automatic cage bedding dispenser and a large autoclave; (5) a necropsy lab; (6) a walk-in feed cooler; (7) a storage room; (8) a veterinary technician's office; and (9) a supervisor's office.

The 8,737 square foot second floor contains 12 animal rooms able to accommodate 95 racks holding 100 cages and 7 procedure rooms and 2 storage rooms.

Mice are housed in the OptiMouse Ventilated caging system which holds 100 cages per rack with up to 5 mice per cage in a carousel configuration with pie-shaped cages. Some mice are housed in larger cages to better accommodate harem breeding programs and multiple litters. These cages are under negative pressure in relation to the room (which is positive pressure to the corridor).

Rats are housed in the OptiRat Ventilated caging system which holds 42 large cages per rack.

All racks, except those in quarantine and the biohazard suites, are under positive HEPA filtered air pressure that is changed 22 times an hour. All rodents are fed irradiated food and acidified (pH 2.5 – 3.5) water in bottles. Rodent cages are changed at least once weekly in HEPA filtered change stations by technicians in clean uniforms using sterile techniques.

C. The Charles River Laboratory

The Charles River Labs provide health monitoring, quality assurance, and diagnostic services support for the PAR and for research.

Available services include:

1. Necropsy, including dissections, fixation and processing
2. Histology, including paraffin and frozen sections, decalcified specimens, special stains
3. Immunohistochemistry
4. Photomicroscopy
5. Morphometry and Image Analysis
6. Hematology and Cytology including automated CBC/ differential, stained smears, Cytospin Preparations, Semi-quantitative Bone Marrow
7. Serum Chemistries (ALT, AST, AP, bili, LDH, CPK, BUN, Creat, TP, Alb, Gluc, Trig, Chol, HDL, LDL, Ca, P, Na, K, etc)
8. Urinalysis, including SG, Prot, Gluc, Sediment, and quantitative Creat, Prot, Ca, etc
9. Serology (ELISA for viruses, bacteria)
10. Microbiology
11. Parasitology

D. In-House Diagnostic Laboratory

Additional rodent sentinel testing is done by the PAR veterinary staff. This includes external physical exam, testing for endo- and ectoparasites, necropsy to fix tissues for histopathology if required.

E. Animal Care and Use Program Consulting Services

The Comparative Medicine Program at Texas A&M University in College Station provides consulting services and support for the PAR animal care and use program. This includes clinical support, investigative and staff training, and provides support when needed for any aspect of the program operations and management.

VI. How to Obtain Access to the Facilities

1. Before access is granted to the animal facility, each individual must:
   Have a valid ID card

Be listed on an approved animal protocol
Have received training from the PAR
Complete CITI Training
Accept or decline enrollment in the Occupational Health Program

*Facility access will be granted to IACUC members on committee business and to approved visitors with approved escort.

2. Personnel working in certain areas such as Biohazard are required to receive additional training on the procedures and policies of that specific area.
3. Monitored access involves more than keeping unauthorized personnel out of the animal facilities. It is also intended to direct traffic to prevent cross-contamination of animals. For this reason, a computerized access system is used and individual facility access is limited to those areas in which each person's animals are housed. Facility access will not be given to a collaborator UNLESS they are listed on the protocol.

4. In general, access is limited to between 7AM and 7PM. This is to allow for an uninterrupted dark cycle at night which is needed for normal mouse behavior and mating patterns. Access outside of this time period should be limited to avoid disruption of the light cycle. PAR availability will also be limited outside of the normal working hours, which are 7:00 am until 4:30 pm.
5. Security and PAR employees have been instructed to refuse access to a facility or room without an ID card and an access card to the animal room door. Access cards and keys cannot be shared with others. Strict penalties have been set for those who do. Please do not ask IBT employees, or others, to grant you access or to open animal room doors. PAR employees are also instructed to keep all animal room doors locked when not being used. Please lock the door when you leave.
6. Do not prop open, or otherwise try to defeat the intent of the doors. To do so puts you, your co-workers, and all of the animals in the facility at risk.

7. Please be familiar with all emergency egress points in the facility. We encourage you to ask the facility manager or PAR staff to point them out to you on your first trip.

8. You cannot get locked into the facilities. All facilities have crash bars or push buttons to open doors if the key card fails and you are alone in a suite or facility. (Phones in each suite dial directly to IBT Security when you pick up the handset). remove

9. Visitors, including untrained personnel, are not allowed in the animal facilities without the approval of the PAR Manager. Children are NEVER permitted in any animal facility.

10. Use of cameras and video equipment is strictly prohibited unless its purpose is within the scope of research documentation. Any investigators who need to document with such media must indicate so in their protocols.

VII. Safety and Occupational Health

A. Hazards in the Laboratories

1. Working with laboratory animals carries several risks. Apart from the obvious physical hazards of bites and scratches, animal research often involves biological hazards that exist because animals can serve as natural reservoirs for infectious diseases including zoonoses, hosts in studies of pathogenic microorganisms, and sources of allergens. These hazards can affect not only laboratory personnel but also laboratory animals. Infectious diseases that remain unnoticed can introduce unwanted variables into research projects and render research results invalid.

2. Physical hazards exist because animals can do physical harm by biting, scratching or crushing; the work environment can contribute to physical harm through accidental falls due to wet, slippery floors; and the work practices can cause physical harm through the use of improper practices. There is some degree of chemical hazard found in disinfectants, cleaning agents, pesticides and as feed and bedding contaminants. Chemicals can cause acute or chronic toxic effects as well as physical harm (fire or explosion).

3. Diseases transmitted to man from animals are called zoonotic diseases. Workers can be exposed to either natural or induced animal diseases. Naturally acquired diseases are usually considered to be more dangerous because they may remain unsuspected and undetected. Zoonotic disease organisms can be classified as viral, rickettsial and chlamydial, bacterial, mycotic, protozoan, helminthic, arthropod borne or transmitted and those caused by bites and scratches. Rodents can carry lymphocytic choriomeningitis, rat-bite fever, and Korean hemorrhagic fever. These diseases may cause sickness if passed to humans and can ruin experimental results. ***Exposure to these diseases can be lessened by (1) purchasing only animals that have been raised in a barrier colony that are free of the infectious agents naturally associated with a particular species, (2) adhering to IBT guidelines.***

4. Investigators should be aware of the hazards of working with experimentally induced disease agents. They know about the organism and the disease produced.

5. There are four primary routes of exposure through which an agent can cause disease. They are: ingestion, inhalation, contact with mucous membranes and direct parental inoculation including ingestion.

6. Laboratory animals can spread disease through urine, saliva, feces, and excretions from skin lesions. Artificial transfer can occur through biopsy, blood or other bodily fluids sampled with needle and/or syringe or during necropsy or surgery. Vectors may facilitate escape of the agent from the animal host.

7. Needles and syringes appear to be the most hazardous pieces of equipment commonly used in biomedical and microbiological laboratories as about 25% of laboratory induced disease is associated with needles and syringes. Accidents with these can be reduced by following some simple rules.

8. **Needles should not be recapped but placed directly into a puncture proof "sharps container" to dispose of them. Direct contact with infected animals is another mode of disease transmission.**

9. Aerosol, small airborne particles of solids or liquids, may serve as the most common mode of infectious

disease transmission. Aerosols are produced by most animal care and husbandry practices and many experimental procedures. These can come from infected animals, tissue culture flasks, and animal bedding or tissue homogenizers. Face masks and biological safety cabinets are the best defense against this type of spread. More information on biological safety cabinets can be found in the CDC/NIH manual Biosafety in Microbiological and Biomedical Laboratories.

10. Animals are also a source of potent allergens to sensitize and sensitized persons. Hypersensitivity to animal allergens has emerged as a significant occupational health hazard for laboratory workers. Substances found in urine, hair, skin, and saliva of mice, rats, rabbits, guinea pigs, and hamsters can cause symptoms ranging from mild rhinitis to debilitating asthma. There is also the potential loss of highly trained personnel because of their allergies to their tools. One way to reduce allergens in laboratories and animal rooms is through engineering controls such as ventilation systems and micro-isolator filters tops.

11. Those most at risk are those who assume there is no risk. Those working with infectious organisms are more careful. Those performing unfamiliar tasks are more careful. Workers may become more vulnerable as they grow older. Those who talk while working are more at risk than those that do not since talking is distracting.

12. Some protective measures include no food or beverages in the work areas; use of personal hygiene and protective clothing; routine housekeeping, decontamination and waste handling; insect and rodent control; and aerosol containment. By observing some simple rules and using common sense, the many hazards associated with working with animals can be reduced or eliminated.

B. Program for Animal Resources Occupational Health Program

1. The Guide for the Care and Use of Laboratory Animals and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) require that an animal care and use program include an occupational health program for personnel with substantial animal contact. The definition of substantial contact and the content of the occupational health program are determined by the institution (Institute of Biosciences and Technology). This Program has been written in conjunction with the IBT Environmental and Physical Safety Department and the Chemical, Radiation, Biological and General Safety Committees.

2. Participation in the Occupational Health Program:
   Texas A&M Health Science Center Occupational Health Program enrollment is required for all individuals listed on an IACUC research or teaching Animal Use Protocol, individuals involved in animal care in TAMHSC animal use rooms housing animals for research and teaching, visitors and collaborators, contract service providers, volunteers, and other individuals who may reasonably be expected to come in contact with human body fluids, human tissues or waste; animals, animal tissues or wastes; or human disease causing microorganisms as part of their job duties.

3. The extent of participation in the OHP is based on a risk assessment that takes into account type of exposure, extent and frequency of exposure, and a review of health history by an Occupational Health Professional. All employees are informed of the zoonoses and other hazards associated with working with animals.

C. Animal Care Personnel, Principle Investigators & Technicians

The Occupational Health Program includes the provision of uniforms and other protective clothing (scrub suits, boots) and devices (gloves, masks or respirators in accordance with the Respiratory Protection Program, eye protection, hearing protection) as the job demands, as recommended by the Occupational Health physician.

It is policy that faculty, graduate students, post-docs, etc, who do not wish to participate in the occupational health program, cannot work with animals until she/he provides the choice to decline in writing. All Physical Plant and Security employees are given the option of participating or declining in writing the occupational health program.

All animal users must complete an annual questionnaire to determine the individual risk each person encounters with animal work. Personnel are notified when it is time to schedule an evaluation and assessment.

If required after review by the Occupational Health Physician, physical examinations for personnel at TAMHSC-Houston are performed by the University of Texas Health Sciences Center Health Services with documentation furnished to the TAMHSC-Houston.

Employees of MD Anderson and Texas Heart Institute participate in institutional occupational health program from their home institution. When individuals from these institutions are added to an animal use protocol verification of OHP participation is obtained and documented within the iRIS system.

D. Allergies

Individuals at risk of developing allergies or experiencing exacerbations of existing allergic reactions include those with pre-existing allergies, asthma, seasonal rhinitis or eczema. The medical examination and history will aid in detecting these individuals. Gloves are required to work with animals and animal handling is performed in a Lab Products Stay-Clean™ L/F Workbench which reduces exposure to allergens.

E. Animal Work Involving Hazardous Agents

1. The PI must complete specific sections of the IACUC application that describes their use of recombinant DNA, infectious biohazards, hazardous chemicals, and radioactive materials. These forms incorporate basic information about what is being used, where it is being used, its containment, how it should be handled and personnel safety. During protocol review, the submission is assigned to Environmental Health and Safety to ensure the exposure control described is appropriate, and to make suggestions for carcass disposal, bedding disposal, etc. It the submission indicated biohazards or rDNA will be utilized, a representative from Biosafety is assigned to ensure the containment is appropriate, and that the activities are covered under an active IBC permit. All infectious agent work involving animals must be done in the Biohazard Suites (ABSL2) of the animal facilities. The IBT Safety Officer oversees all work done with radioactive or hazardous chemicals and provides appropriate training to the individuals performing the experiments.

Any use of hazardous agents (infectious organisms, toxic chemicals, carcinogens, radioisotopes, genetically altered organisms, etc.) in animals must be approved in advance by the IACUC and the Institutional Biosafety Committee (IBC). Their use must meet the requirements set forth in the 5th edition of the Biosafety in Microbiological and Biomedical Laboratories (HHS Publication No. (CDC) 21-1112, 2009, Centers for Disease Control, Atlanta, GA).

The principal investigator, safety officer and various safety committees are responsible for providing training, not only to the research staff but also to involved animal care personnel regarding the dangers of the agent and precautions to be taken while working with the animals or in case of an accident.

In addition, during the Occupational Health Enrollment process, the individual will complete a risk assessment form, which is signed off on by their supervisor. This will identify potential exposure to zoonotic agents, radiation, allergens, etc. The Occupational Health Physician will make recommendations for safe work practices for the individual.

F. Accidents and Safety

1. Working with animals can be extremely rewarding but it also has its hazards. Many animals carry diseases that are easily communicated to people. Some of these can be serious in adults and quite severe in children. Rules have been made that, if followed, should provide an investigator or animal care provider with safe working conditions.

2. Any person involved in an exposure incident must promptly report it to his/her supervisor. All individuals are instructed to notify the Occupational Health staff immediately of any injury, and they are immediately referred to a local Occupational Health Physician at no cost to them. If the accident/injury occurs after hours, they are instructed to be evaluated at the nearest emergency department and will be reimbursed.

Any accident resulting in an overt exposure to recombinant DNA/materials will be reported to NIH/OBA by the Office of Biosafety at TAMU per the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecule*s.

3. **There is to be no smoking, eating, drinking or the application of cosmetics in animal rooms or laboratories. Strict attention should be made to personal hygiene especially hand washing.**

4. Just as animals can transmit diseases to people, people can transmit disease conditions to susceptible animals. If personnel have active cold sores, viral respiratory diseases, or been around people with chicken pox or measles, they should refrain from entering animal rooms for at least 72 hours. If personnel have pet rodents at home they must shower before entering the animal facility. Care should also be used when going from one animal facility to another.

VIII. Maintaining a Pathogen-free Facility

The housing systems and procedures for entering the animal facility are based on operational requirements with the highest level of stringent procedures. We hope to protect animals from disease causing contaminates. Contamination enters animal facilities through:

1. Personnel
2. Equipment
3. Infected Animals/ Biological Materials

A. Personnel Access

1. While it may be necessary for some investigators to maintain their animals at more than one facility, this practice is discouraged since it increases the risk of cross-contamination. If you must work in more than one animal facility on a given day, it is imperative that you enter the IBT animal facility FIRST! **If you have been in any other animal facility within the past 24 hours you MAY NOT enter the IBT PAR. If two facilities (such as Fondren and IBT) must be visited in the same day, you must shower between facilities.**

2. All facilities and rodent rooms require personal protective equipment (PPE) to enter. Experience shows that groups who strictly adhere to procedures avoid contamination of their animals. Contamination most often occurs when there is a failure to use proper technique. In the PAR, the PPE consists of scrubs or disposable gown, which you should wear whenever you are in the vivarium. **Closed-toed shoes** will avoid injury from rolling equipment and sharps. Wear **gloves and mask** whenever you are handling mice to minimize exposure to mouse urinary proteins and reduce your chance of developing hypersensitivity reactions to mouse allergens.

3. Do not bring equipment into a facility if it has been in contact with animals from another facility. Contact veterinarians regarding advice for decontamination of equipment if needed.

B. Animal Facility Dress Code and Personal Hygiene

1. No street clothes, without protective coverings, are allowed in the animal facility. Eating, drinking and the application of cosmetics are permitted only in designated areas. Smoking is not permitted in the buildings.

2. Location of all PPE will be shown during the tour of the animal facility. Scrub suits or a gown will be worn at all times in the animal facility and must be removed before exiting. **All INVESTIGATIVE STAFF** will wear disposable gowns before entering the Animal Care Facility. In addition to the gown, gloves must be worn when working directly with the animals. Gowns and gloves are located directly inside the vivarium. The scrub suits for technicians along with the other necessary items are located on a rack in the PAR laundry room (122). All soiled scrub suits are to be placed in a dirty clothes hamper located in each locker room. Gloves and gowns will be discarded in the trash containers located in the locker areas before leaving the facility. If gloves or gowns become torn or otherwise non-usable, they will be replaced immediately. This may occur more than once per day.

Biohazard Suite: Upon entry into rooms 137-E, you must wear a disposable yellow gown over your street clothes/scrubs which must be disposed of prior to exiting the ABSL2 area. You must wear a disposable gown or dedicated scrubs for the trip from the Biohazard exit to the vivarium exit. You must wear gloves. These gloves must be removed, discarded and hands must be washed prior to leaving the procedure rooms or wash rooms.

After exiting the biohazard suite, you must exit the animal facility without going to any other area of the animal facility. You may not return until after having showered.

Shower: The IBT animal facility has male and female shower facilities for PAR use and upon request by investigative staff. Personnel will be expected to keep the shower facility clean and presentable after use.

Laundry: All used scrub suits and coveralls will be placed in clothes hampers conveniently located in dressing/locker rooms. Personnel from P.A.R. will collect the dirty suits, transport them to the laundry facility where they will be washed, dried and refolded. The scrub suits coming from the biohazard area may require autoclaving prior to removal from the area. Once the suits are clean, they will be returned to on racks in the break room bins in laundry room 122. Used disposable items (caps, masks, shoe-covers, etc.) will be discarded on a daily basis. Those from the biohazard area may require sterilization prior to disposal.

PLEASE DO NOT KEEP COVERALLS IN LOCKERS OR PROCEDURE ROOMS. INSTEAD, GET A CLEAN PAIR EACH DAY.

Visitors: Visitors to the animal facility must also cover their street clothes with a gown. Gowns will be disposed of in the labeled trash container before exiting the animal facility.

C. Health Status of Rooms and Animal Transfers

1. When animals or biological materials are moved from one room or facility to another, contamination may result. The PAR maintains a sentinel program in which mice, provided by the PAR, are housed on the same racks as research mice. These sentinel mice are exposed to bedding from the other mice on each rack. At quarterly intervals they are tested for signs of external parasites, internal parasites, and viral infection. The results of these tests determine the disease status of the room. The results are available in the veterinary technician's office.

2. Under no circumstances are animals to be transferred between rooms of a vivarium or into another vivarium without proper approval. The PAR tracks all animal movements to be sure that animals do not carry pathogens from one room into another. In case of a breach in containment, the PAR uses these records to track animal movements and quickly determine the rooms at risk that need special containment, monitoring, or quarantine.

3. To request that animals be moved, submit a completed **Request for Animal Transfer** form to the PAR Helpdesk at www.ibt.tamhac.edu/par. The animals that are to be transferred must be labeled with green transfer cards obtained from the Veterinary Technician.

D. Quarantine

1. Quarantine is a process in which animals are isolated for an interval of time and, if needs be, tested for the presence of pathogens before they are introduced into the vivarium. A quarantine period is necessary because a mouse newly exposed to a pathogen may not show evidence of disease when it first arrives. The length of the quarantine and the panel of tests performed vary according to the level of bio-security that is required.

2. Quarantine for all animal transfers lasts from a minimum of four weeks up to eight weeks. Guidelines are as follows;

   A quarantine period of 8 weeks is required for all mice coming from non-approved vendor sources.

3. If an animal is found to be contaminated, the investigator will be contacted by a member of the veterinary staff to discuss options.

4. Contact the PAR manager for any questions regarding re-derivation. This may be a preferable (and faster) alternative for some investigators.

5. Quarantine will not be required for animals purchased from IBT approved vendors. However, unapproved vendors may be subject to quarantine requirements as set forth by IBT.

E. Sentinel Monitoring

1. All rodent colonies housed in the IBT will be monitored quarterly for common rodent pathogens using sentinel animals.
2. Sentinel rodents will be obtained from veterinary staff approved vendors and will be negative for common rodent pathogens prior to placing in investigator rooms.
3. Dirty bedding from colony animals will be placed in sentinel cages during routine scheduled cage changes. All procedures will be carried out under a cage change hood following SOP's.
4. At quarterly intervals, a sentinel animal from each cage will be sacrificed and samples for pathogen monitoring will be collected by the veterinary staff.
5. Results of testing will be reported to principal investigators. In the event of a positive test result, the veterinary staff will take necessary immediate action such as colony quarantine.

   Options such as further quarantine, retesting, colony rederivation or other appropriate measures will be discussed with the principal investigator.

IX. Animal Husbandry and Housing

A. Rack Identification

Each rack will have a tag that is attached at the top left corner identifying the animals within the rack. The following information should be provided on these tags:

- **Location.**
- **Investigator Name**. The name of the PI from the protocol should be listed.

B. Cage Cards

1. It is a strict requirement to keep a cage card on all cages at all times. Every cage card must contain the following information: **Investigator name and contact number, center, protocol number, animal species/strain, and animal source**.

2. PAR cage cards are pre-printed cards that are attached to all incoming animal shipments. Pre-printed cage cards are also available to investigators. If you require more than are being provided, please see the manager or supervisor and let them know your needs. These cards are printed with all the required information.

3. To avoid errors in billing, please review the rack ID cards carefully for correct information. The per diems will be charged to the principal investigator listed on the tag. If you have questions concerning billing, contact the PAR manager at 713-677-7452.

C. Schedules for PAR Personnel

1. **NORMAL WORKING HOURS:** there are two shifts available to PAR employees.
   8:00 a.m. - 5:00 p.m. with a one hour lunch, Monday through Friday
   7:00 a.m. – 3:30 p.m. with a half hour lunch, Monday through Friday.

   BREAK TIMES: PAR employees are permitted one morning break, for NO MORE THAN 15 minutes. This break is to be taken between 10:00 and 10:15 a.m.

   LUNCH: for the 8:00-5:00 shift, lunch is from 12:00 - 1:00 p.m. For the 7:00-3:30 shift, lunch is from 12:00 – 12:30 p.m.

   WEEKENDS AND HOLIDAYS: the PAR husbandry staff is generally present in the facility 4 hours each day on weekends and between 4 – 6 hours on holidays.

2. The PAR has established a schedule for servicing the rooms. **During the change out interval for a particular room, PAR personnel need priority access to the hood in the room**. Do not ask them to vacate the hood or leave the room during this interval. If assigned service time is a long-term problem, contact the PAR manager who will determine whether your room can be reassigned to another time slot.

3. Investigators are welcome to remove cages and take them into the procedure rooms where there are hoods if they need to work during this time interval.

D. Standard Operating Policies (SOPs)

1. The PAR has established standard operating policies for the people who work in the animal facilities. You are free to look at the SOPs for the animal husbandry personnel in order to better understand what service these individuals provide. There are daily room logs on the outside of each suite door to show the activities completed in each room every day.

2. If you feel that a PAR person is not following the SOPs, contact the manager.

3. If you want the PAR personnel to follow a different procedure from what is stated in the SOP, you must complete a PAR Work Order that states what special care is to be given to the animals.

E. PAR Work Order - Special Care Instructions

1. No verbal agreements will be made between the Animal Care Attendant and the Investigative Staff for any deviation from the normal husbandry activities.

2. If you want PAR employees to follow a variation from the standard operating procedure a **PAR Work Order** must be completed. This form is used to relay any special needs to the husbandry staff.

3. To avoid errors, the animals referenced on this form must be clearly identified with the appropriate colored card (i.e. Special Diets are marked with a light blue card). Cages receiving multiple special diets and housed in the same cubicle will need extra identifying information.

4. The special instructions must be given to and approved by the PAR Manager. The PAR Manager will fill out the form at that time. In the case of special liquids or diets, these special instructions must also be approved by the IACUC before use.

5. These forms are used for six months, and if the requirement still exists after that time, can be extended in six-month increments.

F. Cage Changes

1. Mouse cages are routinely changed once a week.

2. If you find a cage that needs changing, notify anyone on the PAR staff and they will attend to it.

3. If your room has already been serviced for the day and a cage appears to have been skipped, contact the supervisor or manager so that they can have someone come back and attend to it. If a cage needs immediate attention (for example it is flooded), contact the manager.

4. Clean cages containing feed grid and bottle are located on metro racks (the investigator racks) throughout the facility.

G. Cages Without Food or Water

1. If you find a cage without food or water, contact the person caring for the room and then notify the supervisor or manager. If the caretaker for the room is not available contact the supervisor or manager immediately.

2. **Do not fill the water bottles yourself from the sink**. Carboys of acidified water are available at every hood. The water needs to be acidified to minimize contamination.

3. Husbandry Verification Form – is posted on the outside of the door of each room. This form lists the tasks to be performed by the husbandry person each day. The husbandry person

initials the form to indicate that all required tasks have been completed for the day. Not all tasks on this form are performed each day.

H. Environmental Conditions in the Room

1. The following environmental conditions are monitored:

a) Room Temperatures – room temperatures are maintained at 72 degrees Fahrenheit, plus or minus 2 degrees. Because many animal room temperatures are zone controlled, it is not always possible to make adjustments for individual rooms. Zone control means the thermostat controls 3-4 rooms.

b) Humidity – Normal humidity ranges for animal holding room's ranges from 30 to 70%. If humidity is above or below this range, the humidity will be monitored closely over the next several days to see if the humidity level returns to normal values. Corrective measures will be taken if the humidity does not return to normal.

c) Room Pressures – Air pressures in rooms are maintained at a positive or negative pressure, relative to the corridor. All bio-containment rooms are under negative pressure. Rooms containing disease free animals should be under positive pressure (air is blowing out of the room) and rooms containing animals with pathogens should be under negative pressure (air is blowing in).

d) Lighting – The standard light timer is set on a 12-hour light cycle with the lights coming on at 7 am and off at 7 pm. If alternative light cycles are needed, contact the PAR Manager. If the animals are housed in a multiple user room, it may not be possible to adjust the timer for a single investigator.

I. Health Monitoring

1. The Program for Animal Resources (PAR) provides veterinary oversight to the IBT animal facility. A member of the veterinary staff visits the animal facility daily. A veterinarian from UTHSC will be on call for weekends and holidays. The PAR care staff observes the general condition of the cages and the animals on a daily basis. Animals are closely scrutinized at least twice a week when water or caging materials are changed.

2. If there is a specific condition or complication that needs to be examined by the PAR care staff, or if each cage needs to be removed from the shelf daily and the animals checked, please inform the PAR manager or veterinary technician by e-mail. A Special Instruction Work Order will be created and hung on the front of your cubicle and provide information for the PAR care staff.

3. If sick or dead animals are found, or if a cage is overcrowded according to set criteria, an appropriate colored card will be placed on the front of the cage with the date of observation. Hot pink signifies overcrowded, neon orange notes an animal's death and bright yellow indicates a cage containing sick or injured animals. A HEALTH ISSUE REPORT or an OVERCROWDED REPORT will then be completed. These reports are to be used by the PAR and Investigative Staff to report overcrowded cages, sick, injured or dead animals. This form remains on file with the veterinary technician.

4. If an animal is sick or injured and the veterinary staff feels the animal should be sacrificed, the veterinary staff will put a note on a cage that houses the animal. The investigator will be notified by E-mail. The investigator then has 24-48 hours to sacrifice the animal depending on the severity of the health issue. If this is not done within the time period indicated, the veterinary staff will make an attempt to contact the investigator, and then sacrifice the animal if no one can be reached. If the investigator disagrees with this disposition of the animal,
they must write a memo to the IACUC within the time period for further discussion.

5. A member of the veterinary staff will contact the investigator when an abnormal condition exists with one or more animals. A member of the veterinary staff will recommend an appropriate course of action depending on the condition of the animal(s). This recommendation may range from continued monitoring, treatment, or euthanasia. A member of the veterinary staff and/or PAR management is always available to discuss treatment options with the research staff, and modify clinical treatment as appropriate to maintain research integrity. The research staff is expected to contact a member of the veterinary staff or PAR manager regarding these recommendations within 24 hours of notification. In cases where treatment or euthanasia has been recommended, the PAR care staff will proceed with appropriate actions to maintain humane animal care if the research staff has not responded within 24 hours.

6. If the PI wishes to be notified immediately by the PAR staff when an animal has died, inform the PAR manager or veterinary technician by e-mail. Provide the necessary contact information that will be placed on the Special Instruction Work Order, for use by the PAR care staff.

7. All animals found dead are placed in a bag with the location, date, PI, and initials of PAR staff who found the animal and held in the necropsy refrigerator for three days.

8. A yellow Health Issue Report is placed on the cage and will remain there for five days or as long as the condition exists. The PAR care staff encourages the investigator and staff to visit the facility often if these reports will be of value.

9. The research staff can fill out Vet Notice card and, leave it on the cage, and enter the cage location on the Health Issue and Overcrowding form found on the door of each room to request a non-emergency veterinary consultation. Cards can be found in the red/yellow card boxes located in each procedure room. Remember to use an ink pen and write legibly.

X. Experimental Issues

A. Use of the Hoods in the Procedure Rooms

1. **ALL animal manipulations in the PAR animal facility MUST BE done in a hood**! This includes checking of plugs of females, weaning pups, separating animals into new cages, or adding food and water. This protects both your research animals and those of other investigators.

2. To begin usage of the hood, first put a pair of work gloves on, next disinfect by spraying the inside of the hood, but not the back panel (HEPA filter), with Virkon and then wipe with Wipe-Alls afterwards. Wipe-Alls and gloves are located on top of the hood or in the cabinets near the sink.

3. Next spray alcohol inside of hood also and wipe with a fresh new Wipe-all to ensure that the Virkon has been completely removed. The alcohol is used to clean up any remaining Virkon from inside of the hood. If Virkon is left on the stainless steel, it will corrode the metal. Virkon and alcohol bottles are located on the side railing of the hood. To refill the bottles, please contact a member of the PAR staff.

4. To turn on the hood, locate the power switch for the blower motor on the upper right hand side of the hood.

5. **THE HOOD MUST BE DISINFECTED AND THE POWER HAS TO BE TURNED ON BEFORE CAGES CAN BE OPENED AND THE ANIMALS WORKED WITH.**

6. There is also a light switch located just below the power switch to better aid in viewing the animals under the hood.

7. Upon completion of work, re-clean and disinfect the hood by using the Virkon and alcohol as instructed above.

B. Scheduling a Procedure Room

1. All facilities have procedure room space for investigators and their staff to use. Some facilities have very little space, or the space is in high demand. Therefore, it may be necessary to schedule the space. Contact the PAR manager if this becomes necessary.

2. Leave the area clean for the next user and put all sharp objects in the sharps container. If the area is soiled when you arrive, notify the PAR Manager. When an investigator leaves the procedure room soiled and/or in disarray, there is a minimum $35.00 fee to clean the area. This will be added onto their per diem charge.

3. Investigators using Procedure Rooms 117 – 119 may not leave animals in these rooms overnight. All cages left in these rooms must be labeled with the name of the primary investigator, the protocol number, the name of the person leaving the mice, a contact number for this person, and the times the animals were left and will be removed.

4. After use of the anesthetic machine in the Surgical Suites (Rom 117 & 119), please make sure the Oxygen is turned off on the anesthetic machine (close gently) and the large CO2 tank (close firmly).

C. Using Biohazards

1. The PAR facility has bio-containment housing available. Biohazard housing space is limited, and housing space must be assigned 3-4 weeks before the start of the experiment. Space assignment in these facilities can be arranged by contacting the PAR manager.

2. Obtaining approval to use biohazardous agents:
    a. Protocols involving the use of biohazardous agents in animals require approval by the Animal Biosafety Committee before the IACUC can approve the protocol.

b. Agents that require biosafety approval include infectious agents (even replication-deficient vectors), toxins, carcinogens, recombinant DNA, and chemicals with known or suspected toxicity for humans or animals. The purpose of the review is to ensure the safety of both the animals and the animal care personnel. Use of radioisotopes is reviewed by the Radiation Safety Committee and does not need further review by the Animal Biosafety Committee.

c. Questions regarding the need for biosafety review and approval should be directed to the IBT Environmental Safety Specialist, Stephanie Colman at 713-677-7953.

d. The following agents do not require review/approval by the biosafety committee:

Formaldehyde, paraformaldehyde, or other fixatives used as part of a euthanasia to preserve post mortem tissue
BrdU infused hours prior to euthanasia
Human cells or proteins used in animals unless the human material carries infectious or other hazardous agents.

e. If you do not already have biosafety approval, you can use a link within the animal protocol form found on the share drive / Forms / Protocol Form to find the IBC form. You must then send a copy of the complete IBC form to the Texas A&M University Committee On Infectious Biohazards (IBC) by email or by submitting a paper copy. Supplemental material should be sent directly to the IBC. The PAR does not submit your application for you.

f. The IBT Institutional Biosafety Committee may require revisions/clarification before they issue approval for the use of biohazardous agents.

g. Questions regarding the biosafety approval process should be directed to the IBT Environmental Safety Specialist.

h. Once approval is received, the PI needs to send a copy of the approved IBC form to the IACUC so that a final approval letter for the protocol can be obtained.

i. IBC approval is given for a particular agent used in a particular species. IBC approvals must be renewed every three years.

3. Special personal protective equipment is mandatory whenever entering the bio-containment suite. Contact the Manager to answer any questions you might have and to receive proper training for accessing these areas.

4. Investigators are required to provide a brief training session to PAR staff members so they understand the hazards of the agent being used, and the procedures required to follow when handling the animals and cages.

D. Taking Animals to Your Lab

NO LIVE ANIMALS CAN BE REMOVED FROM THE IBT VIVARIUM WITHOUT IACUC APPROVAL!

1) You may transport euthanized rodents to your lab within the medical center under the following conditions:
    a. Animals should be discreetly carried (i.e. covered with drapes).
    b. Use routes through the building that minimize contact with the public.(Freight elevator when possible)
    c. Animal carcasses may not be kept in the labs. They must be returned promptly (covered when in public areas) to the animal facility and placed directly in the necropsy freezer.

2) Any live animals that have been transferred out of the PAR vivarium may not be returned to the facility without going through the quarantine process.

E. Obtaining Training in Specific Procedures

1. It is absolutely critical that individuals performing procedures on animals be knowledgeable in the techniques. If you would like to perform procedures that are new to you, the PAR has numerous resources to assist you.

2. Please contact the manager to schedule a training session with the appropriate veterinary staff member. This includes but is not limited to: training, surgical procedures, diets, treatments, anesthesia, and contamination prevention.

3. The veterinary staff is available to teach you to perform procedures such as proper restraint, blood collection, injections, and euthanasia techniques.

4. There are also SOPs written to help investigators and their lab personnel with many procedures. These SOP's are available to help with the training process. Ask the manager or the veterinary staff for a copy of the SOP's for the particular question you have.

F. Euthanasia

1. Specific guidelines must be followed in order to perform humane euthanasia of research animals. As you complete a protocol, reference the AVMA panel on euthanasia for approved techniques appropriate for the species under consideration. If these are not compatible with your experimental design, contact a member of the PAR veterinary staff for advice.

2. It is critical that the animals are euthanized using the technique that was proposed by the investigator in the protocol and approved by the IACUC.

3. If using CO2 for rodents, be aware that carbon dioxide is not a poison. It works by displacing the oxygen that the animals breathe. Although they lose consciousness rapidly, **you must keep them in the CO2 chamber for a full 5 minutes** to ensure that they are completely euthanized. **You must ensure cessation of life before placing the carcasses in the bag/freezer.**

4. **For effective euthanasia, place no more than 12 mice in small cage or 30 in a large cage**.

5. Single carcasses should be placed in a Whirl-Pak bag, and sealed tightly, while multiple carcasses should be placed in the provided small black plastic bags. Once the bags are securely sealed they should be placed in the red plastic containers located in the freezer unit.

6. Do not store carcasses in gloves. Use only the Whirl-paks or plastic bags provided. If the carcasses are to be kept in freezer/refrigerator units in the procedure rooms then the bags must be dated and not kept over one week.

7. Euthanasia stations/carts are located in the corridors on both floors of the vivarium. Detailed instructions for use are posted above each cart. If you need assistance with the euthanasia process, please ask any member of the PAR staff. The PAR will also euthanize animals for you at no charge. You must mark the cage with a "SAC" card **with your name on the card**. This is required to insure that the PAR staff euthanizes the correct cages and adjusts the correct inventory.
8. **Animals identified for euthanasia must be provided food and water and should not be crowded!** On occasion the PAR staff will not be able to euthanize the animals until the following day, although they will be removed from your inventory on the day that euthanasia is requested.

G. Weaning and Overcrowding of Cages

When an overcrowding problem is noted, the PAR care staff will place a hot pink card that reads, "Overcrowded" "Separate before , on the cage. An Overcrowded report will be filled out to be

tuned in to the veterinary technician. The investigator will receive an e-mail with notification of the overcrowded cage. The investigator then has 48 hours in which to separate the animals. In other words, the time frame will follow the PAR cage change schedule. For example, on Thursdays, the PAR staff will deal with any overcrowded cages found on the previous Monday, giving the investigator 48 hours to attend to the cage. The PAR staff will deal with those overcrowded cages found on Fridays on the following Tuesdays. For this reason it is important that each investigator, or their staff, monitor their animal's health daily.

Habitual failure to respond to requests to address crowding problems will lead to sanctions. The IBT Director will be notified on the second issuance of overcrowding warning and take appropriate action.

If, by the end of the 48-hour time period (the next change out time for an area), the investigator has not separated the animals, the PAR care staff will separate the animals and the investigator will be charged a $25.00 fee. The PAR care staff will log the date and how many cages they separated into the per diem book, and the overcrowded slip will be saved for verification of separation. The PAR care staff separates animals by identifying the original cage with an alpha letter, i.e., "A", and the number "1". The date of separation is also included. The other cages are labeled with the same letter followed by sequential numbers A-2, A-3 etc. and the date of separation. Animals are separated by gender.

Mice and rats are generally weaned at 21 days of age. The PAR care staff will not write up overcrowded notices for pups needing to be weaned but may not be old enough yet. At such a time when there is no doubt for the PAR care staff that the pups are old enough to wean an overcrowded notice will be drawn up.

If space in the room is insufficient to allow the number of cages required for appropriate weaning, and the investigator has not responded to requests for weaning, the animals may need to be sacrificed to prevent overcrowding.

Number of Rodents per Cage

Per diem rates are calculated, in part, on how frequently cage changes occur. The population of animals housed in each cage dictates how frequently the cage must be changed to insure proper sanitation for the animals. The PAR has calculated per diem rates from these numbers. Alterations in these numbers require approval from the PAR veterinary staff and adjustment in per diem rates.

Cage Size		**# of animals per cage**
MICE		
Standard OPTIMICE Cage 75 Square Inches		5 small mice of the same sex 4 large mice of the same sex or 3 male FVB
*Small Mouse: = up to 25g		*Large Mouse: >25g
Small Breeding Cage (MICE): Standard OPTIMICE Cage 75 Square Inches	All plug dates must be noted on the cage card	1 male and 2 females as long as no litter is present. PAR places a notice on the cage @ 15 days in gestation. Investigator is given 72 hours to remove the pregnant female. If not done within the time frame, PAR will separate for a $25 charge. Multiple separations by PAR will result in the investigator only being allowed to house 1 male and 1 female. **Or** 1 male and 1 female may be housed with no more than 12 pups. Pups must be weaned at 21 days and separated by gender

Large Cage (MICE): 144 Square inches	14 Small Adult mice or 12 Large Adult mice ***NO BREEDING ANIMALS (PREGNANT FEMALES) OR PUPS!***
Large Breeder Cage (MICE): 144 Square inches	***LARGE CAGE WHERE BREEDING ANIMALS ARE PRESENT*** 1 Male mouse and 3 Female mice with pre-weaning age litters (no more than 15 total pups)
RATS OPTIRAT cage 144 Square inches	6 < 100g. juvenile rats 4 101-200g. juveniles 3 201-400g adult rats 2 401-500g adult rats 1 > 500g adult rat
Breeder Cage (RATS) OPTIRAT: 144 Square inches	1 male and 2 females 1 female with a litter

****If animals must be weaned at an age greater than 21 days, please inform the PAR manager or veterinary technician by e-mail. A Special Instruction Work Order will be attached and hang on the front of your cubicle to provide information to the PAR care staff. This should also be noted and justified in your protocol. Weaning should never be delayed beyond 26 days unless there is specific justification for doing so and a Special Care Instruction Form has been completed.

****Breeder males housed with litters during the fourth week will plug young females. It is best to remove the breeder male from the cage before this occurs.

H. If a Rodent Escapes

1. Attempt to catch the animal if you can. If you are unable to do so, notify the PAR staff immediately so they can help.

2. **DO NOT** place the animal back in the cage with the others. This animal may have acquired pathogens from being on the floor and could contaminate your whole colony as well as others.

3. Such animals should be placed in a new cage and then euthanized.

XI. Getting Animals Into the Facility

A. Purchasing Animals

1. All animals purchased for use at the IBT must be done through the PAR.

2. A standard part of the ordering procedure is an assessment of housing space availability. An investigator who bypasses this process may have the animals rejected upon arrival. Space is extremely limited in the facility and may not be available upon the animals' arrival. No animal orders will be placed until it has been verified that the incoming animals are going to an approved protocol and unless adequate and appropriate housing space has been identified and confirmed with the PAR animal care staff.

3. Purchase orders for animals are to be submitted by the departments or centers that are going to use the animals.

4. To place an order for animals, submit an animal purchase request from the PAR Helpdesk. Include the vendor, protocol number, cubicle destination, and the kind(s) of animals to be purchased. A confirmation e-mail will be sent to you after the order is received.

5. Disease outbreaks sometimes occur in vendor colonies. Approved vendors notify the PAR immediately when breaks are detected in colonies. Investigators will be notified immediately if contamination occurs.

6. The PAR manager maintains a list of approved vendors known to provide healthy animals. If you want to order animals from an unapproved vendor, please contact the PAR manager.

7. If you need help in completing a purchase order, see the PAR office associate. Accurate completion of the order will greatly speed up the processing.

8. Animal orders will be placed by the PAR within 72 hours of receipt. Every attempt will be made to place the order more quickly, but the 72-hour interval may be needed to check housing availability, complete administrative tasks, and fix any problems with the order.

B. Transfers Between Rooms Within the IBT Vivarium

See above: section IV.B. Maintaining pathogen-free facilities – Health status and animal transfers

C. Import/Export from Other Institutions

There are three parts to this section: Import of rodents into the PAR, Shipping animals to other institutions within the United States, and Shipping animals to collaborators overseas. For assistance with any animal transfer please contact the animal transfer coordinator, Jonathian Few, at 713-677-7720. No animal imports will be processed until verification that the animals will be going to an approved protocol.

RODENT IMPORT INTO THE PAR ANIMAL FACILITY

The PAR coordinates importation of all rodents at the IBT. The animal transfer coordinator receives health information from other institutions, submits all health information to the PAR veterinarian for review and approval, issues shipping approvals to other institutions, and coordinates final shipping arrangements with those institutions. PAR veterinarian oversee the mandatory quarantine program for all imported rodents. All rodents arriving from non-approved vendor sources are required to

complete a quarantine program before entering the animal facility. Quarantine space is limited, and shipments are scheduled to arrive as space permits. Investigators should allow at least 1 month for completion of paperwork and arrival of shipments. Depending on where the animals will be housed, an additional 5-8 weeks may be required for completion of the quarantine period. Investigative staff should incorporate these times into their schedule when planning experiments on imported rodents.

1. The shipping procedure is initiated by the investigator upon completion and submission of an **Animal Transfer Request** via the PAR Helpdesk.

2. The Animal Shipping Record form provides the following information.
    a. Name and address of PAR Investigator, telephone number, fax and email (if available).
    b. Name and address of the investigator at the Sending Institution, telephone number, fax, and email.
    c. Name and address of the Veterinarian at the Sending Institution, Telephone number, fax and email (if available).
    d. Number, species, strain or line, age, and sex of animals being shipped.
    e. Room location where animals will be housed in the animal facility.

3. Upon receipt of the import request, the process typically proceeds as follows:

    A health report is requested from the sending veterinarian. (Note: written approval to ship MUST be sent to the sending institution before the shipment will be accepted).

    A veterinarian reviews the health report. If the animals are eligible for import, the veterinarian issues approval.

    The Animal Transfer Coordinator then schedules an import date based on space availability in the quarantine facility and requests that the shipping institution contact the PAR with air bill information and delivery date once final shipping arrangements have been made at their end.

    The Animal Transfer Coordinator schedules arrival of the animals with the PAR receiving dock, and sends all pertinent paperwork to the receiving dock.

    When the animals arrive, the PAR staff verifies the number, sex, and strain or line of the animals against the shipping information, visually inspects the animals for health, and notifies the Veterinary Technician of their arrival. Animals are then placed in quarantine. During quarantine, the animals are observed daily for health. After approximately two weeks, samples are collected and sent to Charles River labs for processing/testing. It can take an additional two – four weeks before test results are returned.

    Once received, the PAR veterinary staff reviews the test results. If all results are negative, the animals are transferred into their permanent housing room by the PAR staff. The investigator is then notified by phone or E-mail of their transfer.

4. Animals that test positive are kept in the quarantine facility or moved to the Biohazard suite. The PAR veterinarian will contact the investigator to discuss other options.

RODENT EXPORT IN THE UNITED STATES

The PAR coordinates all shipments of animals to and from the IBT. The Animal Transfer Coordinator provides health information to other institutions, prepares all necessary shipping

documents, receives approvals to ship from other institutions, and makes shipping arrangements with certified animal shipping agents. Investigators should allow at least 2 weeks (10 business days) for completion of paperwork and completion of shipments. The Investigative staff is responsible for clearly marking the animal cages to indicate which animals are being shipped. Animal shipments are made on Monday or Tuesday to ensure that there is adequate time to get the animals to the receiving institution before Friday.

1. The shipping procedure is initiated by the investigator upon completion and submission of an Animal Transfer Request form via the PAR Helpdesk. This form provides the following information:
   a. Name and Address of Receiving Investigator, Telephone number, fax and email (if available).
   b. Name and Address of the Veterinarian at the Receiving Institution, Telephone number, fax and email (if available).
   c. Number, Species, Strain, Age and Sex of animals being shipped.
   d. Location of Animals in the animal facility.

2. Upon receipt of the shipping request, a health report is faxed or e-mailed to the receiving veterinarian, and written approval to ship is requested (Note: written approval to ship MUST be received from the receiving institution before the shipment can be exported to other institutions).

3. When shipping approval is received, the Animal Transfer Coordinator completes the following steps:
   a. The investigator or shipping contact person is notified by email that the animal cages should be labeled for shipping. All animal cages to be shipped must be individually labeled.
   b. A health certificate is prepared, and a member of the veterinary staff examines the animals. The veterinary exam is usually done 1 day prior to shipping. A veterinarian must examine the animals before the health certificate can be signed.
   c. The shipping coordinator makes arrangements with an appropriate animal carrier to ship the animals.
   d. When the health certificate has been signed, the veterinarian delivers the shipping paperwork to the animal transfer coordinator and confirms that the shipment is ready to proceed. The PAR staff picks up the animal cages from the room, packs the animals in an approved shipping container with bedding, food, and shipping gel, and seals and marks the container for shipment. The selected courier picks up the container(s) from the receiving dock.

4. As much lead time as possible should be provided. Two weeks is the minimum amount of time required and delays in contacting the receiving institution often occur. Planning at least 3 weeks in advance is recommended. If timed pregnant or surgically manipulated animals are being shipped, the investigator should contact the animal transfer coordinator to make arrangements well in advance to ensure a timely shipment of animals.

5. Personal vehicles may not be used for the transport of any animals between facilities unless specifically approved by the PAR and the IACUC.

INTERNATIONAL RODENT EXPORT

The steps required to make an international shipment of animals are similar to those required for domestic shipments, but the shipping regulations of the receiving country must be met. Regulations concerning the importation of animals into other countries vary widely and change frequently. Some countries have very precise requirements for the importation of animals while others have no identifiable import restrictions. The following is a step-by-step procedure for shipping animals to foreign countries. Each step must be completed in order for the process to proceed.

1. The shipping procedure is initiated by the investigator upon completion and submission of an Animal Transfer Request form via the PAR Helpdesk. This form provides the following information.
    a. Name and address of receiving investigator, telephone number, fax and email (if available).
    b. Name and address of the veterinarian at the receiving institution, telephone number, fax and email (if available).
    c. Number, species, strain, age and sex of animals being shipped.
    d. Location of animals in the animal facility.

2. The best source of information is the receiving institution. The investigator that requests an international shipment must provide the name, telephone number, and fax number of the appropriate institutional representative at the receiving institution. The Animal transfer coordinator frequently needs to contact the institutional representative, and communication through E-mail greatly facilitates this process; so an e-mail address is very helpful if available.

3. Upon receipt of the shipping request, a health report is faxed to the receiving veterinarian, and written approval to ship is requested (Note: written approval to ship MUST be received from the receiving institution before the shipment can be exported.)

4. When shipping approval is received, the animal transfer coordinator completes the following steps:
    a. Upon receipt of approval to ship, the IBT investigator or shipping contact person is notified by email that the animal cages should be labeled for shipping. All animal cages to be shipped must be individually labeled.
    b. The Animal transfer coordinator makes arrangements with an appropriate animal carrier to ship the animals.
    c. The health certificate is given to a PAR veterinarian, and the animals are examined prior to shipment. The veterinary exam is usually done 1 day prior to shipping. A veterinarian must examine the animals before the shipment is authorized.
    d. When the health examination is complete, the veterinarian delivers the shipping paperwork to the Animal transfer coordinator and confirms that the shipment is ready to proceed. The PAR staff picks up the animal cages from the room, packs the animals in an approved shipping container with bedding, food, and shipping gel, and seals and marks the container for shipment. The selected courier picks up the container from the PAR receiving dock.

5. A minimum of three weeks is required to process requests for international shipment, so investigators are advised to allow for adequate time.

D. Allocating Space in the Facility

1. The PAR Manager, determines the policies for space assignment and works with IBT investigators to allocate space and resolve any possible conflicts.

2. MDACC space allocations are currently managed by Mr William Atkinson.

3. Cages cannot be stacked on top of another cage, placed on the floor, or placed on carts within the room. Investigators with inappropriately stored cages will be contacted by the PAR staff to cull their colony as necessary to prevent room overcrowding. Cages that remain inappropriately stored will be sacrificed as needed to prevent overcrowding

4. Space is very limited and investigators are urged to manage their colonies efficiently to minimize the amount of space needed.

E. *Per diems* and Inventories

1. *Per Diem* is the cost an investigator pays the PAR to care for the animals for one day. Rodents are counted on a per cage basis.

2. The time an animal is housed is called an Animal Day. If you have 10 animals (or cages) for 30 days, you would pay for 300 Animal Days.

3. In many cases, the PAR provides different housing methods for the same species. For example, mice can be housed in a small cage, a large cage, or a breeder cage. Obviously, the large cage can house more mice, but more food, material, and labor is used, resulting in a different *per diem* rate. The PAR manager can provide you with a *per diem* list.

4. An investigator's animals are counted daily, using the Animal Cage Count sheets that are kept in each suite's Per Diem book. There is a sheet for each rack and each sheet tracks all the various cage types. If a rack is shared by multiple investigators, there is an individual sheet for each investigator.

5. The PAR conducts a physical inventory every day of the month.

6. Billing is done at the end of each month.

7. Whenever an investigator or staff member permanently removes or adds animals to the room, the inventory is adjusted that day.

8. Bills are generally sent to the department administrator. Investigators should contact their administrator for a copy of the bill, or ask them to forward it to them.

XII. Penalties for Infractions of Rules Regarding Use of Animals and Animal Facilities

The following rules must be observed at all times. The rules have been divided into two categories, Level A and Level B. Level A and Level B differ in the extent to which infractions place everybody's animals at risk and in the consequences of non-compliance.

Level A Rules

1) Each person entering the vivarium must have his or her own access card.
2) Do not compromise the security of any animal facility by propping open entry doors, letting someone in who does not have a key card or guest pass, or using emergency exits during non-emergency times.
3) Do not bring mice from other institutions, or return live mice from your laboratory, into the PAR facilities.
4) Do not work in the PAR after having been in any other animal facility without showering before entry in any 24-hour period.
5) Do not bring any dirty equipment or supplies, such as cages that are likely to have been in contact with animals, into the PAR facility.
6) Do not conduct experiments that are not described in your approved animal protocol.
7) Do not use hazardous agents in animals unless you have an approved animal biosafety protocol. The measures described in that protocol must be followed precisely.
8) Euthanize animals using the procedure identified in the approved animal protocol applicable to the animal. Complete the euthanasia in a humane manner. This applies to all species within the facility.

Level B Rules

1) Respect the time schedules for PAR husbandry personnel. During their scheduled time, PAR husbandry personnel have priority use of the hoods.
2) Always provide food and water for animals that are moved into a new cage, even if the animals are scheduled to be euthanized. If the water bottle was inverted, be sure that it is returned to the upright position when you finish moving the cage.
3) Do not leave cages unmarked in the facility. Every cage must have a cage card in the holder.
4) Wean rodents according to the SOP. If you must deviate from the SOP, complete a Special Handling form and carry out the weaning according to that protocol.
5) All cages with animals must be on racks. Do not leave cages with animals on the floor or on carts in any facility unless explicitly approved by PAR.
6) If you want an animal moved from one room to another within the vivarium, or to another facility, do not move it yourself unless you have explicit prior approval from the PAR to do so. The PAR will perform all transfers after you submit an Animal Transfer Request.

7) Do not implant tumors or cell lines that are not listed in your approved animal protocol.

Level A infractions carry serious penalties because they place everyone's animals at risk. After the first Level A infraction, the individual, their PI and all members of the lab must be retrained, and the departmental chair will be notified. In addition, the PI will have to pay for any expenses incurred by the PAR that are directly related to the infraction. For example, additional testing of a colony may be necessary following an infraction to ensure no pathogens have been introduced. After a second Level A infraction, by the same individual, within 12 months of the first, the VP for Research will meet with the departmental chair and the PI to decide on the penalties. Depending on the circumstances of the infractions, the penalties may be as severe as barring the individual from the animal facility for one year, termination of the employee, and/or doubling of the per diem rates for all of the investigator's animals for an interval of time.

Level B infractions are also serious, but the first infraction will be handled by re-training only the individual involved. After the first infraction, a written notice will be sent to the PI and the individual must be retrained. After a second infraction, by the same individual, within 12 months, the entire lab, including the PI must be retrained along with the individual. After the third infraction, within 12 months, the VP for Research will meet with the departmental chair and the PI to decide on the penalties. Depending on the circumstances of the infractions, the penalties may be as severe as barring the individual from the animal facility for one year, termination of the employee, and/or doubling of the per diem rates for all of the investigator's animals for an interval of time.

XIII. Reporting Concerns About Inhumane Treatment of Animals

A. Policy

All laboratory animals must be handled, housed, treated, cared for, and transported in a humane and ethical manner. Any employee who has reason to question the treatment of animals at Institute of Biosciences and Technology is encouraged to report incidents involving the improper treatment of laboratory animals with complete assurance of confidentiality and without any fear of retaliation.

B. Procedures for Reporting Animal Concerns

Individuals who have concerns about animals receiving proper treatment or care or about personnel being adequately trained are encouraged to share these concerns with the PAR staff, supervisor, or manager, the Consulting Veterinarian, IBT Institutional Official, or any member of the IBT Institutional Animal Care and Use Committee (IACUC). Contact information is posted conspicuously at the facility entrance.

One of the above persons will meet with the employee to discuss the incident and prepare a written report. The employee's name will be held in the strictest confidence throughout the process.

Concerns can also be reported in writing. All inquiries will be treated confidentially.

The incident will be reviewed by the IACUC.

If the incident is found to be inconsistent with the policy of the PAR or applicable regulations regarding the humane and ethical treatment of animals, actions to remedy the problem will be taken.

Regardless of the outcome of the review, the reporting employee will be contacted by the initial interviewer and informed of all steps taken to correct the problem.

It is a violation of the policies of the Program for Animal Resources, and may be a violation of applicable laws, for any person to take any retaliatory action against an employee who reports an incident involving improper treatment of laboratory animals or who participates in a review of such an incident. Any person who feels that he/she has been subjected to retaliatory actions should report the same to any of the individuals listed above or to the Human Resources Office.