

CASE COMPREHENSIVE CANCER CENTER

Athymic Animal Facility (AAF) Contact Information

Athymic Animal Facility (AAF)

**216-368-3169 (Wolstein)
216-368-0074 (BRB)**

Dr. Lili Liu, Director, AAF

216-368-5696
216-368-1166 (fax)
email: lili.liu@case.edu

Ms. Pamela Steele, Technician, AAF

216-368-0074

Dr. Nanette Kleinman, Veterinarian for AAF
Associate Director, ARC

216-368-3375
email: nkh@case.edu

Animal Resource Center (ARC)

216-368-3490

Dr. W. John Durfee, Director, ARC

216-368-3490
email: jwd7@case.edu

Cancer Center Administration

216-844-8797

Revised: Fall, 2006

INTRODUCTION

Athymic Animal Facility (AAF)

The Athymic Animal Facility (AAF) is a core facility of the Case Comprehensive Cancer Center, funded by the National Cancer Institute. As such, the AAF receives part of its support from the NCI Cancer Center Support Grant to the Cancer Center and the remainder from user fees charged to each investigator. It is maintained by two technicians who are trained in the handling, breeding, and experimental use of these specialized animals. The AAF is overseen by the Director who operates under the aegis of the Cancer Center Director, along with a veterinarian from the Animal Resource Center. These individuals are identified on the face page of this manual.

The AAF provides housing for any class of immunodeficient rodent that requires the highest level of protection from infectious agents. The AAF maintains a breeding colony of athymic nude mice for sale to investigators (currently, the NCR nu/nu colony from the Frederick Cancer Center of NCI), as well as NOD/SCID mice (Jackson Labs). In addition, investigators can purchase other immune-deficient classes of animals from external vendors and have them housed in the AAF for subsequent experiments after a suitable quarantine period.

Because there are no hazards to investigators using the facility but many potential hazards to these immuno-incompetent animals, the AAF must use the most stringent set of regulations and protections to guarantee that the several thousand animals housed in the AAF are exposed to minimal danger. Therefore, it is absolutely critical to the success of this facility that **every investigator follow the protocols described in this manual RIGOROUSLY and that every investigator cooperate to the fullest with AAF technicians.** For these reasons, access to the AAF is restricted to properly trained individuals via keycard access that is controlled by the Director of the AAF. More than 25 principal investigators use the AAF, putting considerable strain on the scheduling of facilities and technician time, and adding to the potential for breakdown in sterility conditions. We are seeking your full cooperation for the benefit of all AAF users.

ATHYMIC ANIMAL FACILITY (AAF)

Table of Contents

Contact Information	i
Introduction to the Athymic Animal Facility	ii
Table of Contents	iii
I. Guidelines for the Use of the Animals Housed in the AAF	1
A. Regulations for Entering the Facility	
B. General Regulations	
C. Preparation of “Stay-Clean” Workbench	
D. Handling of Microisolators and “Stay-Clean” Workbench	
E. Disposable Items and Specimens	
F. AAF User Training	
1. General Information for Investigators	
2. Autoclave/Quality Control Protocol	
a. Sterilization Cycle for Microisolators and Prefilled Water Carboys	
b. Preparation of Microisolators for Sterilization	
c. Preparation of Water Bottles for Sterilization	
d. Preparation of Water for Sterilization	
e. Spore Testing with <i>Bacillus stearothermophilus</i> Ampules	
II. Husbandry, Housekeeping and Sanitation	5
A. General Protocols	
B. Design of the AAF Facility	
C. Security System; AAF Location	
D. Environmental Conditions-Environmental Monitoring	
E. Caging and Bedding	
F. Food and Water	
G. Maintenance of Rooms in Pathogen-Free Conditions	
H. Husbandry Procedures for SCID-HU Mice	
III. Animal Production	8
A. Breeding System	
B. Mating System	
C. Quality Control	
D. Records	

ATHYMIC ANIMAL FACILITY (AAF)

Table of Contents (continued)

IV.	Ordering Immunodeficient Animals	9
	A. From Breeding Colony	
	B. From Outside Vendors	
V.	Transferring Animals from the AAF	11
VI.	Animal Handling Procedures	11
	A. Restraining Procedure	
	B. Sexing	
	C. Ear Notch-Punch Code for Identification of Rodents	
VII.	Tumor Growth and Humane Endpoints	15
VIII.	Special Procedures	15
IX.	Anesthesia	16
X.	Euthanasia	17
	AAF Mouse Purchase Form	18
	Facility Floor Plans	
	IACUC Guidelines for Tumor Inoculation in Rodents	

I. Guidelines for the Use of the Animals Housed in the AAF

A. Regulations for Entering Facility

1. Access to the facility (see Section II.C.) is restricted to investigators directly involved in ongoing experiments. Accompanying persons may be allowed to enter the facility if they are being trained in experimental procedures. In such cases, the investigators are requested to notify AAF technicians in advance by calling 216-368-3169. Entry into the facility is restricted by key card access and to only those individuals that have received the two-hour training session with AAF technicians.

2. All investigators must provide a copy of an approved and activated experimental protocol by the Case Institutional Animal Care and Use Committee (IACUC). Approved protocols may be activated by presenting a copy indicating (a) the types of animals to be used in the AAF, (b) the exact types of experiments being conducted, and (c) the names of all individuals in the principal investigator's laboratory who will be handling animals. When new personnel are recruited to labs or new procedures added to a protocol, the IACUC and AAF must be provided copies of approved addenda for these new investigators from the IACUC Office (216-368-6979, IACUC@case.edu)

3. Investigators are asked to make an appointment for use of a workbench located in laboratory rooms. They are asked to call at least one day in advance to confirm availability of a workbench for scheduling with AAF technicians.

4. Investigators must not have any contact with other experimental animals the day they enter the facility. They must also avoid contact with household pets the morning prior to an experiment.

5. All supplies brought into the facility by investigators should be placed in plastic containers with covers. These containers should be sprayed with disinfectant Clidox before entering the facility. Paper boxes are prohibited.

6. Investigators entering the AAF must wear surgical shoe covers, a sterile gown, cap, mask and sterile gloves (these items are provided by the AAF). When exiting the facility, the lab uniform should be removed and placed in the designated containers. Wall hooks are provided for hanging gowns if returning to the AAF is anticipated on the same day.

B. General Regulations

1. Investigators are prohibited from entering the breeding room (only AAF technicians are permitted in this room).

2. Investigators are requested to completely fill out the information cards which are located on each microisolator. Be sure to include your current IACUC protocol number, telephone and fax contact numbers.

3. When removing animals from the AAF permanently, investigators are asked to update the number of animals which are housed in the microisolators for that principal

investigator. The number of animals inside the microisolator should be equal to the number of animals specified on the information card. Date and description of all experimental procedures should be recorded on the animal cage card.

4. Animals to be euthanized must not be taken from the facility without first recording their removal in the animal log located in the laboratory.

5. Investigators are prohibited from taking the cages out of the facility. If animals must be taken from the AAF, plastic containers are provided or investigators can use their own microisolators if animals are going to be in an experiment outside the AAF. These microisolators can be brought into the corridor between the double doors (but mice must be transferred in the “Stay-Clean” workbench).

6. Inventory forms for each principal investigator are provided to update the number of animals in the laboratory. These inventory forms will be used for assessing charges to the principal investigators for housing animals in the AAF on a monthly basis.

7. Each investigator entering the facility must sign the log book located in the laboratory, noting the date he/she entered the facility, the type of experiments he/she performed, and the number of animals used and/or euthanized.

C. Preparation of “Stay-Clean” Workbench

1. Activate work light by turning on the light switch on the control panel.

2. Turn on the blower motor selector switch to the high position for approximately 5 minutes to insure proper balance within system.

3. Spray workbench top and inside the transparent walls with Clidox. **DO NOT SPRAY THE BACK WALL WHICH CONTAINS FILTERS.** The back wall may be wiped with a Clidox-damp rag.

4. After pressure balance has been achieved, turn the motor blower selector switch to the low position.

5. Use one towel at a time from the sterile towel pack. Repack the rest and put in proper place for later use.

6. Place sterile instruments and supplies on the sterile towel. Use heat-sterilized instruments only; 70% EtOH bath is not sufficient.

D. Handling of Microisolators and “Stay-Clean” Workbench

1. The microisolators can be opened ONLY INSIDE the operating “Stay-Clean” workbench.
2. Animals, instruments and supplies must not be taken outside the workbench during experimental procedures. A newly autoclaved set of instruments must be used for each cage.
3. The surgical instruments must be resterilized when using them with multiple animals housed in the same microisolator (otherwise, other animals can be cross-contaminated).
4. Gloves should be sprayed with Clidox repeatedly to keep them constantly wet.
5. Investigators are required to leave the “Stay-Clean” workbench perfectly clean. The workbench should be sprayed with Clidox, wiped and sprayed with water, wiped again, dried and turned off.

E. Disposable Items and Specimens

1. Extreme care must be taken when disposing of any item that contains or has contacted biological specimens. Special precautions are required for the items listed below.

NEEDLES – place in box marked for disposal of contaminated needles (red needle disposal container).

SYRINGES – place in the trash can marked for disposal of contaminated glassware.

2. Investigators are required to clean instruments after experimental procedures. Instruments should be soaked with Clidox for at least 3 minutes, then washed with water and dried. Clean instruments should be left on the designated cart.

NOTE: In case of questions or comments, investigators should seek the assistance of AAF personnel.

F. AAF User Training

1. General Information for Investigators

a. All investigators must provide the AAF with a description of planned studies (IACUC-approved protocol) and notice of protocol approval and activation by CWRU IACUC before they may be trained and can subsequently order animals from the breeding colony or outside vendor.

b. Investigators will complete a form to indicate their level of experience with animal experimentation and their experimental plan (part of IACUC protocol). The completed form will indicate:

- whether facility personnel will be needed for training in the techniques used with the planned investigators;
- number, sex and age of animals which are going to be housed in the AAF;
- period of time animals will be kept for experiments in the AAF;
- expected (subject to change as experiments proceed) frequency of ordering new animals from outside vendors and/or from the AAF.

c. Investigators will be given a copy of:

- Guidelines for the Use of the AAF (this manual);
- Laboratory Products “procedures for using the microisolator system”, and;
- Recommended literature.

d. Investigators shall be trained in AAF standard operating procedures by AAF technicians after they have reviewed all the training materials provided above (Section c).

e. When training is complete, the investigator’s security key card will be activated and the investigator may begin work in the AAF.

2. Autoclave-Quality Control Protocol

Model: Finn Aqua 72 x 84 x 72 Pass-thru

Installed: 2004

a. Sterilization Cycle for Microisolators

Sterilization: H₂O 253°F for 2 hours 30 minutes
Eagle Series Sterilizer 250°F 20 minute sterilization,
20 minute exhaust

For cages: 20 minute sterilization and 20 minute exhaust

b. Preparation of Microisolators for Sterilization: After removing dirty bedding and washing, microisolators are assembled with clean corncob bedding and Teklad 8760 High Protein diet. Autoclave indicator tape is placed on the front of each unit (you should fasten filter top to cage bottom).

c. Preparation of Water Bottles for Sterilization: Dirty water bottles are emptied and placed in the upright position in a bottle rack for subsequent cleaning and sterilization.

d. Spore Testing with *Bacillus stearothermophilus* Ampules

Frequency: 3 times/week. First load.

Technique: Dated ampule of spores is placed on bedding in the center of a microisolator or in the approximate center of a group of water bottles. Package wrapping is labeled to notify AAF personnel. AAF personnel retrieve ampules and submit to ARC veterinarian for incubation and interpretation. Records of spore testing shall be maintained in the ARC office for a period of 3 years with reports also being sent to the Director of the AAF periodically.

II. Husbandry, Housekeeping and Sanitation

A. General Protocols

Nude mice (Figure 1) and other immune-deficient rodents (e.g., SCID mice) are extremely sensitive to environmental pathogens. Therefore, pathogenic agents normally tolerated by mice with a normal immune system may lead to severe illness and death in nude mice. For these reasons, the AAF is strictly isolated from the rest of the animal quarters at the School of Medicine. Strict isolation of athymic mice prevents undesirable infections by pathogens from other animal species. To insure isolation, positive air pressure exists within the AAF with respect to the adjacent hallways.



Figure 1. Nude mouse (hairless) in “Stay-Clean” workbench.

B. Design of Athymic Animal Facility (AAF)

The Health Sciences Animal Facility (HSAF) is currently being renovated, with an estimated completion date in the spring of 2008. A floor plan of the facility after renovation is included at the end of this manual.

For the duration of the renovation period, the AAF will be located in the Wolstein Animal Facility (WAF), room SB162. A floor plan of the WAF is also included at the end of the manual.

For a description of the WAF, including instructions on how to obtain access to the facility, see the Animal Resource Center website at <http://labanimals.case.edu/>.

Wolstein SB162 is equipped with 1 laminar air flow workbench, one class 2A biohazard hood, and cage racks with microisolators, carts, and equipment needed for cleaning. Once animals are removed from the room, they are never returned. .

C. Security

To be allowed access to the AAF, one must: 1) be named on an approved protocol as an investigator, co-investigator or research assistant, and 2) be trained by AAF personnel.

D. Environmental Conditions – Environmental Monitoring

Temperature (74° F – 78° F/28° C) and humidity (55%) are continuously monitored using a Fisher 7-day RH and Temperature Recorder. A 24 hour-a-day temperature and humidity monitoring system is also electronically connected to the CWRU Engineering Department facility.

A uniform light-dark cycle (12 hours of each) is maintained with light intensity of 30 ftc.

There are >15 air changes per hour.

E. Caging and Bedding

1. Caging: Microisolator caging units are used in this facility. They consist of standard autoclavable, transparent plastic cages that permit visual inspection of animals without opening them. The shape and size (26 x 16 cm, and 13 cm high) of the cages insure comfort of animals. Up to 5 adult mice may be housed in one microisolator.

2. Bedding: Sterilized corncob bedding is used in the facility.

F. Food and Water

P3000 Autoclavable high-protein diet is used. Autoclaved tap water is used for most experimental animals and the breeding colony. In select cases where warranted, penicillin VK (800 units/ml) is added to water to control *Corynebacterium spp.* associated with hyperkeratosis and pre-weaning mortality. Both food and water are sterilized before use.

G. Maintenance of Rooms in Pathogen-Free Conditions

Microisolator cages are opened and animals are handled only within the “Stay-Clean” laminar air flow workbench using standard microisolator techniques. Cages are changed once a week or as often as necessary to maintain a clean, dry environment. All microisolators are examined every day by AAF technicians. During experiments, investigators are expected to check their own animals at least twice a week, or more frequently if requested by AAF technicians. Cages with animals are changed once a week and sterile water bottles are changed twice a week.

Dirty cages are delivered to the entrance doors located in the corridor immediately outside the facility. They are hand-carried from the “clean” cart which belongs to the AAF. They are placed onto the “dirty” cart belonging to the ARC which is kept outside the entrance doors. These two carts do not pass the entrance doors.

The cages are washed and sanitized in the ARC washing facility, assembled with corncob bedding and autoclavable P3000 high protein food. Water bottles and caps are autoclaved in rat microisolators. Once inside the AAF, water bottles are filled in the “Stay-Clean” workbench.

Racks and other movable equipment inside the animal rooms are cleaned weekly with Clidox Sterile-Disinfectant. Floors are washed with disinfectant every week. Air filters are changed every month and pre-filters in the workbench are vacuumed and cleaned every week.

H. Husbandry Procedures for SCID-HU Mice

Because SCID-HU (Severe Combined Immune Deficient when reconstituted with a Human immune system) mice can be unwitting hosts for human pathogens such as HIV, OSHA standards for handling materials potentially contaminated with blood-borne pathogens must be followed when handling these mice. These include:

1. Cages housing SCID-HU mice should be clearly identified by placing orange biohazard tape on which is written "SCID-HU mice" on a card on the front of the cage.
2. Cages housing SCID-HU mice should be opened and mice handled only within a Class II biological safety hood.
3. During husbandry procedures, mice should be handled with forceps rather than gloved hands.
4. During routine cage changing, soiled bedding from cages housing SCID-HU mice should be placed in biohazard bags and autoclaved prior to disposal. Gross soil from the surface of the dirty cages should be removed with a paper towel and placed in a biohazard bag. The cage surface should be sprayed with Exspor® or a 10% solution of Clorox prior to returning to the ARC for cleaning.

III. Animal Production

A. Breeding System

Athymic nude mice with BALB/c background bought from Frederick Cancer Center, National Cancer Institute are bred in the AAF at Case. An outbreeding system is used for this purpose. To correct for genetic drift, the breeding colony is replaced every 3-4 years. This was recently done in 2002. The correct nomenclature for these mice is NCR: Athymic Nude nu/nu.

B. Mating System

For production of nude mice, mating is established between heterozygous (nu/+) females and homozygous (nu/nu) males. This produces 50% of homozygous (nu/nu) and 50% heterozygous (nu/+) mice.

C. Quality Control

The Animal Resource Center via Dr. Nan Kleinman (our overseeing veterinarian) monitors the health status of animals in the breeding and experimental rooms of the Athymic Animal Facility. Quarterly serology, parasitology and bacteriology tests are done; gross pathology observations are also made.

Five nude heterozygotes are housed in microisolators in each room for six to twelve weeks. The Charles River Professional Services Tracking Profile is used for serological assessment. Serological evidence of exposure to Sendai, PVM, MHV, MVM, GD-7, REO-3 and M. Pulmonis is tested. Parasitological screening consists of visual inspection and skin scrapes for ectoparasites and fecal flotation, perineal cellophane tape preps and duodenal/fecal saline preps for detection of endoparasites. Nasopharyngeal flushes and swabs of fecal contents are submitted for aerobic bacteriologic assessment. Skins of animals are also tested for possible *Corynebacterium* contamination for subsequent treatment with penicillin in drinking water.

D. Records

To provide complete information on the breeding system, integrity of the stock, and biological performance, proper animal records are maintained by AAF personnel and are available upon request.

IV. Ordering Immunodeficient Animals

A. From the Breeding Colony

Athymic nude mice (NCR nu/nu) and NOD/SCID mice from our breeding colony are available for primary investigators using them for their studies. Both male and female animals between the ages of 4-8 weeks can be purchased from the AAF by calling the facility personnel at 216-368-3169. We will try our best to provide you with your special request regarding weight, age, or sex. Advance notice of 15-30 days will help us give you the availability of animals if the order is for more than 25 animals of the same age at one time. The current charge for a 6-week old male or female animal is \$14.00. The price will be slightly higher if you want to buy older animals.

To order animals from our breeding colony, the investigator must fill out a “Nude Mouse Order” form as shown on page 19 of this manual. The order **MUST BE SIGNED BY THE PRINCIPAL INVESTIGATOR** in order to be valid.

B. From Outside Vendors

1. All animal orders from outside vendors should be placed with the Animal Resource Center (ARC) after obtaining a signature of an AAF technician. In addition, a copy of any outside order must be supplied to AAF technicians at the time of its placement so that they can plan for caging and space requirements in the AAF. If this is not done, AAF technicians will not accept these animals for further study. An example of an ARC requisition form is shown on page 11.

2. As a source of athymic mice and rats we recommend:

Frederick Cancer Center (FCC)
Frederick, MD
Phone: 301-846-1649

NOTE: NIH grants or contracts are required to order animals from the FCC.

REQUESTER TO EXTRACT COPY NO. 2 (BLUE) AND SEND REMAINDER
 OF SET INTACT TO ANIMAL RESOURCE CENTER, SCHOOL OF MEDICINE,
 10900 EUCLID AVE., CLEVELAND, OHIO 44106-4925

Case Western Reserve University Animal Resource Center Requisition

All white areas must be filled in completely to expedite your order

Gray areas are for ARC use only

ARC # 20046 B		PO #		PI Last Name, First Initial		Department		Extension		Protocol #	
Req Date		Shipment to Arrive		Acct Name				Housing Rm		Acct #	
Vendor		Special Instructions								<input type="checkbox"/> Microisolator Housing	
Quantity	Gender	Weight	Age	Stock/Strain Name & #				Quoted Price		Actual Price	
Date Ordered		Vendor Acct #		Vendor Contact				Surcharge			
								Handling/Box			
Entered By		Receipt Verification	Control #	Confirmation #	Confirmed Arrival Date		Freight				
							Total				

Authorized Signature _____

Return Priced Copy (Green) to: _____ Building _____ Room _____

V. Transferring Animals from the AAF

Transferring animals from the AAF to ARC or any other location is left to the principal investigator. However, when this happens, these animals will never be permitted back into the AAF. The investigator should bring clean, sterile microisolators to the AAF to transfer animals. The animals must be transferred to the cages under the “Stay-Clean” hood. Once the athymic animals are taken from the AAF, they cannot be returned. The investigator must notify the Animal Resource Center (216-368-3490) prior to transferring mice to the ARC to arrange for their placement.

VI. Animal Handling Procedures

A. Restraining Procedure

Mice may be picked up by grasping the tail with fingers or rubber-tipped forceps. If inspection or manipulation is intended, the mouse is lifted by the tail, placed on a rough “toe-gripping” surface, grasped on the scruff of the neck by thumb and forefinger (see Figure 2) and inverted; the tail is held between the palm and the little finger (see Figure 3). Grasping and pulling the tip of the tail may result in stripping the skin from the tail. Tail vein injection of mice is shown in Figure 4 on the following page.

Figure 2. Restraint of mouse by grasping neck and tail.

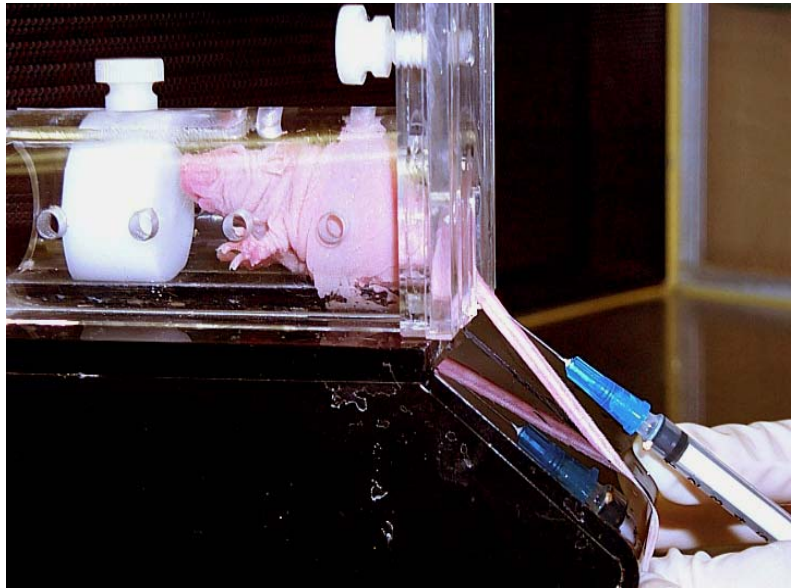


Figure 3. One-handed restraint of mouse for injection or bleeding procedure.



For tail vein injection of mice, the animal is placed in a specialized apparatus with a hole on the cap for the tail to be extended. Another hole on the other end helps the animal to breathe easily.

Figure 4. Tail vein injector with mouse ready.



Rats may be restrained with a method that utilizes autoclaved protective gloves to grasp the animal with the palm of the hand over its back; with the thumb and forefinger, fold the forelegs across each other and under the animal's chin. With the legs held correctly, the animal cannot depress its chin to bite.



Figure 5. Restraint of rat technique.

B. Sexing

Neonatal females may be distinguished from males by a large genital papilla and a conspicuous row of nipples 7-9 days after birth (see Figure 6). The males may be distinguished from females by a greater anogenital distance and the pale testes visible through the abdominal wall (see Figure 7).

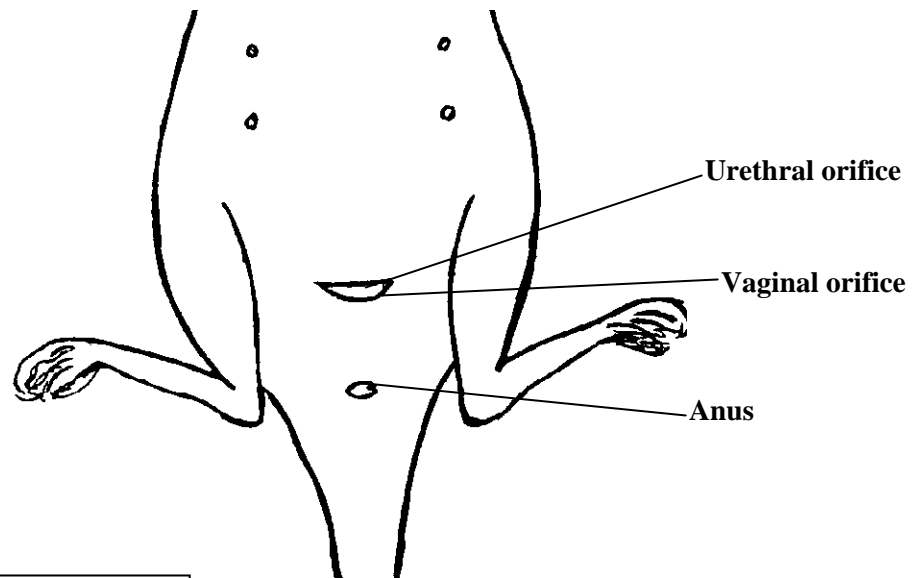


Figure 6. External genitalia of female mouse.

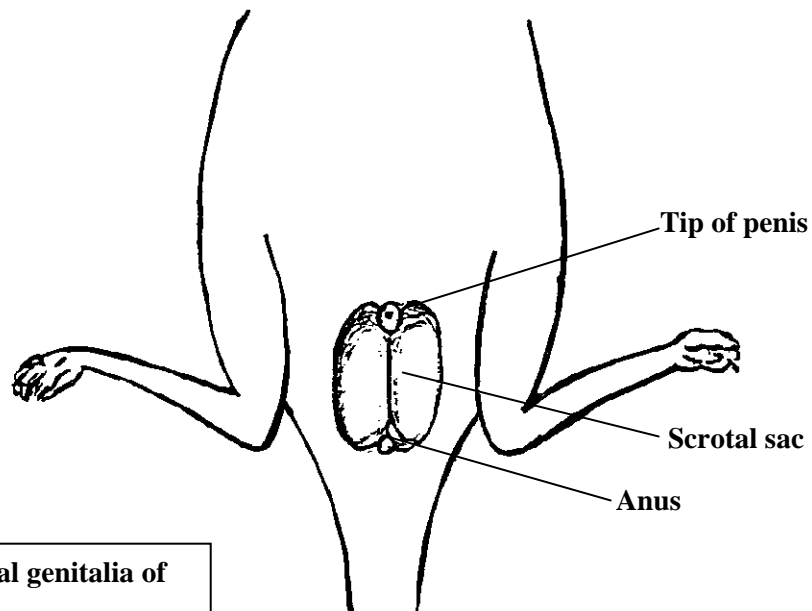


Figure 7. External genitalia of male mouse.

C. Ear Notch-Punch Code for Identification of Rodents (Figure 8)

These number codes are used in various combinations to produce the desired number, but always with single units on right ear and tens units on left ear.

Examples

Left Ear (Tens)

Right Ear (Single Units)

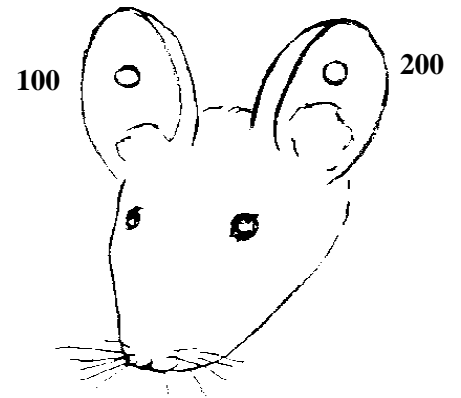
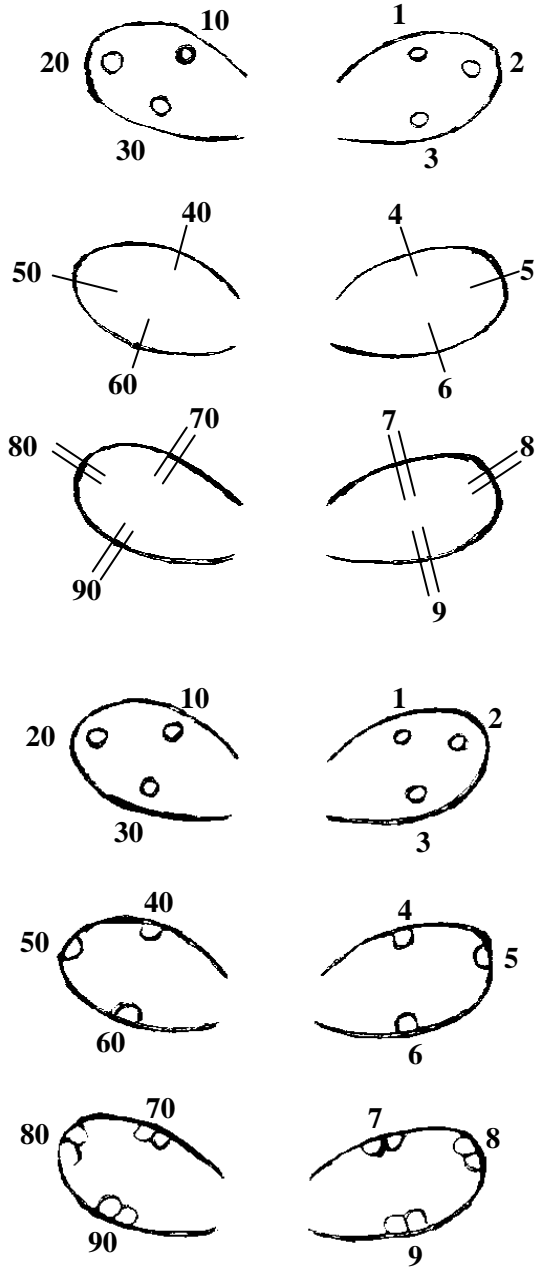


Figure 8. Ear notch-punch code for mouse identification

VII. Tumor Inoculation: IACUC Guidelines are included at the end of this manual.

Tumor Growth and Blood Withdrawal Guidelines

Tumor Growth and Humane Endpoints

No precise quantitative guide can be given as to the acceptable upper limit of tumor burden, since the adverse effects on the host depend on the biology of the tumor, the site, mode of growth, and the nature of the associated treatments. However, tumor burden should not exceed 10% of the host animal's normal body weight. Considerable care should be given to the judicious choice of endpoint for tumor growth. Predictable indications of pain, distress or significant deviation from normal behavior should be taken into account. Unless specified otherwise and approved specifically in a relevant IACUC protocol, animals should be sacrificed before:

- predictable death occurs;
- they get into poor health conditions, including lethargy, cachexia, or abnormal behavior;
- the tumor mass becomes too large (>10% body weight), likely to ulcerate or unacceptably limits normal behavior.

The collection, processing, dilution and storage of rodent blood or sera intended for testing must follow an established protocol if the results are to be meaningful.

VIII. Special Procedures

Presently, the facility is NOT set up for:

- use of radioisotopes
- radiation exposure*
- experiments using hybridoma cells

*The Cancer Center's Radiation Resources Core Facility (Biomedical Research Building, Room 339) maintains and operates ionizing radiation-producing devices for cancer researchers. Contact John Mulvihill, MS, technical manager of the Radiation Resources facility, at 216-368-6435 (email: jwm5@po.cwru.edu) for more information.

IX. Anesthesia

All drugs in the AAF are kept in a locked cabinet and will only be dispensed to investigators on an “as-needed” basis. When used, investigators must record the following information on a log for that particular drug cocktail: date, principal investigator, user of drug, and amount of drug used. These rules are governed by IACUC, NIH, and USDA oversight. The following cocktail recipes are used for short-term anesthesia following IACUC and ARC guidelines.

Recipe for Rodent Anesthesia Cocktail

(Provides 30-60 minutes of general surgical anesthesia)

For rats:

Ketamine HCl	150 mg (100 mg/ml)	1.5 ml
Xylazine HCl	30 mg (20 mg/ml)	1.5 ml
Acepromazine	5 mg (10 mg/ml)	0.5 ml
	Total	3.5 ml

Dose: 0.5-0.7 ml/kg IM or SC

For mice:

Ketamine HCl	15 mg (100 mg/ml)	0.15 ml
Xylazine HCl	3 mg (20 mg/ml)	0.15 ml
Acepromazine	0.5 mg (10 mg/ml)	0.05 ml
Sterile water or saline		1.4 ml
	Total	1.75 ml

Dose: 0.1-0.2 ml/25 gram mouse IP

NOTE: Animals must not be permitted to come into contact with liquid anesthetics. Anesthetized animals are particularly susceptible to cold, and therefore, should be kept warm using a heat lamp during the procedure until fully conscious.

X. Euthanasia

Euthanasia is the humane killing of an animal. Implied in humane killing is a painless, free-of-fear and distress, quick, quiet transition from consciousness to unconsciousness and death. Euthanasia should be carried out by a trained person using methods, substances, and facilities permitted under IACUC policy.

The method of euthanasia varies with the species and number of animals to be sacrificed. The common method in our laboratory is the intravenous or intraperitoneal injection of an overdose of Sodium Pentobarbital. A concentrated barbiturate solution specifically intended for euthanasia is available to the investigators through the AAF.

0.1 ml/10 g of body weight of the mouse is administered intraperitoneally. This will stop the respiration and heart beat in a few minutes.

CASE WESTERN RESERVE UNIVERSITY
ATHYMIC ANIMAL FACILITY

NUDE MOUSE ORDER

DATE: _____

INVESTIGATOR: _____
PRINT NAME

ACCOUNT NO: _____ PROTOCOL NO.: _____

ORDER: (Please indicate number in appropriate box)

MALES # _____	FEMALES # _____	EITHER # _____
---------------	-----------------	----------------

Additional notes regarding order.

INVESTIGATOR'S SIGNATURE*
***order cannot be processed without the principal investigator's signature.**

Filled by: _____ Date: _____