UNIVERSITY STANDARD ON RODENT IDENTIFICATION

Introduction

PURPOSE
The purpose of this standard is to ensure that the advantages and disadvantages for animal identification methods in addition to the required training for certain techniques are communicated to animal users.

SCOPE OF APPLICABILITY
All research personnel engaged in the identification process for rodents within their animal colony.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Standard

There are several IACUC approved methods used to identify rodents. Each method has both advantages and disadvantages. In long-term studies it is important to choose a method that is permanent and easily read. All methods must be described in the approved protocol prior to use in animals. Some techniques require specific training and certification by either Office of Animal Care and Use (OACU) Training and Compliance Team, or by Laboratory Animal Coordinator (LAC). The IACUC Mouse Handling & Techniques and Rat Handling & Techniques packets should be referenced for acceptable techniques and methods.

Long Term Methods:

Ear Notching – This method is frequently used in both mice and rats. There are several tools that may be purchased to achieve this. Most resemble a hole puncher and are not expensive to purchase. There are previously created maps that serve as a numbering system, or the researcher may create a map. This technique requires certification prior to approval.

A) Advantages – Ear notching can be done quickly causing very little pain or distress. The instruments are not costly and can be obtained easily.
B) Disadvantages – This method cannot be applied until the ears are fully developed. This may be too late for those that use young rodents. This method may not be a reliable permanent way to identify individuals in strains that are prone to fighting. Rips or tears caused by fighting may leave the pattern indiscernible. Tools used to notch ears dull easily so they must be replaced frequently.

Ear Tagging – Ear tags can be purchased with numbers and/or letters. Correct placement of the tag makes them fairly easy to read. Ear tags should be placed in the lower half of the ear to facilitate normal ear position and on the outer third of the ear to avoid the area with the highest concentration of capillaries. This technique requires certification prior to approval. Alternatives to metal bar tags are button & bar code ear tags (see Reference section.) (There is a tattooing technique for identification that can be used as an alternative method to ear tagging. (see JAALAS article in ingentaconnect and also southpointsurgical tattooing system in Reference section.))

A) Advantages – Ear tags are inexpensive and are fairly easy to apply. This method does not require the use of anesthesia. Tagging can be done quickly and does not seem to cause pain and only minor distress.

B) Disadvantages – Tags can fall out if not applied properly. They can also be lost if ears are ripped or torn in strains that fight. Different sized tags are available for different species. Tags are relatively heavy for weanlings and may cause young mice to tilt their head even when the proper sized tag is applied or cause the ear pinna to fold over if placed too high. Some strains are prone to scratching the tagged area which can lead to infection, hematomas, and granulomas. DCM Veterinary Services have also reported seeing tag reactions with masses developing on ear pinna. Other issues seen have been folded pieces of ear pinna within the tag, body tissue included in the ear tag due to being placed too close to the base of the ear, and ear tags placed too close to the center of the ear. Therefore, it is important to remember that incorrect placement of ear tags can lead to health issues that can affect both the overall health of the animals as well as the study results.
Microchipping – Microchips, electronic transponders, are safe and reliable.

A) Advantages – Microchips may be applied without the use of anesthesia. Applying microchips seems to cause little or no pain. Even though the chip may migrate to a different area, they are not lost so they prove to be a reliable method for permanent identification. Animals can be identified (by reading the microchip/transponder) without handling and removing them from the cage. Some microchips are designed to provide other information such as core body temperature and heart rate.

B) Disadvantages – The equipment used to read the chips is fairly expensive. Despite what manufacturers say, the chips can be reused. In order to reuse the chips they must be sterilized by ethylene oxide. If not implanted properly there is a slight risk of infection. In a very limited number of cases, microchips may stimulate tumor growth.

Tattooing – This method seems to be growing in popularity. It is both permanent and fairly easy to apply. There is a tattooing technique for identification that can be used as an alternative method to ear tagging. (see JAALAS article in ingentaconnect and also southpointesurgical tattooing system in Reference section.)

A) Advantages – This method works in all strains, even the more fractious. The cost is very reasonable after the initial expense. Tattoos can be applied to rodents of any age. The markings are easily read, especially when applied to the tail of light colored rodents. When placed in the proper area, it is not necessary to handle the animal to read the tattoo. Tattooing causes only minor pain and distress and does not require the use of anesthesia.
B) Disadvantages – The identifying marks may be a little difficult to read in young pigmented mice. This improves as the mice age. The initial cost is rather expensive. There is a small chance of inducing infection if the tattoo is not applied correctly.

Toe Clipping – Toe clipping must be scientifically justified in an approved IACUC protocol. Adequate justification does not include cost, convenience or lack of formal training. When utilized as a method of identification, toe clipping should only be used when no other identification methods are feasible and should be combined with genotyping.

Toe clips must be performed on or before 10 days of age. The ideal timing is between 5-7 days of age when the toes are separate and the bone not calcified and the toes are no longer webbed. Toe clipping beyond 10 days of age is considered painful and is not an acceptable procedure for either identification or genotyping. If tissue for genotyping is required beyond 10 days of age, alternative methods of collecting tissues should be considered. Contact DCM veterinarians for additional information.

The IACUC allows toe clips on a maximum of 4 toes and no more than 2 toes per foot. It is preferable to remove toes from the hind feet rather than the front feet. Any identification numbering system should be designed to minimize the number of toes clipped per animal. The hallux (also referred to as dew claw or thumb) may not be cut as this may decrease the rodent’s grasping ability.

Procedures:
- Obtain adequate training from Principal Investigator (PI) and/or Laboratory Animal Coordinator (LAC).
- Restrain the animal for the minimum amount of time required for the procedure. With a sharp instrument, remove the toe(s) at the most distal joint of the toe (i.e., remove the last phalangeal [toe] bone; P3).
- Sharp scissors are recommended for toe clipping in neonatal rodents.
- Instruments must be clean and disinfected initially, and blade surfaces should be cleared of debris and wiped with 70% alcohol between animals.
- Apply pressure to the exposed tissue with gauze or other clean and absorbable material to ensure hemostasis. Monitor animals continuously until bleeding has stopped.
- During the procedure, the neonatal rodents should be handled gently and then placed back with the mother as quickly as possible.

A) Advantages – Toe clipping can be done at a very early age. The tissue can be used for genotyping. No anesthesia is needed. This seems to cause little or no pain when performed early enough. The young react to being removed from their mother, but do not react to the clipping of the toe.

B) Disadvantages – Toe clipping may not be performed after post-natal day ten. The young show signs of distress when removed from their mother and siblings. This may cause a small amount of pain. There is a small possibility of infection. A reduction in the number of toes may reduce the ability to grasp objects.

Short Term Methods:

Hair Clipping – Trim patterns in fur. Keep a record or picture to identify rodents.

A) Advantages – Causes no pain.

B) Disadvantages – This is very temporary. The hair will grow back within ten days and must be repeated. In animals as small as mice, removal of much hair can affect their ability to maintain body temperature.
Permanent Markers and Fur Dyes – It is easy to apply marks or dyes to different body parts. Temporary identification can be achieved with non-toxic stains or dyes that come in a variety of colors.

**A) Advantages** – This method is non-invasive. It causes only minor stress due to restraint.

**B) Disadvantages** – This method can be time consuming because it must be redone soon after application. If you are working with nursing pups, the mothers will groom the neonates excessively and the markings may disappear overnight. This could result in a loss of identity.

If you would like to inquire about equipment used, or training provided, in the methods discussed above, please email the OACU Training & Compliance Team. You may send questions to the general IACUC email account iacuc@med.unc.edu.

**EXCEPTIONS**
Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

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**Definitions**

- **IACUC**: Institutional Animal Care and Use Committee
- **DCM**: Division of Comparative Medicine
- **University Standard**: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

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**Related Requirements**

**EXTERNAL REGULATIONS AND CONSEQUENCES**

**UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES**

For more detailed guidance, please refer to the [University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes](https://www.ingentaconnect.com/contentone/aalas/jaalas/2016/00000055/00000002/art00010?crawler=true).

**REFERENCE**


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Policy Title: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD ON RODENT IDENTIFICATION
Effective Date: 04/22/05
Last Revised: 05/11/07; 05/2015; 11/2018
https://www.rarc.wisc.edu/animal_health/experimental_techniques/rodent_methods_of_id.html

Bar code tags: https://rapidlab.com/mouse-ear-tags-product-info/

Button tags: https://www.stoeltingco.com/neuroscience/misc/animal-identification/ear-tags.html

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**Contact Information**

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<th>Subject</th>
<th>Contact</th>
<th>Telephone</th>
<th>Email</th>
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<tr>
<td>Veterinary Services</td>
<td>DCM</td>
<td>919-962-5335</td>
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<tr>
<td>IACUC Protocol and Training</td>
<td>OACU</td>
<td>919-966-5569</td>
<td><a href="mailto:iacuc@med.unc.edu">iacuc@med.unc.edu</a></td>
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**Important Dates**

- Effective Date and title of Approver: 04/2005; UNC IACUC
- Revision and Review Dates, Change notes, title of Reviewer or Approver: Revised 05/017; UNC IACUC; Revised 05/15, Revised 11/2018; Transferred to new standards template, updated toe clip description, ear identification options and updated contact information; UNC IACUC

Approved by: UNC IACUC

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Dr. Roland Tisch
UNC IACUC Chair