Joslin Diabetes Center

POLICY AND PROCEDURE MANUAL

LABORATORY ANIMAL HOUSING AND CARE

RESEARCH DIVISION

JOSLIN DIABETES CENTER

BOSTON, MASSACHUSETTS

March 2009

(Minor administrative changes made to March 2007 version)

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I. INTRODUCTION

A. General Facility Introduction

While the primary goal of the Joslin Animal Facility is to maintain healthy, virus-free animals for Joslin's investigators, it also has the responsibility to ensure that research animals are treated humanely. These multiple goals are accomplished by purchasing quality, disease-free animals, using proper husbandry techniques, providing training and guidance, and monitoring investigative procedures.

Rodents housed in the research animal facilities create an intense concentration of animals susceptible to infection. Joslin Diabetes Center has made a large investment in security measures intended to prevent adventitious murine infections that may affect experimental results. This document sets out the conditions and procedures for housing rodents free of these diseases which otherwise interfere with research.

This manual also outlines the procedural policies of the Animal Facility at the Joslin Diabetes Center. They meet federal regulations as outlined in 1996 NRC publication "*Guide for the Care and Use of Laboratory Animals*" as well as accepted norms of state and federal licensing organizations.

Persons using the JDC Animal Facility should familiarize themselves with the information contained in this manual. Compliance with these policies should result in a clean, virus-free facility providing the best possible environment for successful research. For further information about regulations, investigators may access the NIH Guide online at:
http://www.nap.edu/readingroom/books/labrats/chaps.html.

B. Contact Personnel

Dr. Laurie Goodyear, x4383 – Chairperson of the IACUC
Leigh Read, CIP, x4329 - IACUC Program Administrator
Dr. Richard Hurley, x4470 – Attending Veterinarian
John Stockton, x4388 - Animal Resources Manager

Animal costs and per diems are coordinated through the Office of Sponsored Research.

Copies of the *Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act, and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* can be obtained from the Director of the Animal Resources Facility.

II. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

A. Responsibility & Authority

The responsibility for administering the animal care and use program of the Joslin Diabetes Center rests with the Director and Manager of the JDC Animal Resource Facility and the Director of the Office of Sponsored Research. The Director of the Office of Sponsored Research also has the authority to implement policies for the Research Division of the Joslin Diabetes Center. The IACUC has the role and authority from the Institutional Official to oversee the animal program, facilities and procedures, and review, approve and request modification of proposed and ongoing research involving the use of animals at this institution. The IACUC is also responsible for reporting and making recommendations on all matters pertaining to the PHS Assurance of Compliance on Humane Care and Use of Laboratory Animals to the Director of the Office of Sponsored Research.

The Public Health Service mandates that all research projects that use animals must be reviewed by an Institutional Animal Care and Use Committee (IACUC) before public funds could be awarded. Strict established guidelines define the role and responsibilities of an institutional animal care committee, charging it to oversee all animal research within the institution, and to make sure that established norms for all aspects of protocols including sedation and anesthesia, housing, feeding, pain control, and euthanasia ("Public Health Service Policy on Humane Care and use of Laboratory Animals", Public Law 99-158) were followed.

Furthermore, it was mandated that frequent inspections of the facilities be carried out to ensure that established policies were being followed, and that the committee terminate research which has not been approved or that deviates from an approved protocol. Finally, proof of protocol acceptance by the committee was required to accompany any grant application to the NIH; a similar policy is now in place at many private organizations including the American Diabetes Association and the Juvenile Diabetes Foundation. The Joslin Diabetes Center files a yearly Assurance of Compliance with the Office of Laboratory Animal Welfare (OLAW). This file number is important on many grant applications.

Additionally, Joslin maintains an active registration as a research facility with the United States Department of Agriculture (USDA) in accordance with the federal law, the Animal Welfare Act (current revision, as amended). While the Animal Welfare Act that the USDA enforces does not cover mice of the genus *Mus* and rats of the genus *Rattus,* it does require that registered research facilities maintain an IACUC.

The IACUC makes a significant effort to achieve compliance with all laws, policies, and guidelines for the care and use of animals involved in research activities. This includes meeting on a monthly basis to review and approve all new research proposals, conducting continuing review of approved ongoing research, and requesting modifications and clarifications of ongoing projects. The IACUC also conducts semi-annual reviews of the animal care and use program and the animal facility for compliance and submits reports to the Institutional Official with recommendations, concerns or problems.

The institution and the IACUC make a concerted effort to ensure that all individuals involved in the care and use of laboratory animals understand their individual and collective responsibilities with all laws, policies, and guidelines for the care and use of animals involved in research activities for compliance. This includes participation in an institutional training program for all new employees who are involved in research with animals and annual review for all research staff.

B. Membership

The IACUC of the Joslin Diabetes Center is composed of ten members: one chairperson, one vice-chairperson, six affiliated members (4 scientists, 2 non-scientists, 1 veterinarian), and one non-affiliated member.

Members of the Joslin Diabetes Center Animal Care Committee are appointed by Joslin's Institutional Officer.

The Committee's Chairperson and Vice-Chairperson are:

Laurie Goodyear, Ph.D. – Chairperson
Section Head, Section on Metabolism, Joslin Diabetes Center
Phone: 617-732-2573 or x4383 / e-mail: laurie.goodyear@joslin.harvard.edu

Susan Bonner-Weir, Ph.D. – Vice Chairperson
Senior Investigator, Joslin Diabetes Center
Phone: 617-732-2581 or x4368 / e-mail: susan.bonner-weir@joslin.harvard.edu

Complete membership lists are available upon request from the IACUC Program Administrator in the Office of Sponsored Research.

C. IACUC Protocol Review & Approval Procedures

Before obtaining animals or initiating a study, investigators are required without exception to have prior approval from the Institutional Animal Care and Use Committee.

Complete information regarding the IACUC protocol submission and approval process can be found in the Joslin IACUC Handbook, maintained by the Office of Sponsored Research.

Briefly, once an "Application for Approval of Protocol for Animal Experimentation" is completed, it is given a four-digit protocol number and placed on the agenda for the next scheduled meeting. After review of the completed application at the scheduled IACUC meeting, one of the following actions will be taken:

- Approval (as submitted)
- Approval (pending modifications/clarifications)
- Deferral
- Disapproval

The IACUC is required to annually monitor ongoing, approved activities that involve the use of animals. Each active protocol must undergo an annual renewal process. Investigators are sent a reminder approximately 4 to 6 weeks before their protocol needs to be reviewed, along with the "Annual Protocol Review" form. The completed form must be submitted to the IACUC by the appropriate deadline and then is placed on the agenda for the next scheduled meeting. After review of the completed protocol renewal at the scheduled IACUC meeting, on of the following actions will be taken:

- Continuation Approved (as submitted)
- Continuation Approved (modifications/clarifications requested)
- Suspension of Study

Every three years after the initial review and approval, every protocol must be submitted for a *de novo* review. At this time, Investigators must follow the process for protocol submission and approval as above.

III. FACILITIES

The bulk of Joslin's Animal Resource Facility is located on the 5th floor, consists of approximately 7300 sq. ft and is divided into a barrier and non-barrier side. Both areas house specific pathogen free mice, exclusively. There is also an additional 1150 square feet of conventional housing space in the basement that houses rats and mice for short-term experiments.

A. Animal Housing

1. Location

The Joslin Diabetes Center houses animals on site (5th floor and Basement), as well as at Harvard School of Public Health, Children's Hospital in Boston, Harvard Institutes of Medicine, Brandeis University, Charles River Laboratories, Taconic, and Jackson Laboratory. All of these off-site facilities are accredited by the Association for the Assessment and Accreditation for Laboratory Animal Care, International.

The rodents at Joslin are free of many of the recognized pathogens of mice and are maintained in such a way as to minimize the introduction or spread of disease organisms. The 5th floor consists of Barrier and Conventional areas and the Basement houses rats and mice being held for short-term experiments or that need to be transported to Joslin laboratories. All areas have a policy of restricted access.

2. Classification of Animal Housing

Several acronyms are commonly used to describe the health status of rodents. Among them VAF (virus antibody free) and SPF (specific pathogen free) are the most frequently used. These acronyms refer to the idealized health status but are not specific as to the security of a barrier, or the methods of housing, nor the frequency of testing nor the agents tested. Rodents may be housed using arrangements with varying degrees of security against microbial infection. These security levels may be designated as barrier maintenance, and conventional housing.

a. Barrier (5th Floor)

The barrier systems consist of architectural, mechanical, procedural and operational arrangements designed to maintain a closed environment around the animal in order to minimize exposure to infectious agents. Barrier systems can be maintained at the level of the institution, facility, cage rack and cage. Mice in the JDC Barrier are housed in positive individually ventilated (PIV) microisolator cages. Microisolators are special cages in which the animals' ambient air is filtered from room air. All animal manipulation (including cage changing) is done in a Class 100 laminar airflow workstation. To secure the prevention of rodent infections, JDC uses microisolator housing inside the Barrier facility as well as irradiated food and autoclaved bedding. Using this system, all physical contact with the animals must be done using sterilized (Clidox) gloves and instruments. Microisolator cages extend barrier protection to the level of the mouse cage. The level of protection greatly decreases the spread of pathogens. This cage system may also serve as the most important barrier to infection in an otherwise contaminated environment.

With microisolator housing, the animals are protected from airborne pathogenic virus particles only while the filter top is in place. To maintain strict barrier status within the room, the cages should be opened for cage change or manipulation of the mice only under a biosafety cabinet with laminar airflow that protects the animals from airborne pathogens within the room.

Personnel access is highly restricted and mice that are removed from the facility cannot return.

b. Conventional (5th Floor)

Mice are housed in positive individually ventilated (PIV) microisolator cages (same as barrier) and are changed in laminar flow workstations. Access is limited and animals that are removed cannot return, except for animals housed in Room 585, Isolation. Details on Isolation Room use are found in Appendix XV.

c. Basement

Mice are housed in static microisolator cages and are changed in the room; rats are maintained in positive individually ventilated (PIV) microisolator cages. Access is limited and animals can be removed and returned to the facility with prior IACUC approval.

d. Traffic Flow

Due to the restricted nature of the facilities and different housing methodologies, the Animal Facility does maintain adherence to a strict traffic flow policy. The areas, in order of entry, are the 5th floor Barrier, 5th floor Conventional, and then the Basement. Therefore, people, animals, and equipment must follow this pattern within the facility. Once people or items leave an area (i.e. the 5th floor Barrier), they cannot go back that same day. Caging from the Basement area, however, is brought up to the 5th floor Conventional area for cleaning and sanitation since there is no cleaning area in the Basement.

3. Security & Animal Facility Access

a. Gaining Access

Computerized access cards secure the entrance to the Animal Facilities. Access is limited to one area at a time for any individual. **The Animal Resources Manager, Attending Veterinarian, and/or Group Leader must train all personnel prior to entering animal facilities**. Orientation training will cover proper entry procedures for animals, equipment and personnel, facility and animal husbandry overview, location and availability of clean cages, dirty cage drop off, microsolator cage changing techniques and procedures for requesting materials and services from animal care staff. No one, including facilities maintenance and HVAC maintenance personnel, may enter the barrier facility unless trained in required personal protection equipment usage and other procedures appropriate to their use of the facility (except in an emergency situation). New personnel will be asked to sign an agreement to adhere to the operating procedures before access is granted.

Animal facility access is granted when a person completed the required training and check-off list. The check-off list can be obtained from the IACUC Program Administrator in the Office of Sponsored Research. This includes:

- Online training modules from the AALAS Learning Library
    - The Research Facility Environment (ALAT 2)
    - Mice (ALAT 16)
    - Rats (ALAT 17)
    - Aseptic Technique, Surgical Support, and Anesthesia (LAT 11)
    - Overview of the *Guide for the Care and Use of Laboratory Animals*
    - Post-Procedure Care of Mice and Rats in Research: Minimizing Pain and Distress
    - Essentials for IACUC Members (IACUC Members)
- Review and understanding of the current Policy and Procedure Manual for the Animal Facility (this manual).
- Review of the OLAW educational pamphlet "What Investigators Need to Know about the Use of Animals" (NIH Publication No. 06-6009)

- Receive and review with the Principal Investigator the IACUC approved protocols for the studies to be performed.
- Completion of on-site orientation/training given by the Animal Resources Manager or Attending Veterinarian.
    - Topics covered include:
        - Entry procedures for humans and animals
        - Personal Protective Equipment and safety procedures
        - Equipment and personnel
        - Facility and animal husbandry overview
        - Location and availability of clean cages
        - Dirty cage drop off
        - Procedure room usage
        - Cage card procedures
        - Cage change technicians
        - Procedures for requesting materials and services from the animal care staff
    - Training for the 5th Floor Conventional and Basement areas is given every Wednesday at 10:30am and begins at the Animal Resources Manager's Office, Room 561.
    - Training for the 5th Floor Barrier area is every Wednesday at 1pm and begins at the Animal Resources Manager's Office, Room 561.

Once the training has been completed, the completed check-off list is to be given to and kept by the Animal Resources Manager. Animal Facility management will notify security that access to the Animal Facility has been granted and an appropriately coded key card will be given by security to the staff member.

b. Unauthorized Users

Unauthorized individuals are allowed in animal facilities only if they have received special permission from the Animal Resources Manager or Director and are escorted by an authorized user and follow all applicable guidelines. No one shall provide access to the barrier to relatives, friends, children, co-workers, or rotating students. It is not permissible for unauthorized personnel to piggyback into the facility with those who do have legitimate access. Card keys can only be used by the personnel to whom they are issued.

c. Limited Access

In limited circumstances, researchers may be granted access to Room 594, which serves as an area for using radioactive materials. Those individuals must complete the same steps as above, but only the animal facility specific modules need completion, an abbreviated training session with no animal handling is needed, and a notation is made in the training records regarding the fact that the researchers are not allowed to use or handle animals. Special lab coats are provided for individuals using this room. If animals are to be used in this room, please contact Joslin's Safety Officer, Wes Straub.

The IACUC Program Administrator keeps the training records for individuals with access to the animal facility.

4. Operational Conduct

All animals, which come to the Joslin, must be ordered and/or approved by Animal Facility management (Dr. Hurley or John Stockton) (see description of ordering animals, Section V.A.).

All research covered by Joslin protocols must be carried out on Joslin property. No animal should be kept outside the Animal Facility overnight (this includes all laboratories at Joslin).

Laboratory areas outside the Facility to be used for animal surgery or euthanasia must be indicated in protocol applications and according to NIH guidelines must be available for inspection by the Animal Committee. Protocols should be submitted to the other institutions, whenever and wherever applicable.

No one should enter the JDC Animal Facility after having been in another animal facility, or in a dirty, suspect, or biohazard area earlier that same day. **Under no circumstance shall any person enter an animal housing area or clean area after having been in a room where animals are isolated, sick or suspect.**

Animals must receive every consideration for their bodily comfort. They must be treated humanely, properly fed and watered, and maintained in the best possible sanitary conditions.

5. Cage and Animal Identification

All animals and/or cages should be easily identifiable with cage cards. Each cage card **must** contain:

- Label stating the investigator's name
- IACUC protocol number
- Cost center
- Contact person and phone #
- Sex and strain of the animals (Recommended)
- Weight and/or date of delivery and supplier (Recommended)

Investigators will be contacted regarding cages not labeled with P.I., cost center and Protocol # for immediate attention and correction.

Animals can be individually identified where appropriate by tail marking, tattooing, ear tags, or ear punching.

It is the **responsibility of each investigator** to insure that all personnel using animals as part of their research are trained in the care and handling of the animals, and that all procedures are carried out as required by the Animal Care Committee. This program has several components: 1) Completing on-line training; 2) Receive and review Protocol with P.I.; 3) thorough reading of this Manual; 4) An interview with John Stockton for orientation. **New personnel added to a project must also read the protocol approved by the Committee together with any reservations expressed by Committee members.**

6. Procedure Rooms & Areas

Procedure rooms and areas are available for use in the Animal Facility. The Animal Facility will keep the rooms stocked with minimal cleaning supplies, sharps containers, and trash receptacles. Individual laboratories may keep supplies in the rooms if they are properly labeled, maintained, and stored.

Animal Facility microscopes in the procedure rooms can be used but must be cleaned, turned off, and covered with the provided plastic cover after use.

7. Surgical Procedures and Monitoring

All surgical procedures must be carried out under an approved protocol and by an investigator qualified by experience or training. When the animal is expected to recover from anesthesia, aseptic techniques must be employed and appropriate steps should be taken to minimize the risk of postoperative infection and discomfort. Where the study does not allow recovery, the animal must be euthanized in a humane manner. Animals under anesthesia **must** be monitored

at all times. Monitoring includes observations of heart rate, respiratory rate, mucous membrane color, responses to reflexes, and/or monitoring by electrocardiograms or pulse oximeters.

8. Record Keeping
Record keeping is an essential part of any animal experimentation. When collection of data regarding animal weight, blood glucose, response to surgery or administered agents is part of an approved protocol, such information must be systematically recorded and be available for inspection by the attending veterinarian and/or members of the IACUC.

9. Animal Restrainers
Animals placed in restraint should be conditioned to such equipment before starting the research; the Joslin IACUC must approve unusual methods of restraint. Please contact the Animal Resources Manager or Attending Veterinarian for instruction on this. Animal restrainers need to be cleaned and sanitized after each use.

10. Animal Disposal
Animals should be disposed of by approved methods. Carcasses should be placed in the freezer used for that purpose only (in the Procedure Rooms). Dead animal freezers are for dead animals only. Any other waste from the procedure should be placed in a proper waste depository.

A death, infection, or infestation in any animal, which is not experimentally induced, must be promptly reported to John Stockton. Animals will be isolated, if deemed necessary, and all diagnostics and/or treatments required by the veterinarian will be carried out irrespective of how it may affect the research.

Animals from labs from terminal procedures are appropriately bagged brought to freezer in facility of origin. There is a designated freezer on the 6th floor for the disposal of animal carcasses for certain labs. There is a designated freezer on the 4th floor (room 435) for carcasses exposed to radiolabelled materials during terminal procedures. When disposing such carcasses, the date, isotope, and amount of activity must be documented. The waste is disposed of by a company designated by the Joslin Safety Officer.

11. Eating, Drinking, and Smoking
Eating, drinking and smoking are not permitted within animal or procedure rooms. Food and drinks are not permitted in animal or procedure rooms. Human food used as part of a study must be appropriately labeled and covered to prevent human consumption and should be removed at the end of the study. Food may not be stored in procedure room refrigerators.

12. Clean-up Procedures
Persons using the Animal Facility are responsible for cleaning up after themselves. After using any area in the Facility, all debris, used supplies, animal parts, etc. should be disposed of as appropriate. The counters should also be washed down and then wiped down with the Clidox disinfectant provided in each room. Sharps containers are provided in each room for the disposal of sharps materials. Sharps containers are disposed of by the animal facility staff in accordance with the Joslin Biosafety Manual

Trash containers, cleaning supplies, brushes and brooms are kept in each room. Mops and buckets are kept in the janitor's closet.

To minimize the risk of airborne transmission of contagious diseases, soiled litter from rodent cages should not be disposed of in garbage cans within the animal rooms. Rodents are transferred from dirty to clean cages in their housing room under laminar flow hood on the 5ht floor, and the dirty cages are then taken and emptied in the "dirty" side of the wash area.

13. Hazardous Agents

Use of radioisotopes, toxic chemicals, or infectious agents requires special arrangements to be made in consultation with John Stockton, Dr. Hurley and Michael Lanner (Safety Office), and should be part of an approved protocol. Approval from Joslin Biosafety Committee is required. Staff from the laboratory running the project will provide the daily care for these animals. Guidelines for the use of Adenoviruses within in the Animal Facility are located in Appendix XII. Please refer to the Joslin Biosafety Manual for further guidance.

Use of infectious agents or vectors requires approval of the Harvard Biosafety Committee and special arrangements for isolation must be made.

14. Restricted Areas

Areas containing experimental animals are "restricted areas" and should not be entered by unauthorized persons. Locked doors should not be unlocked under any circumstance. People who have not received orientation should not be in any animal area.

B. Environmental Monitoring

The entire facility is air-conditioned, and lighting in the areas housing animals is controlled by separate time clocks for each room with the lights on from 7 a.m. to 7 p.m. The ventilation system is electronically monitored by the Johnson Controls' Emergency Management System 24 hours a day, 7 days a week. Any failures are immediately relayed to the Facilities Supervisor or a designee, who is on call 24 hours a day, 7 days a week. The Facilities Supervisor is responsible for correcting the problem and contacting Animal Facility management.

Individual room light cycles may be adjusted to enhance reproductive performance so long as it does not interrupt another investigator's research. Please contact the Animal Resources Manager and/or Director for further information.

Humidity is monitored on a weekly basis by facility (i.e. Barrier, Conventional, and Basement). The range for humidity values remains between 30 to 70%. Digital monitors in each room monitor temperature. The range for animal room ambient temperature is $71 \pm 3°F$. Each room in the animal facility has at least 10 air changes per hour. Cages in ventilated racks have 30 to 50 air changes per hour, as recommended by the manufacturer.

Temperature, humidity, and air room pressure are electronically monitored through the Emergency Management System. The Facilities Supervisor is alerted to any readings in these environmental factors through the system.

All animal rooms, except for 579 (BL II) and 585 (Isolation), are maintained at a positive pressure relative to the corridors. Room 569 (transgenic laboratory) can be at either negative or positive pressure depending on its use. Rooms 579 and 585 are maintained at negative pressure relative to the corridors. Corridors are maintained at a relative pressure to the outside of the facility. Alarms and controls are located outside each room in the 5^{th} animal facility areas. The Facilities Supervisor is also alerted through the Emergency Management System to any abnormalities in air pressure.

Back up power is supplied to the animal facility through Joslin's emergency power system. This system operates off an oil system and can sustain back up power for a minimum of 4 days.

IV. VETERINARY CARE

A. Routine

1. Health Surveillance Program

Every rack of rodents contains a cage of sentinel animals that receive samples of dirty bedding from each cage on the rack and the time of cage changing. Mouse Sentinels are tested on a monthly basis for serological evidence of exposure to the following disease agents:

- Sendai virus
- Pneumonia virus of mice (PVM)
- Mouse hepatitis virus (MHV)
- Minute virus of mice (MVM)
- Mouse Parvovirus (MPV)
- GD-7 virus
- Reo-3 virus
- Epizootic diarrhea of infant mice (EDIM or Mouse Rotavirus)
- *Mycoplasma pulmonis*

Rat sentinels are testing on a monthly basis for serological evidence of exposure to the following disease agents:

- Sendai virus
- Pneumonia virus of mice (PVM)
- Sialodacryoadenitis virus/Rat coronavirus (SDAV)
- Kilham rat virus (KRV)
- H-1 virus
- Reo-3 virus
- *Mycoplasma pulmonis*
- Rat parvo virus (RPV)
- PARV NSI

Also on a monthly basis, the sentinel animals that are bled will have anal tape tests performed to screen for pinworms (*Syphacia*). This screening is done internally under the direction of the Attending Veterinarian.

At least twice a year, live sentinel animals from each room will undergo more extensive serological testing, which will include the following pathogens for mice:

- Sendai virus
- Pneumonia virus of mice (PVM)
- Mouse hepatitis virus (MHV)
- Minute virus of mice (MVM)
- Mouse Parvovirus (MPV)
- Lymphocytic Choriomeningitis virus (LCMV)
- Ectromelia virus (Mouse Poxvirus)
- GD-7 virus
- Reo-3 virus
- Epizootic diarrhea of infant mice (EDIM or Mouse Rotavirus)
- K virus
- Polyoma virus
- MAV 1 & 2
- Mouse cytomegalovirus (MCMV)
- Hantavirus

- *Encephalitazooan cuniculi*
- CARB
- Mouse Thymic Virus (MTV or MTLV)
- *Mycoplasma pulmonis*

Mice will also be tested directly for endo- and ectoparasites; microbiological cultures will be obtained from respiratory and intestinal systems and a gross necropsy performed. Histopathology samples will be taken if deemed necessary by the veterinary pathologist. Sentinel animals are replaced every 6 months.

The twice yearly serological testing for the following pathogens of rats will also be conducted. Serological evidence of the following pathogens will be tested for:

- Sendai virus
- Pneumonia virus of mice (PVM)
- Sialodacryoadenitis virus (SDAV)
- Kilham rat virus (KRV)
- H-1 virus
- GDVII virus
- Reo-3 virus
- *Mycoplasma pulmonis*
- Lymphocytic choriomeningitis virus (LCMV)
- MAV 1 & 2
- Hantavirus
- *Encephalitozooan cuniculi*
- CARB
- Rat parvo virus (RPV)

Rats will also be tested directly for endo-and ectoparasites; microbiological cultures will be obtained from respiratory and intestinal systems and a gross necropsy performed. Histopathology samples will be taken if deemed necessary by the veterinary pathologist. Sentinel animals are replaced every 6 months.

Sentinel animal testing is performed by reputable diagnostic laboratories specializing in rodent health monitoring programs. Joslin's sentinel animal program operates under an approved IACUC protocol and the Attending Veterinarian is the Principle Investigator.

Results of the animal health monitoring program have been negative for rodent viruses since the Fall of 2001 (except for MPV in room 574 in November 2006).

2. Sick/Dead Animals

Animal care staff and investigators need to use the green "Animal Concern" cards available from the animal facility to alert the veterinary staff regarding a veterinary/animal concern. Any sick animals or ones that have died unexpectedly should be reported to the Group Leader, Animal Resources Manager, Veterinary Technician, or Attending Veterinarian. In many instances, the veterinary technician can prescribe routine treatment for sick animals or euthanize them in conjunction with the investigator. Otherwise, the veterinary technician in conjunction with the Attending Veterinarian will examine the animal and determine the appropriate treatment, and discuss the plan with the investigator.

The treatment will be recorded on the "Animal Concern" cards in the provided area. After the animal's course of treatment has ended, or when the animal is euthanized, the cards will be kept on record by the veterinary technician or Attending Veterinarian

Moribund animals will be immediately sacrificed after observation. Animals with spontaneous tumors that are large enough to affect the mobility of the animal or are infected and/or ulcerated will require euthanasia. Untreated diabetic animals need to be carefully observed for adverse clinical signs (emaciation, lethargy, extreme polyuria/polydipsia), which can necessitate euthanasia.

4. Controlled Substances

The Animal Facility will not order controlled substances for investigators. All laboratories must coordinate their own purchase and use of controlled substances.

5. Diagnostics

JDC has limited in-house diagnostic capability and utilizes the Laboratory Animal Diagnostic Service at Charles River Laboratory. Prompt reporting is essential in the timely identification of health issues, which may have an impact on the colony as a whole.

The Attending Veterinarian will investigate unexpected animal illnesses and deaths as necessary and will perform necropsies and other diagnostics in cooperation with the investigator. Results will be reported back to the investigator and evaluated for overall health significance to the colony.

6. Holiday and Weekend Veterinary Care

Animal care staff is present in the facility on weekends and holidays to care for animals. Animal health concerns discovered during those times maybe communicated to the Attending Veterinarian who is on-call 24 hours a day, 7 days a week. In the event that the Attending Veterinarian is out of town, an appropriate back-up veterinarian will be identified and contact information will be provided to the animal care staff.

Emergency contact information for the Animal Resources Manager, and animal care staff is posted in the break rooms for the 5th floor Barrier and Conventional areas.

B. Movement of Animals Between Rooms (Internal Transfer of Animals)

If animals are to be transferred to a different room within the Barrier, or be transferred from the Barrier to the Conventional or Basement facility, a “Request for Internal Transfer ” form should be completed and given to John Stockton by 5 PM on Mondays. This form can be found in the “Forms” section of the Animal Facility on Joslin’s intranet.

When transferring animals between rooms in the Barrier or from the 5th floor Barrier to the 5th floor Conventional, animals are tested (Murine ImmunoComb and pinworm) on Tuesdays of the week of transfer. Animals are only transferred once a week. Animals will only be transferred if negative test results are obtained. Any positive test results will be investigated.

Animals being transferred from the 5th floor Conventional facility to the Basement are subject to testing and approval must be granted prior to transfer.

Once testing has been completed, the Animal Resources Manager or Veterinary Technician will approve the transfer and the investigator can arrange for the movement of the animals. No animals can be moved without prior approval from the Animal Facility.

C. Animals from Harvard Medical School

Animals from Joslin colonies at Harvard, and animals from the transgenics core at the Brigham and Women’s Hospital animal facility, may be brought into the Joslin Animal Facility after careful review of sentinel animal results and recent serology results from the animals to be moved. Animals may be pinworm and ImmunoComb tested once they arrive at Joslin. The “Request to Import Animals from HMS” form can be found on Joslin’s intranet.

Please note that while the Animal Facility will send out the serology sample for animals coming from HMS to Joslin, the proper forms must be completed before samples will be received and processed. Please contact John Stockton, or Maria Petruzzelli for further information.

D. Quarantine

1. Arranging for Quarantine

Animals from sources other than approved vendors will need to undergo a period of quarantine. This will consist of isolation for a period of 6 to 8 weeks and subsequent testing of sentinel animals. The quarantine program will be conducted off-site at Charles River Laboratories in Wilmington, MA. A "Charles River Laboratories Quarantine Project Initiation" form needs to be completed and submitted to the Animal Resources Manager along with a health report from the originating institution. Quarantined animals found to have been exposed to pathogens or mice from conventional facilities will need to be rederived into Joslin Animal Facility.

2. Quarantine Protocol

The protocol of testing to be performed while animals are in quarantine is as follows:

a. Add 6 (3 HO and 3 HE) female contact sentinels into the isolator of the quarantined animals within 2-3 days of arrival.
b. After 2 weeks, the sentinels are moved from direct contact and housed together on bedding soiled by the quarantined animals for 4 weeks.
c. Animal pups born to sentinel animals during this time are euthanized.
d. After a total of 6 weeks of exposure, 2 HEs and 2 HOs are submitted for health monitoring.
e. Both HEs are submitted for the Serology Assessment Plus profile, endoparasite screen, and ectoparasite screen.
f. One HO is submitted for the full bacteriology (no *Helicobacter*), endoparasite, and protozoan screen. The other HO will receive the endoparasite and protozoan screen.
g. The remaining sentinels will remain alive until the initial health monitoring results are obtained and reviewed.
h. The remaining sentinels might be tested, at the discretion of the Joslin Veterinarian depending on the results of the first four sentinel mice tested.

If animals are not shipped to Joslin at the completion of quarantine, health monitoring will continue according to the above protocol on a monthly basis.

All movement of animals must be approved by the Manager or Director prior to the movement occurring. Forms and detailed instructions are available through the Joslin Intranet on the Animal Facility site pages.

3. Isolation Room

Room 585 in the 5th Floor Conventional Facility is designated as an Isolation Room. Animals may be brought into this room after approval and completion of "Request for Isolation" Form with virus free health screens from their originating facilities. Further instructions on the use of this room can be found in Appendix XIV.

E. Tumor Cell Lines/Transplantable Tissues

Any product of murine origin or which has been passed in mice (i.e. tumor cell lines, bone marrow) needs to be tested for the presence of pathogens prior to being used in mice at JDC. *In vitro*, PCR , multi-analyte profiles, and other methodologies are available and arrangements can be made with the Director of Animal Resources for testing.

F. Animal Exportation

1. Procedures

When an investigator needs to ship animals to another facility, the "Request for Animal Export" needs to be completed and returned to John Stockton, Animal Resources Manager or Dr. Hurley. This form can be found in the "Forms" section of the Animal Facility on Joslin's intranet.

The current Joslin Health Monitoring should be sent to the receiving institution.

When the receiving institution grants approval for the shipment, the investigator will be notified and can arrange for shipment. The investigator is responsible for contacting a courier, making the appropriate financial arrangements, and arranging for the shipping date. Animal facility assistance is available for helping to pack the animals for shipment. A listing of animal couriers can be found on the Joslin intranet under the "Animal Vendors and Animal Export" sections.

If serology needs to be performed on mice being exported, please contact the Animal Resources Manager or Veterinary Technician. No serology can be performed unless the export form has been completed and returned to the Animal Resources Manager.

2. Health Certificates

A health certificate may be required for shipment of the animals, please ask the selected animal courier whether or not one is needed. Health certificates can only be completed by a licensed and accredited veterinarian. Some international shipments may require additional documentation. Please coordinate health certificate needs with the Attending Veterinarian/Director.

V. ANIMAL HUSBANDRY

A. Animal Procurement

All Joslin animal movement, including animal purchases and orders must be coordinated through the office of the Animal Resources Manager (Mr. John Stockton). An animal order form must be completed and placed in box outside Room 561 by noon of the requested order day. **No animals are to be ordered directly by an investigator**. This assures that animals are being ordered under an approved protocol and cost center, that there is sufficient space for incoming animals, and allows us to bulk order so that the most favorable shipping fees are obtained. When animals arrive at the Facility, they will be logged in and placed in cages usually within three hours. **The animals should be given plenty of time (usually the day of arrival) to become acclimated and should not be used until the next day at the earliest**. Animals arriving without prior approval will not be accepted. This includes any animals that are gifts. Any special instructions for the handling of animals should be discussed with John Stockton before the animals are delivered. When animals are ordered the yellow copy of the request form is sent to the investigator noting the arrival date. If there is any problem with the delivery, the investigator is notified immediately.

Animal order forms are not available online. Please contact John Stockton for the forms.

B. Ordering from Approved Vendors

A key objective is to keep the Animal Facility disease-free. Several strategies have been adopted to accomplish this, the major one being that all animals entering the facilities must be pathogen free (See section IV.A.1. of this manual for a description of excluded pathogens). In most cases, companies that are known to frequently screen their animals for pathogens and provide regular reports of the results can meet our needs. Joslin's designated "approved vendors" are Taconic, Harlan, Charles River Laboratories and Jackson Laboratories.

Animals obtained from these suppliers will be placed in the general population upon arrival unless prior arrangements are made with John Stockton for special handling.

The requirements for an approved vendor will be: 1) frequently updated serologic and parasitic data showing that the animals come from a pathogen-free facility; and 2) experience from our facility or facilities in the area which corroborates that the animals are indeed pathogen-free. If this has been demonstrated to not be the case, special precautions may be taken.

When investigators anticipate the need for a strain of animal, which is new to the Facility, they should give Mr. Stockton as much notice as possible so that a reliable supplier can be found.

C. Daily Care

1. Water and Food

Purina mouse and rat chow (Section IV.5) is provided *ad libitum*. Food is added as needed -- the receptacles are changed and new food provided on an as needed basis. No vitamin or other dietary supplementation is otherwise given or needed. Filtered water is provided *ad lib* -- bottles are checked daily to make sure that every cage has a sufficient supply. Clean bottles are provided every week or more often if needed such as with polydipsic diabetic animals. If a protocol calls for fasting (food and/or water), the animal facility supplied "Special Needs" card should be put on the cage stating this with the date(s) and investigator listed. **Animals should always have access to water.** Any empty water bottle found in an animal cage will be filled, regardless of the instructions provided by the researcher.

Water bottles should never be "topped off". Clean bottles can be found on the "clean" side of cage wash for the Barrier, clean cage storage for the Conventional are, and the anteroom/storage area in the Basement.

2. Special diets

If a research project entails dietary or fluid manipulation, the investigators will under most circumstances be responsible for this part of the animal's daily care. Please note that there are significant differences between rodent maintenance chow and diets used for breeding purposes.

When animals are to be fasted (which must be approved as part of the IACUC protocol), the investigators are responsible for initial removal of food, and for labeling the cage with the "Special Needs" card about how long the fast is to continue. The "Special Needs" cards for that purpose are available from Lead Animal Care Technician and within each animal housing and procedure room.

3. Bedding

Animals in the Barrier are housed on contact, autoclaved, deep sani-chip bedding. Animals in the 5th floor Conventional and Basement areas are housed on deep contact pelleted paper bedding. Bedding is changed and the cages sanitized once a week, or more often as needed.

Due to animal manipulations and/or breeding, it may be necessary for an investigator to change the bedding. If this is the case, the "Special Needs" should be notified to alert animal care staff. Bedding that is unacceptably dirty and/or wet, however, will be changed and the cage sanitized.

4. Environmental Enrichment

Nesting material is provided in each animal room for animal care staff or investigators to place in animal cages for breeding or other animals. Animals on soft diets should be supplied with a nyla/gumma bone to help with chewing needs and to decrease the incidence of malocclusions. Other environmental enrichment devices may be used.

Environmental enrichment devices should be ordered from a reputable supplier. Nesting materials need to be changed when soiled, however, the material may be kept with dams and their litters to decrease cannibalism and other aversive behaviors towards the pups. Solid devices need to be cleaned and sanitized on a regular basis, along with the cages, or as needed if soiled.

Environmental enrichment devices that are deemed dangerous to animal or human health (i.e. sharp edges), or that cause animal or human injury will not be allowed. Please contact the Animal Resources Manager or Director for further detail and information.

5. Food, Bedding, and Environmental Enrichment used at Joslin Diabetes Center

Animal Facility purchases food, bedding, and environmental enrichment materials for the mice and rats housed in the facility. Materials that are needed by an investigator that do not appear on this list may be procured after consultation with the Animal Resources Manager/Director.

The following table lists the food and bedding currently used in the Animal Facility:

Facility Area	Food	Bedding
5th Floor Barrier	Lab Diet # 5058 Picolab Chow	Northeastern # NE Beta Chip 1030
5th Floor Conventional And Basement	Lab Diet # 5020 9F Mouse Chow	Shepherd # CB1010 Paperchip
	Lab Diet # 5001 Rodent Chow	
	Lab Diet # 5008 Formulab	
	Lab Diet # 5001 M Rodent meal	
	Lab Diet # 5020 M 9F Meal	
All		Shepherd # SS Envirodri (enrichment)

6. Cleaning and Sanitation of Caging and Water Bottles

All solid bottom cages are sanitized a minimum of once per week – food receptacles every two weeks – racks, trash bins and cleaning supplies every thirty days.

Rodents are transferred from dirty to clean cages in the housing room under hoods (5th floor facilities) and given new food and clean water bottles. The dirty cages and water bottles are then taken to the washing facilities, emptied, and washed in the tunnel wash. Dirty litter should not be disposed of in the garbage cans in the animal housing areas – litter aerosolization can be a major source of disease transmission and allergy formation in animal handlers. Caging materials in the Basement are brought up in the dedicated cargo elevator using a covered rolling rack to the 5th floor Conventional area so that the items can be brought into dirty cage wash.

The washing system has a final rinse temperature that is held at 180° F for 3 minutes to sanitize the equipment. This is documented by temperature strip indicators that are used at least once weekly. Used indicator strips are kept in a logbook. After washing, clean cages and racks are stored in a room separate (clean cage storage) from any animal housing area.

Polydypsic rats may be housed on wire flooring that is elevated from the bedding and the discretion of the animal husbandry crew or Animal Resources Manager. This enables the animals to be away from excessively soiled bedding that is a result of their diabetic condition. Studies and reports have demonstrated that rats can safely tolerate this type of housing for up to 3 months.

7. Storage of food, bedding, and supplies

Unopened chow bags and bedding are stored off the floor and away from the walls on sturdy plastic pallets. Open chow bags are closed and then kept in vermin-proof containers with tight-fitting lids in the animal housing areas. Care should be taken to make sure that tops are replaced securely. All food, bedding, and supply bags, along with their contents, will be disposed of when expired, as determined by the expiration date on the bags.

8. Working With Adenoviruses and Other BLII or BLII+ Agents

Use of such agents requires specific animal husbandry procedures. Please refer to Appendix XII for specific guidelines.

VI. TRAINING

A. Introduction

Animal Facility staff training is key to the growth and development as well as proper functioning of the Facility. Staff receive the required training to access the various areas of the facility as described in Section III.A.3. Staff also receive regular and required training sessions as outlined below. Additionally, the Animal Facility will directly provide training or will host opportunities for laboratory animal related training at Joslin.

B. Animal Facility Staff Training

1. Regular Training

Approximately twice a month, the animal care staff is trained by the Attending Veterinarian in didactic sessions. These sessions follow the basic outline of the Assistant Laboratory Animal Technician (ALAT) program as set forth by the American Association for Laboratory Animal Science and include the following topics:

- Basic physiology, Anatomy, Cell Structure, Integument System, Skeletal System
- Circulatory System, Lymphatic System, Respiratory System, Digestive System
- Urinary System, Reproductive System, Nervous System, Endocrine System
- Nutrition, Genes & Chromosomes, Reproduction, Breeding, Mating, Animal ID
- Animal Rooms & Facilities, Bedding, Caging, Water, Cage Wash Hygiene
- Animal Procurement, Health & Disease, Disease Treatment
- Euthanasia, Experimental Design, Research Methodology
- Mice
- Rats, Hamster, Guinea Pigs
- Rabbits
- Dogs (including the “Working with the Laboratory Dog Video”) and Cats
- Non-Human Primates and Amphibians
- Ruminants, Swine, Birds, Fish

Additional training sessions that are rotated in this schedule include the “Training in Survival Rodent Surgery – General Training” CD Rom, Rules and Regulations Governing the Use of Research Animals Parts I & II, Allergies & Zoonoses, and Review Sessions. Staff members are also encouraged to request training on various topics. The goal of this training is to enable animal care staff to sit for and pass the ALAT exam.

2. Special Training Sessions

From time to time, invited guests may be brought to Joslin to give directed training to the animal care staff. Past topics have included disinfecting and sanitation, mouse breeding, caging products, and nutrition. The Animal Resources Manager and Director are responsible for arranging these sessions. Approximately two a year are offered.

3. Training Documentation

Animal Facility access training documentation is maintained by the IACUC Coordinator on an electronic database. Documentation for regular and special training sessions for animal facility staff are maintained by the Animal Facility Director. The training files include the training session titles and outlines, signatures of attendees, date of the session, and acknowledgement by Animal Resources Manager. Animal care staff may have access to their files upon request and the files are open to review by the IACUC and Joslin management.

B. Institution Wide Training

1. Regular Training

Animal Facility access training is provided on a regular basis. IACUC members have additional online modules required for training. Other AALAS Learning Library Modules have been identified as recommended or required for re-training by the IACUC.

2. Special Training Sessions

The Animal Facility may host internal training sessions for research animal users. These sessions will include rat and mouse models of diabetes and other related diseases, food, bedding, and breeding methodologies. These sessions are informal and not required but serve to enhance the knowledge level of the animal users.

3. One-on-One Training

The Attending Veterinarian/Director, Animal Resources Manager, and the Veterinary Technician are available for one-on-one training for various topics upon request. These topics include animal handling, injections, test article administration, surgery, anesthesia & analgesia, blood collection, necropsy, and protocol/study design. The training subjects available are posted in the Animal Facility area of the Joslin intranet. Please contact the Director for further information.

C. Outside Training

1. Professional Organizations and Meetings

Attendance at professional meetings is documented and maintained in the training files in the Director's office. Such meetings include AALAS, NEBAALAS, and the AVMA. The Animal Resources Director and Manager are members of AALAS and the Director, Manager, Veterinary Technician, Animal Care Supervisors, and IACUC Administrator are members of the New England Branch of AALAS.

2. Other Meetings and Tuition Reimbursement

Training at local seminars or training courses (i.e. Charles River Laboratories and Jackson Labs) is encouraged and documented in the training files. Joslin Human Resources also administers a tuition reimbursement program that animal facility employees may take advantage of, with the approval of the Animal Facility Director. Please contact Human Resources for further details.

VII. HEALTH AND SAFETY

A. Introduction

The goal of the occupational health and safety program (OHSP) at the Joslin Animal Facility is to prevent occupational injuries and illnesses of employees and visitors by avoiding, controlling and eliminating hazards in the workplace. A successfully program will depend on participation and compliance of all employees using the Joslin Animal Facility.

1. The IACUC's Role

The IACUC will review animal care and use protocols to ensure that a safe working environment is addressed. The IACUC will work with the Animal Facility staff and Joslin Safety Officer to ensure that the animal care and use program is functioning according to current regulations and standards. The IACUC also requires online training regarding occupational health and safety for all Animal Facility users.

2. The Investigator's Role

The investigator is responsible for ensuring that research is conducted in accordance with Joslin policies and good safe laboratory practices. The investigator is responsible for completing all appropriate hazardous agent protocols (i.e. radiation, chemical, or biological agent) and the IACUC protocol prior to research initiation. The investigator, and/or designee, should also coordinate the procurement of necessary safety equipment and procedures with the management of the Animal Facility and/or Joslin's Safety Officer.

3. The Institution's Role

Additional information regarding occupational health and safety may be obtained by contacting sources within Joslin. This includes the Human Resources office (incident reporting forms), Beth Israel Deaconess Medical Center Occupational Health Services at 185 Pilgrim Road, Farr Building, (pre-employment risk assessments, vaccinations), and the Joslin Safety Office.

4. The Employee's Role

The employee is responsible for conducting all animal work in a safe and humane manner in accordance with Joslin policies and safe laboratory practices. The employee is responsible for reporting to his or her supervisor, investigator, Safety Officer, Animal Facility management, or the IACUC any unsafe or hazardous conditions. The employee is also responsible for reporting any work-related injuries or incidents and wearing requiring personal protective equipment in accordance with Joslin policies.

5. Training

In addition to the training provided to animal facility users on a one-on-one basis, all animal facility users must complete the online training modules that have information on laboratory hygiene, zoonoses, animal allergies, and occupational health in general. This training is documented and the IACUC Coordinator maintains the documentation.

B. Health Evaluations

It is the policy of the Joslin Diabetes Center that new staff members participate in the Center's "New Staff member Health Assessment Program." This consists of completed the "Employee Placement Occupational Evaluation," seeing the Employee Health Nurse in the Beth Israel Deaconess Medical Center Occupational Health Services, and having the required tests performed. A confidential medical record will be established in your name. No staff member may commence service until cleared by Employee Health. All staff members means all staff, whether payroll or non-payroll, and students.

The standard screening tests ordered are: PPD (unless documentation of a negative PPD within the past three months is presented), Hepatitis Surface Antibody and Antigen, Rubella titer, Varicella, and tetanus. The Employee Health Nurse will also cover the appropriate steps to be taken with regard to health and safety that a staff member might encounter while performing his or her job.

If interested, Joslin staff members are eligible to receive the following immunizations: Rubella, Varicella, tetanus, and hepatitis (Requiem bivax) if a titer indicates a lack of immunity. Immunization is recommended but not required. Animal Facility staff still work within a human health care environment and some may be exposed to tissues or other materials of human origin (please refer to the Joslin Biosafety Manual for further information).

Any positive PPD tests will be followed up appropriately with radiographs and other medical care as deemed necessary by the Employee Health Nurse.

C. Personal Protective Equipment (PPE)

PPE are those garments worn to decrease or eliminate an employee's exposure to potential disease agents or allergens within the Animal Facility. PPE is required in all areas of the Joslin Animal Facility. The PPE must be worn at all times while in the Animal Facility.

The PPE for the 5th floor Barrier area is located in anteroom to the facility. The PPE for the 5th floor Conventional area is located to the left hand side of the door just as you enter the facility. Cloth lab coats are found in the lockers. The PPE for the Basement area is located in the anteroom to the facility. Cloth lab coats are found in the lockers outside of the facility, across the hallway.

1. General Staff

At orientation, employees learn about general health and safety procedures within Joslin from the Safety Officer. This will include a discussion of required and acceptable PPE within the building and laboratories. In general, this includes lab coats (not to be worn outside the laboratory), closed toed shoes, and gloves (appropriate for the type of use).

2. Animal Facility Users

PPE is described in detail for animal facility users at their one-on-one orientation given by the Animal Resources Manager, Director, or Group Lead. The PPE for each facility is required. Please note that lab coats worn in research laboratories may not be worn into and/or used within the Animal Facility.

a. 5th Floor Barrier

- Disposable Coveralls (only those supplied by the Animal Facility are permissible)
- Shoe Covers
- Head Cover
- Gloves when handling animals and/or performing procedures (S, M, L with no powder provided – other types are provided upon request)
- Closed toe shoes
- Face Masks (if needed)

b. 5th Floor Conventional and Basement

- Cloth Lab Coat, buttoned at all times
- Shoe Covers
- Head Covers
- Gloves when handling animals and/or performing procedures (S, M, L with no powder provided – other types are provided upon request)
- Closed toe shoes

- Face Masks (if needed)

3. Animal Facility Staff

Animal facility staff has dedicated scrubs and shoes that are to be worn only within the facility. Scrubs can be worn in lieu of the disposable coveralls and cloth lab coats. Scrubs must be kept clean and in good repair. The Animal Resource Manager can purchase all needed scrubs. Disposable arm covers are available to cover exposed forearm areas. Facemasks should be worn when changing cages or working in the cage wash areas.

4. Disposal of PPE

PPE donned while in the Animal Facility may not be taken and worn outside of the facility. Gloves are to be removed and disposed of in the room of use. Users should change gloves between rooms and not wear the same gloves from room to room. Disposable garments (coveralls, shoe covers, head covers, arm covers) are to be thrown in the trashcans provided at the exits to each area. For the 5th floor Barrier area, coveralls may be saved and reused the same day. However, head and shoe covers need to be disposed of after every use. Dedicated cloth lab coats need to be disposed of at least weekly in the provided bins by facility exits so they can be laundered. Dedicated coveralls and lab coats for the Animal Facility may be stored in designated areas within the Animal Facility.

D. Facility Operations

1. Emergency Shower Stations

Emergency shower stations are located in the hallways of both the Barrier and Conventional areas.

2. Animal & Waste Disposal

Animal carcass and wastes are disposed of in designated areas within each Animal Facility area. Carcasses are bagged and placed within the designated freezers in the Barrier, Conventional and Basement. One to two times a week, the bags are removed from the freezer and placed into a biohazard container that is then picked up by a biohazard waste disposal company. Animal waste (primarily food and bedding) is bagged in biohazard bags and placed in the designated trash containers. The trash container is placed in the designated room outside of the facility on the 5th floor and is emptied one to two times a week by a contracted biohazard waste disposal company. Animals from labs from terminal procedures are appropriately bagged and given to an animal care technician on the Conventional side of the facility. There is also a designated freezer on the 6th floor for the disposal of animal carcasses for certain labs. There is a designated freezer on the 4th floor (room 435) for carcasses exposed to radiolabelled materials during terminal procedures. When disposing such carcasses, the date, isotope, and amount of activity must be documented. The waste is disposed of by a company designated by the Joslin Safety Officer.

3. Storage of Needles and Syringes

Needles and syringes need to be appropriately stored in locked cabinets, designated shelf areas, or in locked carts.

4. Controlled Substances

Controlled drugs and other anesthetics are maintained in a locked cabinet for use by the veterinarian and veterinary technician only. The Animal Facility does not order controlled substances for investigators. All laboratories must coordinate their own purchase and use of controlled drugs. The Animal Facility cannot store controlled substances for investigators.

5. Pest control

Pest monitors and traps are placed throughout the facility. The pest control program is monitored monthly by a commercial vendor, Waltham Pest Control, and by facilities staff.

6. Sharps

Sharps containers are located near each work bench in each animal housing and procedure room. Sharps are to be disposed of in accordance with the Joslin Diabetes Center Biosafety Manual. When containers are full, a designated sharps disposal company will replace full containers with empty ones. Do not overfill sharps containers.

7. Hazardous Agents

Other than cleaning chemicals for Animal Facility use and formalin for diagnostic use, the storage of hazardous agents is not allowed. Chemicals for cage wash are stored in a barrel dollar to contain any possible leaks. Spray bottles with Clidox® in animal rooms are appropriately labeled. Hazard agent use within an animal research protocol needs to be approved by the IACUC. Room 579 is designated as a BL2 room.

E. Work-Related Injuries and Illnesses

1. Bites & Scratches

Bites and scratches are hazards associated with all laboratory animals. A thorough understanding of species-specific rodent behaviors and habits is the best preventative measure against bites and scratches. All personnel handling animals are required to go through species-specific training according to the requirements sent forth by the IACUC and this manual. If any person feels that they need additional animal handling training, please contact the Animal Resources Manager.

Injured and sick animals, along with certain strains of mice and rats, may display unusually high levels of aggression towards one another and humans, and even experienced animal handlers must exercise caution. All bite wounds and scratches should receive immediate first aid and an evaluation for more extensive medical care may be needed. Diseases such as rat-bite fever are transmitted through bites and scratches as well. Please report all incidents, and seek proper medical care, through Joslin's Work-Related Injuries and Illnesses/Workers Compensation policy.

2. Zoonoses

Zoonoses are diseases that are communicable from lower animals (i.e. rats and mice) to humans under natural conditions.

a. Mice

There is no known risk of zoonotic disease development from typical exposure to the microbial floor of laboratory reared mice. All mice at the Joslin Diabetes Center have non-pathogenic, well-defined microbial flora as determined by either the vendor and/or quarantine services. Two diseases of concern when working with mice are lymphocytic choriomeningitis virus (LCM) and hantavirus. Animals brought into the Animal Facility are known to be free from these diseases and the Animal Health Monitoring program evaluates for these agents on a regular basis.

b. Rats

There is no known risk of zoonotic disease development from typical exposure to the microbial flora of laboratory rats. All rats at the Joslin Diabetes Center have been raised commercially and have a non-pathogenic, well-defined microbial flora. Two diseases of concern when working with rats are hantavirus and "rat-bite fever." Animals brought into the animal facility are known to be free from Hantavirus and the Animal Health monitoring program evaluates for the presence of this virus on a regular basis. "Rat-bite fever" is caused by two bacteria, *Streptobacillus moniliformis* and *Spirillum minor*. These bacteria are present in the upper respiratory tract and mouths of rats[1]. Rats are asymptomatic. Commercial vendors have virtually eliminated these bacteria from their animals[1].

[1]NRC (National Research Council). *Occupational Health and Safety in the Care and Use of Research Animals.* National Academy Press. (1997).

3. Allergies

a. Laboratory Animal Allergy Development

Allergic reactions to animals are among the most common conditions that adversely affect the health of workers involved in the care and use of animals in biomedical research [1,2]. The development of laboratory animal allergies (LAA) commonly begins with the inhalation of allergens, such as animal dander and urinary proteins. Skin and ocular contact with allergens can also result in symptoms. Most animal allergens are found in urine, dander, hair, serum, and saliva. Coexisting allergies and tobacco smoking can exacerbate the development and symptoms of LAA[3]. The objective of the Joslin Animal Facility is to decrease or eliminate the exposure of personnel to allergens when working with laboratory animals.

b. Symptoms

Symptoms of LAA can range from minor to life threatening. Rhinitis (a runny nose), conjunctivitis, asthma, or other breathing difficulties, fever, skin rashes, bumps (atopic dermatitis), and gastrointestinal disorders can all be the result of LAA. Please be ware that symptoms can be delayed up to 12 hours after animal exposure[3]. Promptly report any suspicious clinical symptoms through Joslin's Work-Related Injuries and Illnesses/Workers Compensation policy.

c. Guidelines

Guidelines of the Joslin Diabetes Animal Facility to help decrease and/or eliminate exposure to laboratory animal allergens include the following:

- Wear required and provided PPE at all times when working with animals
- Do not wear PPE outside of the animal facility
- Wear gloves at all times when handling animals
- Do not distribute animal bedding in your immediate work environment
- All cage cleaning should be performed in accordance with Animal Facility Policies and Procedures
- Ensure that animal cages are properly fitted into ventilated racks and that static microisolator cage lids fit properly
- Do not overpopulate animal cages
- Work with your animals in a ventilated hood or biosafety cabinet when required and whenever possible
- Work with animals in well ventilated areas, especially when you are not working under a hood/cabinet
- Clean and disinfect all equipment after use
- Wash your hands frequently and always after handling animals
- Avoid touching your face when working with animals
- Keep your work area clean
- Keep animal cages and transport containers properly covered at all times
- Avoid handling common times (i.e. door knobs) with gloved hands that have had animal contact

[1]Wolfle, T.L, and Bush, K.R. The Science and Pervasiveness of Laboratory Animal Allergy. Institute for Laboratory Animal Research. *ILAR Journal* 42: 1-3(2001).
[2] NRC (National Research Council). *Occupational Health and Safety in the Care and Use of Research Animals.* National Academy Press. (1997).
[3]Bush, R.K. Assessment and Treatment of Laboratory Animal Allergy. Institute for Laboratory Animal Research. *ILAR Journal* 42: 55-64 (2001).

4. Physical Hazards & Ergonomics

Risk factors for development musculoskeletal injuries when working in the animal facility include repetitive motion injuries (moving cages, moving animals, filling/emptying water bottles, scraping cages), lifting improperly, awkward postures, uncomfortable environment, heavy/fast work pace, and pulling or pushing large, heavy, or bulky items[1]. Symptoms can include low back, upper back, neck, wrist, and knee pain. While a team approach, well-maintained equipment, and job rotation are used within the Animal Facility to help decrease such injuries, staff should be aware of the hazards, work smart to avoid such hazards, report uncomfortable conditions to management, and properly reportinjuries[1,2].

[1]Echlin, E.T. Ergonomic Issues in Animal Research Facilities. CareGroup Occupational Health network at New England Baptist Hospital. April, 2003.
[2]Jones, R.L., and Eagleson, D. Ergonomic Considerations in the Laboratory. *Animal Lab News.* 2002.

5. Reporting Work-Related Incidents

In the event that a staff member experiences a work-related injury or illness, no matter how minor it may seem at the time, it is important that the incident be reported in a timely manner and that the appropriate medical care be provided. When a work-related injury or illness occurs, it must be promptly reported to the immediate department supervisor on duty (or another person in authority if a supervisor is unavailable) who will complete a Joslin Work-Related Incident Report. All incident reports are reviewed by Joslin's third party case management company as well as the Joslin Safety Office for statistical purposes and to assess and correct any safety concerns.

6. Occupational Health Services

The Joslin Diabetes Center contracts with the Beth Israel Deaconess Medical Center Occupational Health Services to provide treatment for work-related incidents. The Incident Report has instructions on it for locating care centers. In the event of a serious incident, staff should see immediate care at a hospital emergency room listed on the Incident Report. In the event of an eye injury during regular working hours, staff should seek immediate care at the Beetham Eye Center on the first floor of the building.

APPENDIX I

RECOMMENDED ANESTHETICS AND ANALGESICS IN RODENTS

The PHS *Policy* requires that "Procedures that may cause more than momentary or slight pain or distress to the animals **will** be performed with the appropriate sedation, analgesia, or anesthesia..." The choice of which drug regimen to use depends on the species, length and type of procedure, and personal experience and expertise. The following are recommended dosages for common compounds used in laboratory rats and mice. This list is not intended to be all-inclusive, but as a guide. Any questions regarding appropriate anesthesia/analgesia can be directed to the Attending Veterinarian (x4470). A link to the website for the PHS is available on the Animal Facility section of the Joslin Intranet.

MICE

1. Anesthesia
   a. Inhalation (must be used with proper scavenging of waste gas*)
      - Isoflurane, Metafane: To effect
   b. Parenteral
      - Avertin (tribromoethanol): 125-240 mg/kg IP
      - Ketamine/Xylazine: Ketamine 80-100 mg/kg and Xylazine 10 mg/kg IP
      - Pentobarbital: 50-80 mg/kg IP
      - Ketamine/Midazolam/Butorphanol:
        Ketamine 40mg/kg, Midazolam 2mg/kg and Butorphanol 0.1mg/kg IP. (Mix 0.4ml each of ketamine and midazolam with 0.01ml of 10mg/ml butorphanol and administer 0.8ml/kg)
2. Analgesia
      - Butorphanol: 1-5 mg/kg SC, every 4-6 hours
      - Buprenorphine: 0.05-0.1 mg/kg SC, every 8-12 hours
      - Flunixin Meglumine (Banamine): 0.5-2.0 mg/kg SC, once or twice a day.

RATS

1. Anesthesia
   a. Inhalation (must be used with proper scavenging of waste gas*)
      - Isoflurane, Metafane: To effect
   b. Parenteral
      - Ketamine/Xylazine: Ketamine 50-80 mg/kg and Xylazine 10 mg/kg IP
        Ketamine 90 mg/kg and Xylazine 10 mg/kg IM
      - Ketamine/Medetomidine: Ketamine 60-75 mg/kg and Medetomidine 0.25-0.5mg/kg IP (DO NOT MIX)
      - Pentobarbital: 30-50 mg/kg IP
      - Ketamine/Midazolam/Butorphanol:
        Ketamine 40mg/kg, Midazolam 2mg/kg and Butorphanol 0.1mg/kg IP. (Mix 0.4ml each of ketamine and midazolam with 0.01ml of 10mg/ml butorphanol and administer 0.8ml/kg)
2. Analgesia
      - Butorphanol: 0.2-2.0 mg/kg SC, every 4-6 hours
      - Buprenorphine: 0.01.0.05 mg/kg SC, every 8-12 hours
      - Flunixin Meglumine (Banamine): 0.5-2.0 mg/kg SC or IM, once or twice a day.

Administration Routes:

- IP = Intraperitoneal
- SC or SQ = Subcutaneous
- IM = Intramuscular

* Please note that due to insufficient waste gas scavenging, anesthetic gases should not be used in the Basement. Anesthetic gases may be used in the certified Class II hoods in rooms 585, 583, and 579, as well as the work tables in the procedure rooms in the Barrier and 5th Floor Conventional areas with slotted exhaust benches.

APPENDIX II

ALLEVIATION OF PAIN AND DISTRESS

Introduction

Two of the U. S. Government's Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training are as follows: "Proper use of animals, including the avoidance or minimization of discomfort, distress and pain when consistent with sound scientific practices, is imperative" and "Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with the appropriate sedation, analgesia, or anesthesia...". In addition a definition of a painful procedure can be found in the "Guide for the Care an Use of Laboratory Animal" and is as follows: "In general…it should be assumed that procedures that cause pain in humans also cause pain in animals." The Joslin Diabetes Center adheres to all of the Principles set forth by the U.S. Government and all procedures using animals must be done in such a way as to minimize or eliminate any pain or distress.

Any person who feels that an employee of Joslin Diabetes Center is causing inappropriate pain or suffering to an experimental animal or is inappropriately using radioisotopes, toxins, infectious materials, etc. is encouraged and required to report their concern to the senior investigator involved, John Stockton, Dr. Hurley or to the Chairperson of the Animal Care Committee. After conducting an investigation, the Animal Care and Use Committee will determine what actions if any should be taken.

Methodology

It is the policy of the JDC IACUC that Investigators incorporate the precepts below when developing an experimental protocol:

1. Reduce the number of animals to the minimum needed to obtain statistically significant results
2. Refine experimental procedures such that pain and distress are minimized
3. Replace animals with non-animal systems whenever possible and use the least advanced appropriate species when animal use is justified
4. Use appropriate anesthesia and post procedural analgesia as needed
5. House animals appropriately
6. Provide scientific justification when pain or distress cannot be minimized

Alleviation of pain and distress may be achieved by non-pharmaceutical means. This may include the separation of animals from other aggressive animals, provision of environmental enrichment, administration of fluids, warming animals, provision of special diets, etc. Please contact the Attending Veterinarian with any questions.

Animals that are experiencing pain and distress will typically exhibit overt clinical signs. These signs include abnormal activity levels, vocalizations, self-trauma, aggressiveness, isolation from cage mates, unkempt or greasy fur, porphyrin staining around the eyes and nostrils, hunched posture, cyanosis, pale mucous membranes, soiled anogenital area, respiratory distress/dyspnea, weight loss, emaciation, dehydration, and anorexia.

APPENDIX III

ASEPSIS IN SURVIVAL RODENT SURGERY

Introduction & Definitions

A basic tenet of laboratory animal regulations and philosophy of use is that pain and distress be minimized. Non-survival surgery (i.e. tissue collection while the animal is anesthetized but still alive with death being the outcome) it is considered non-survival surgery) does not require asepsis, but does require clean instruments. Aseptic procedures for all survival surgery is essential in the effort to eliminate the potential pain and distress associated with post-procedural infections. Proper surgical technique also plays a critical role and is of equal importance. Good technique will reduce tissue trauma and the time the animal is under anesthesia. This will minimize the exposure of tissue to adverse conditions and impairment of local blood supply, both of which contribute to tissue devitalization and hypoxia and increase the chance of post-procedural complications. The Public Health Service *Policy* and the *Guide for the Care and Use of Laboratory Animals* require that aseptic technique be utilized for all survival rodent surgery. What comprises aseptic technique, as it applies to rodents, is not expanded upon in these regulations. The IACUC approves the following methodology as it pertains to rodent surgery and they must be adhered to, unless a specific exemption has been described in the approved animal use protocol.

Surgery Area

Rodent surgeries do not require a dedicated space, but do need to be performed in a clean uncluttered area. No other activities may occur in this area while surgery is being done. Surfaces should be wiped down with a surface disinfectant (alcohol*, clidox, 10% bleach, etc) and accommodations made to keep the animal warm.

Animal Prep

Surgical area needs to have the hair removed, at least 1 cm surrounding incision site**. Rats need to have the area cleaned with surgical scrub and rinsed with alcohol or sterile water and have a final application of alcohol or an antiseptic solution (betadine, nolvasan). Mice should have three applications of alcohol or 2 applications of alcohol and one antiseptic solution. Draping the animal is highly recommended and should be done in such a way as to ensure the animal can be adequately monitored during surgery.

Surgeon Prep

The surgeon should clean hands with soap and water and wear sterile surgical gloves, a clean lab coat and cap (masks are not required, but are recommended). If only sterile instruments handle tissue and the surgeon's hands do not come in contact with the surgical field, exam gloves, which have been thoroughly wiped with alcohol, can be used.

Instruments

All procedures need to begin with sterile instruments (autoclaved, ethylene oxide, appropriate time in cold sterilant or dry heat, i.e., hot glass beads). Alcohol, betadine or nolvasan will not sterilize instruments. If multiple procedures are planned for the same time, two sterile packs could be used. The instruments from one would be wiped clean with sterile gauze and placed in a disinfectant (Alcohol, betadine or nolvasan are adequate here) or glass beads after the first animal and the second pack would be used on the next animal. This alternating of the packs would continue until the procedures are completed. Hot instruments need to be allowed to cool and chemically disinfected instruments need to be rinsed with sterile water or saline before use.

Post Procedure

Animals need to be kept warm and OBSERVED while recovering and also need to receive postoperative analgesia. A one or two word description of the procedure, date of procedure and

analgesia administration must be recorded on cage card. Ten to fourteen days after surgery, sutures or wound clips need to be removed.

*70%-92% ethanol or isopropyl alcohol

**Simple, quick procedures in mice may be able to be performed by disinfecting the hair and surrounding skin, parting the hair and using careful surgical technique to prevent entrapment of hair during closure. This would have to be stated in the protocol and specifically approved by the IACUC.

Please contact the Attending Veterinarian with any questions regarding surgical preparation and procedures. A CD on survival rodent surgery training from NIH is available to research staff.

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APPENDIX IV

BLOOD COLLECTION

Blood Volume

The maximum amount of blood collected from any animal cannot exceed 25% of their total blood volume in a 2-week period of time. It is recommended that no more than 15% of the blood volume be taken at one collection. If 15%-20% must be collected (needs IACUC approval), fluid replacement must be administered. No more than 20% can be taken at one time in a survival procedure.

Every species has a different blood volume and is commonly based on body weight. The following formulas can be used to determine total blood volume in mice and rats:

- Rats whole blood volume = 58ml/kg
- Mice whole blood volume = 78ml/kg

Collection Sites

The following is a list of recommended collection sites:

- Mice
  Tail (lancing of vein/artery, or via tail tip, or via needle/syringe)
  Retro-orbital*
  Cardiac puncture**
- Rats
  Tail (lancing of vein, via tail tip, or via needle/syringe)
  Retro-orbital*
  Cardiac puncture**

All blood collection methodology needs to be described in the approved protocol and must be performed by personnel trained in the procedure. The greatest volume of blood can be obtained from cardiac puncture; retro-orbital blood collection should be used if volumes desired NEED to be reliably more than 100ul; tail vein can reliably produce volumes in the 50-150ul range and tail tip is used if a drop or two of blood is desired.

* Only permitted with the use of anesthesia and justified in the animal use protocol and approved by the IACUC

** Only permitted as a terminal procedure while under general anesthesia.

APPENDIX V

CAGE SPACE REQUIREMENTS

Policy

The Joslin Diabetes Center adheres to the minimal space requirement as set forth in the National Research Council's "*Guide for the Care and Use of Laboratory Animals*". The following guidelines will be used to determine if a cage is in compliance with the "Guide".

Mouse

- Barrier Caging
  Mice should be housed no more than 5 per cage (4 per cage if > than 25g). There should be no more than one female with litter per cage (male mouse can also be included). Neonates should be weaned at 3 weeks of age. Obese and/or polyuric mice should be housed no more than 2 per cage. Overcrowded cages will have a notice attached and continued overcrowding will be brought to PI's and/or IACUC 's attention.

- Conventional Caging
  Same as above except housing should be no more than 4 per cage (3 if >than 25g).

Rat

- Rat Caging
  Standard housing for rats is 2 per cage. Polyuric animals may need to be housed in cages with floor inserts to keep them off the contact bedding. These animals may need water and bedding changed daily. Severely polyuric animals need to be housed one per cage or euthanized. Large rats (>500g) need to be housed one per cage.

APPENDIX VI

EUTHANASIA

Introduction
Euthanasia is the killing of an animal by methods that induce rapid unconsciousness and death without pain or distress. All requests to perform euthanasia must be included in the IACUC protocol that is reviewed and approved by the IACUC. Euthanasia may be performed only as described in the protocol to terminate a study, or as a means to relieve pain or distress that cannot be alleviated by other means (i.e. pharmaceuticals, fluids, change in environment, etc.). It is essential that only skilled personnel, whom the IACUC has pre-determined, perform euthanasia and that death is confirmed by the complete absence of a heart beat before disposal of the animal remains.

Policy
The Joslin IACUC adheres to the recommendations of the 2000 AVMA Panel on Euthanasia (2001. JAVMA, 218(5): 669-696.). A link to the 2000 AVMA Panel is found on Joslin's Intranet in the Government and Regulatory Agencies Section of the Animal Facility. Generally, inhalant or injectable agents ($C0_2$, inhalant anesthetics, barbiturates, etc.) are preferable to physical methods (cervical dislocation, decapitation). Physical methods may be used subsequent to the administration of $C0_2$ or anesthetic and the loss of consciousness by the animal. This would be one method that would assure death. However, the use of physical methods alone may be approved with scientific justification and with implementation limited to identified, trained personnel.

Special Consideration for Neonatal Rodents
Neonatal rodents have underdeveloped lungs and can be insensitive to the effects of carbon dioxide. Carbon dioxide might only "anesthetize" or "sedate" the animal. Therefore, once a neonatal animal has been "anesthetized" or "sedated" by carbon dioxide, another method of euthanasia should also be used to properly euthanize the animal. This includes administration of a barbiturate (i.e. pentobarbital), decapitation, or cervical dislocation. Always confirm that death has occurred (the heart has stopped beating) prior to disposing of the animals. This should be done for animals younger than 14 days of age.

APPENDIX VII

EXPIRED PRODUCTS AND CONTROLLED DRUGS

Policy

Medications, diets, intravenous solutions, drugs, etc. have an acceptable time frame for use. Once an agent has passed the date of expiration issued by the manufacturer, it may exhibit a reduced efficacy, safety or the accumulation of toxic byproducts. These, in turn, may compromise the health and well being of the animal and/or introduce undesirable variables into controlled studies. No expired compounds may be used in animals unless the practice is outlined in the protocol, justified and approved by the IACUC.

In addition, controlled substances need to be secured in a locked area and records maintained in accordance with all federal and state regulations. The Animal Facility maintains a limited supply of controlled drugs for veterinary staff use only. The Animal Facility cannot order drugs for research staff.

APPENDIX VIII

FOOD OR FLUID RESTRICTION

Policy

Any food or fluid restriction, beyond an overnight fast (16 or less hours), needs to be included in the Animal Use Protocol and have scientific justification. In addition, a monitoring plan, with endpoints that would necessitate the removal of an animal from a fast, needs to be described in the protocol and approved by the IACUC. While a cage is on food or fluid restriction, a cage card indicating when the fast began, how long it will last and a contact name and number must be placed on the cage.

Animals on special diets or treated water should also have a yellow “Special Needs” cage card indicating the name and phone number of the individual responsible for feeding or watering the animals.

Yellow “Special Needs” Cage cards can be obtained from John Stockton at x4388, Lead Animal Care Technicians, animal housing rooms, and animal procedure rooms.

APPENDIX IX

MULTIPLE MAJOR SURVIVAL SURGERIES

Definition

Major Surgery is defined as any surgical procedure that penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (NIH *Guide for the Care and Use of Laboratory Animals, 1996).* Orthopedic surgeries can also be included as major survival surgeries.

Policy

All surgical procedures must be described in the IACUC approved animal use protocol. While multiple major survival surgeries on a single animal are generally not allowed, exceptions may be made if the procedures are scientifically justified and approved by the IACUC. The interval between surgeries and the number of animals involved must also be included in the description.

APPENDIX X

PHYSICAL RESTRAINT

Definition
The use of manual or mechanical means to limit some or all of an animal's movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation *(Guide for the Care and Use of Laboratory Animals,* NRC, 1996).

Policy

- Brief periods (< 5 minutes) of restraint for conducting routine clinical or experimental procedures do not require justification or description in the animal use protocol. If a restraint device is used under these circumstances, it should be constructed in such a way as to eliminate potential pain or discomfort and be of a material that can be easily sanitized.

- Prolonged restraint must be described and justified in the animal use protocol and be approved by the IACUC. Animals should be acclimated to the restraint and endpoints must be defined that would necessitate the temporary or permanent removal of an animal from the restraint device.

NRC Guide
The following are guidelines found in the NRC *Guide for the Care and Use of Laboratory Animals:*

- Restraint devices are not to be considered normal methods of housing.

- Restraint devices should not be used simply as a convenience in handling or managing animals.

- The period of restraint should be the minimum required to accomplish the research objectives.

- Animals to be placed in restraint devices should be given training to adapt to the equipment and personnel.

- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.

- Veterinary care should be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates temporary or permanent removal of the animal from restraint.

APPENDIX XI

REPORTING AND HANDLING ANIMAL CARE OR USE CONCERNS

General Policy

The Joslin Diabetes Center requires all animal care and use to conform to all applicable Federal and State laws and regulations. The National Research Council's "*Guide for the Care and Use of Laboratory Animals*" is the principal document providing standards of care and use of animals at Joslin. It is the responsibility of Principal Investigators to ensure that their staff uses proper procedures and techniques and that training is provided. By signing the "Application for Approval of Protocol for Animal Experimentation", this responsibility is acknowledged and accepted by the Principal Investigator.

Reporting Procedure

Any employee of the Joslin Diabetes Center who has a concern regarding an observed animal care or use activity is encouraged to report such activity to one of the following individuals:

- Dr. Laurie Goodyear, IACUC Chairperson x4383
- Dr. Richard Hurley, Attending Veterinarian x4470
- Leigh Read, IACUC Program Administrator x4329
- John Stockton, Animal Resources Manager x4388
- Any IACUC Member

Center Policy, as well as Federal law, protects individuals from any discriminatory or reprisal measures being taken for reporting animal care or use deficiencies. The Joslin Diabetes Center will also take all steps possible to protect the anonymity of any person who, in good faith, reports such a deficiency.

An initial evaluation of all reports shall be conducted under the direction of the Chair of the IACUC or her/his designee. If it is determined that the activity was within the parameters of the approved protocol, the results will be recorded in the IACUC minutes and no further action is necessary. If further action is indicated, the incident will be investigated by the IACUC and disciplinary actions may be taken which could include temporary or permanent restrictions on the use of animals by the personnel involved. The individual who made the initial complaint will be notified of the decision of the IACUC and, if there is evidence of a serious noncompliance, appropriate Federal agencies need and will be notified.

APPENDIX XII

GUIDELINES ON THE USE OF ADENOVIRUSES AND BL II AGENTS

The Harvard Committee on Microbiological Safety - Committee for the Regulation of Hazardous Biological Agents must approve any procedure involving adenoviruses and other BL II or BL II+ agents. A copy of the approval must be on file with the Joslin Safety Office before the research protocol is submitted to the Joslin Institutional Animal Care and Use Committee for approval. The Joslin Safety Office can provide guidance in the COMBS process.

- Adenovirus use may occur in room 594 in the 5th Floor Conventional Facility. This room can only be accessed with a key, available from John Stockton, Animal Resources Manager. The Adenovirus/BL II Agent Use Request Form must be completed and approved before a key to the room is issued and the procedures are started.
- The approved IACUC protocol must explain the adenovirus procedure.
- Only one investigator may use the room at a time.
- Animals inoculated with Adenoviruses are to be isolated in room 594 for a minimum of 48 hours following inoculation. The inoculation should be performed in the Baker Class II hood that is in the isolation area using BSL-II practices. These animals are to be housed in polycarbonate microisolator caging. Longer isolations may be required depending on virus strain and shedding times.
- The Investigator must feed and water the animals during the isolation period.
- At 48 hours post-inoculation, the animals should be removed from their cages, placed in new cages, and returned to general housing in their source room (BL-1 conditions). The adenovirus contaminated caging should be put into biohazard bags and sprayed down with Clidox. Animal facility personnel will autoclave the bag after the spraying and before sanitation.
- Protective garments (lab coat, gloves, head cover, shoe covers) must be worn while working in the room. Protective garments and any non-animal waste should be placed in an appropriately labeled Biohazard box.
- Animals that die before the 48-hour post-inoculation time point must be sprayed with the provided disinfectant and then double bagged prior to placing the carcass in the freezer.
- When using other BL II agents in Room 594 (i.e. BL II+ level Lentiviruses) the same general procedures must be followed. Isolation times may vary depending on viral strain and origin as well as shedding times.
- Room 569, the transgenics Procedure Room, may be converted to a BL II area according to the guidelines set forth in Biosafety in Microbiological and Biomedical Laboratories (1999) after approval from the Joslin IACUC, Joslin Safety Officer, and Animal Resource Facility Management. The same general procedures as above are to be followed along with any modifications as deemed necessary.

Contact Information: John Stockton, Animal Resources Manager, Room 561, Ext. 4388.

For additional biosafety information, please visit http://www.hms.harvard.edu/orsp/coms/.

APPENDIX XIII

RODENT DOSING GUIDELINES

Below are volume recommendations for the safe, acute administration of fluids in adult mice (average weight of 20 grams or above). The recommended needle sizes are 25 or 27 gauge, however, larger needle sizes may be necessary for injecting viscous materials. Special gavage needles are needed for *per os* administration of compounds. These needles can be ordered from a commercial supplier or can be obtained from the veterinary staff.

- Subcutaneous (SC or SQ)
  - 2-3 mL total, maximum of 0.5 mL per site
- Intraperitoneal (IP)
  - 2-3mL
- Oral (PO)
  - 0.4mL; 40mL/1 Kg
- Intradermal (ID)
  - 0.05 mL/site
- Intramuscular (IM)*
  - 0.05 mL/site

* Intramuscular injections are generally not recommended in mice because of the lack of muscle mass, which can lead to necrosis and other problems subsequent to an injection.

APPENDIX XIV

GUIDELINES FOR USING THE ANIMAL FACILITY'S ISOLATION ROOM

The isolation room is located in Room 585 of the 5th Floor Conventional side of the Joslin Animal Facility. Housing is limited to a maximum of 35 microisolator cages for mice (including one cage for sentinel animals) in the room. A hood is located in the room for animal manipulations.

- Researchers wishing to use the room must submit a "Request for Isolation Room Use" form to John Stockton, Animal Resources Manager. The use or the room must be approved by the Animal Facility prior to entry of research animals. Only animals with virus free health screens will be allowed to come into the facility.
- Once animals enter the room, they can leave to be transported back and forth from an investigator's lab. However, animals cannot be taken into other rooms within the animal facility.
- A carbon dioxide tank and euthanasia chamber is available in the room for euthanizing the animals. After being appropriately bagged in plastic bags, which are available in the room, euthanized animals may be put in the freezer in the procedure room in the animal facility. The bag should be sprayed with disinfectant prior to leaving the isolation room.
- Please wear the appropriate lab clothing (dedicated lab coat, booties, hair bonnet) when in the room.
- Do not enter another animal facility room after being in the isolation room in the same day. Please visit other rooms the following day.
- Animals entering from Harvard or Joslin facilities may not have to have serology testing prior to entering the room. Please consult with either John Stockton or Dr. Hurley regarding this.

APPENDIX XV

TOE CLIPPING IN MICE FOR IDENTIFICATION

The *Guide for the Care and Use of Laboratory Animals* stresses the importance of proper animal identification in sound research and humane animal care in the section on Identification and Records:

"…room, rack, pen, stall, and cage cards with written or bar-coded information; collars, bands, plates, and tabs; colored stains; ear notches and tags; tattoos; subcutaneous transponders; and freeze bans. Toe clipping, removal of the first bone of certain toes, corresponding to a predetermined number code, as a method of identification of small rodents, should be used only when no other individual identification method is feasible and should be performed only on altricial neonates."

The IACUC has determined that under certain circumstances, toe clipping for the purpose of identification may be necessary. Toe clipping is considered a potentially painful procedure that should be done when no other methods of identification are feasible and with prior approval of the IACUC. The Principal Investigator must provide justification to the IACUC for the use of this method of identification in the written protocol (for example, permanent marking of animals is needed, early determination of genotype is required) and why alternative methods cannot be used.

After approval has been obtained, the following guidelines must be followed:

- Toe clipping can only be performed on mice prior to weaning (less than 21 days old).
- Must be conducted in the most painless and humane manner as directed by the IACUC and attending veterinarian. The foot should be cleaned with antiseptic treatment (i.e. betadine) and sharp sterile scissors should be used.
- Only one toe per animal can be clipped unless otherwise justified.
- Must only be performed by properly trained individuals.
- If toe clipping is to be used, this must also be the initial method of genotyping. If additional genotyping is needed, tail clipping is allowed.
- Should acceptable alternative methods become available in the future, the PI must examine these methods for feasibility and make every effort to eliminate the use of toe clipping.

APPENDIX XVI

Joslin Diabetes Center

Institutional Animal Care & Use Committee

General Animal Facility Rules and Procedures

1. Access to the facility is restricted by card access to personnel who have been trained and authorized. Each individual is granted access to only one facility (barrier, non-barrier, or basement).

2. Colored lab coats (green for basement, blue for non-barrier, coveralls for barrier) or uniforms, booties or dedicated shoes, and hair bonnets must be worn at all times in the Animal Facility. White lab coats cannot be brought into the Facility.

3. Disposable gloves must be worn when handling animals or animal waste.

4. All procedures must be performed in the Animal Facility procedure rooms unless prior approval has been obtained from the IACUC.

5. If approved, mice that leave the facility must be transported in moist food containers, all cages must remain in the Facility.

6. Animals cannot be removed from the Facility and then returned to the Facility without prior IACUC approval.

7. Investigators must have a dedicated cart/storage cabinet for work in the Facility. Equipment cannot be transported back and forth to laboratories without prior approval of the Animal Resources Manager.

8. All animal arrivals must have prior approval of the Animal Resources Manager. Animals arriving without prior approval will not be admitted.

9. Cages, bottles, and other animal supplies from non-Joslin facilities cannot be brought into the Joslin.

10. For mice only one litter per cage, and litters must be weaned at 3 weeks of age. Cage overcrowding will result in euthanization of mice.

All animals must be treated with compassion, empathy and consideration.

APPENDIX XVII

ANIMAL ORDERING SCHEDULE/GUIDLEINES

To ensure that animals will arrive as requested a completed Request for Animal Purchase forms need to be submitted to the Animal Resources Manager as detailed below.

This will allow for proper approval, transmission and processing of the requests.

VENDOR	REQUEST DEADLINE	DELIVERY DAY
TACONIC	MONDAY AT 2 PM	THURSDAY
TACONIC	THURSDAY AT 2 PM	FOLLOWING TUESDAY
CRL	MONDAY AT 2 PM	THURSDAY
CRL	THURSDAY AT 2 PM	FOLLOWING TUESDAY
JACKSON LABS	THURSDAY AT 2 PM	FOLLOWING TUESDAY
HSD	THURSDAY AT 2 PM	FOLLOWING WEDNESDAY

Please note that some strains from JAX can be wait listed from weeks to months and certain rat strains, such as Zuckers, can have weeks to months to delivery.