A. Animal Restraint

The use of proper restraint and handling techniques reduces stress to animals and also to the researcher. Stress represents an experimental variable and should be minimized whenever possible. Animals can inflict serious injuries to humans and to themselves as a result of improper handling.

i. Animals experience stress as a result of shipping. All large animals must be allowed to acclimate to the facility. During this time, they should not be experimentally manipulated. Acclimation periods of up to one week are recommended for all animals.
ii. If a study will involve significant handling of animals, it is recommended that the animals be acclimated to the handling.
    - ✓ Prior to experimental manipulation, handle the animal on a regular basis in a non-threatening situation, e.g. weighing, petting, and giving food treats.
    - ✓ Most animals, even rodents will respond positively to proper handling and will learn to recognize individuals.
iii. Handle animals gently. Do not make loud noises or sudden movements that may startle them.
iv. Handle animals firmly. The animal will struggle more if it sees a chance to escape.
v. Use an assistant whenever possible.
vi. Use restraint devices to assist when appropriate.
vii. Chemical restraint should be considered for any prolonged or potentially painful procedure.
viii. Restraint devices should be suitable in size, design, and operation to minimize discomfort, pain, distress, and the potential for injury to the animal and the research staff.
ix. Restraint devices should not be considered a normal method of housing, and must be justified in the animal use protocol.
x. The period of restraint should be the minimum required to accomplish the research objectives.
xi. Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel while those that fail to adapt be removed from the study.
xii. Handling may require the use of anaesthetics to prevent unnecessary pain and suffering of the animals in the animal house.

B. Handling Methods

Animal handling methods depend on the type and size of the experimental animal, type of research and mode of treatment administration.

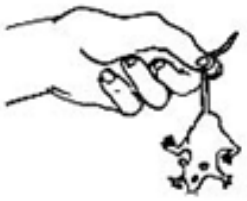
The methods described below will assist with performing basic manipulations. Alternate techniques may be needed for special procedures.

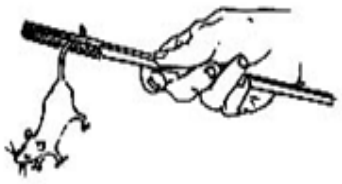
a. Mouse

i. Tail restraint, as described below is adequate for examining animals and transferring them to another cage.
When a mouse is handled, movements should be slow and gentle. They are lively animals, but they will rarely bite unless they are afraid or mishandled.

- ***By hand:***
- Grasp the middle of the tail between the thumb and index finger, and lift the mouse.
- Frequently, the animal will hang quietly and can be carried in this manner. Occasionally, a mouse may turn and crawl into the palm of the hand; it should be allowed to do this.

- ***Using thumb forceps:***
- Thumb forceps are shaped somehow like an eyebrow tweezers.
- The tip must be covered with a rubber or polystyrene tubing to prevent damage to the mouse-tail.
- Grasp the middle of the tail with the forceps and apply just enough pressure to lift the mouse.
  - Too much pressure may cause pain or even crush the tail.





By Hand **Hand forceps**

[Reference: www://ahc.umn.edu/rar/restraint/hmouse.jpg]

ii. To perform minor, non-painful procedures such as injections or ear tagging, the following approaches may be used.
The mouse is held gently at the nape with the two ears held gently together

- One-handed method
  - Follow the step above.
  - Then place the mouse-tail between the last two fingers of the hand that is holding the nape.

- Two-handed method
  - Place the mouse on a rough surface holding the tail firmly.
    Note: Smooth surface will frighten the mouse because it cannot get a foothold.
    This may cause it to turn and try to bite in its attempt to escape.
  - Grasp the nape gently and firmly with your free hand and lift the mouse.

One-handed **Two-handed**

[Reference: www://ahc.umn.edu/rar/restraint/1mhold.jpg and www://ahc.umn.edu/rar/restraint/2mhold.jpg]

b. **Rat**

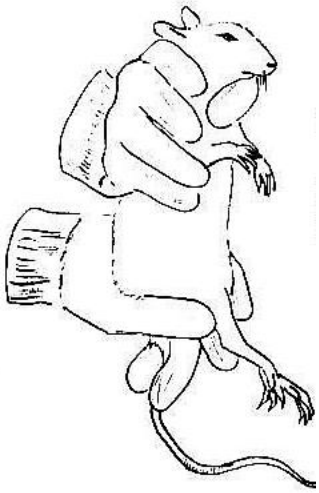
May be handled in similar manner to the mice; similar precautions to those used for mice, with emphasis on only grasping the tail base.

Note: *Holding the tail distal to the base can result in a de-gloving injury to the tail that will require surgical repair or euthanasia.*

For injection administration

i. Pick up the rat. Rotate the wrist of your right hand to expose the mid-section.

ii. Extend the rat's hind leg, grasping one hind leg between your thumb and index finger and the other between your index and second finger.



C. Identification Methods

Animal identification is a means of body markings dated back to the 18th century.

Basis for animal identification:

i. To track specific animals.
ii. To verify and claim ownership.
iii. To reduce the risk of infection transmission.
iv. For dating during breeding period.
v. For research purposes.
   - It is absolutely essential for experimental design and its execution. Lack of identification can cause mix-up and misinterpretation of data.

The choice of a particular identification method is based on:

a. Research purpose
b. Age of the animal
c. Experimental design
d. Duration of the experiment

Types of identification methods

These methods can be classified as either short-term [temporary] or long-term [permanent].

a. Short-term - utilizes non-toxic markers on the fur, tail, head or skin of the animals. The marker should last for between 3-4 days without the need to remark again unless the experiment extends beyond the period.

b. Long-term - requires the expertise of a known user. Examples are: Ear punches, Microchips and tattooing

i. ***Ear punches***

They are of different types. The procedures should be sterile prior to initial use. A punch should be placed approximately 3 mm from the edge of the ear pinna. The ear punch should be appropriately sanitized e.g. using 70% ethanol.

ii. Microchip transponders

*A **microchip implant** is an identifying integrated circuit placed under the skin of an animal. The chip, about the size of a large grain of rice, uses passive RFID (Radio Frequency Identification) technology, and is also known as a PIT tag (for Passive Integrated Transponder).*

Each microchip is coded with a unique, non-replicable number. This is implanted subcutaneously by a veterinarian or technician. The procedures on the sterile package should be followed closely by expert. The chips are read with a portable, handheld scanner

iii. Tattooing

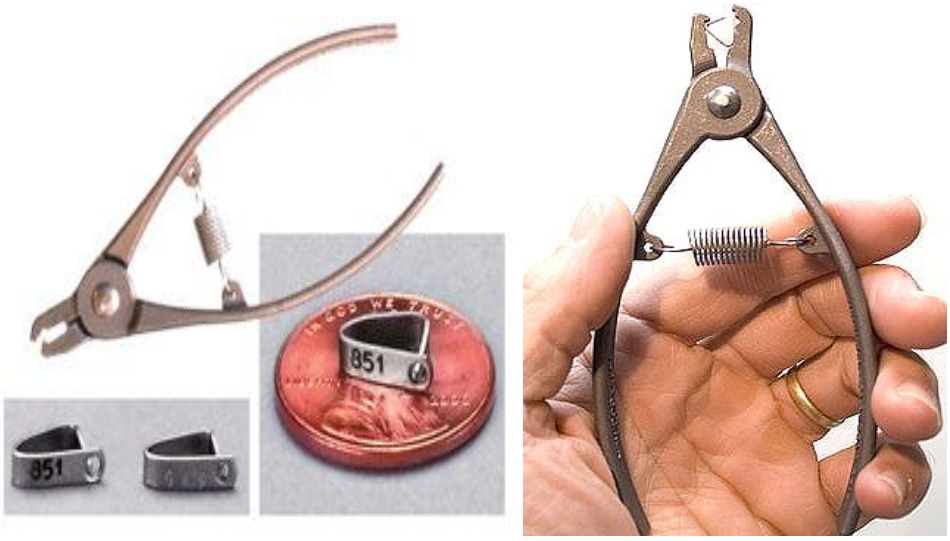
It is a method which can be employed on young and adult animals. It uses a sort of specialized type of machine that writes numbers or other characters on the tails and footpads of young and adult mice. An example of such application is the ***Aramis*** *tattoo system*

[Ref: Animal Identification and Marking and Marking Systems (AIMS™, 2017), https://animalid.com/neonate-rodent-tattoo-identification/32]

iv. Ear tags

This is another means of identifying mice or other species of animals like cows. It is imprinted as digits or letters on the ear lobes using a special ear tag applier that must have been sanitized. Proper placement of the ear tag is necessary so as to prevent detachment from the ear during fight or when caught in wire mesh of cage.

Ear tagging requires a special applicator to place an “earring” or metal tag with an identification number in the pinna of a mouse or rats ear. Since tags are relatively large and mouse ears develop quickly, ear tagging is primarily used on weanling and older mice. The applicator tip and ear tags should always be disinfected before use.



Stainless Steel Lab Animal Tag Applicator (Ref: https://nationalband.com/la-1005-1/)