

University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

Rodent Identification

Policy: All primary enclosures must be identified with a cage card produced by the Comparative Medicine Research Unit (CMRU). Exceptions for unusual cage types should be outlined in an IACUC-approved *Proposal*. Methods of individual identification must be described in an IACUC-approved *Proposal*. Acceptable means of rodent individual identification include ear tags, ear notch or punch, tail or toe tattoo, subcutaneous transponders, and temporary marking with a non-toxic dye or ink. Identification with *toe-clipping requires specific justification* and may only be applied under circumstances described below. If toe-clipping is approved as a means of rodent identification, the tissue should be used for genotyping. Because picric acid is a highly sensitive explosive detonated by heat, flame, shock, or friction, its use for temporary identification of animals is *not allowed*.

Rationale: This policy was designed to guide investigators on appropriate means of identifying rodents. The Guide¹ outlines acceptable means of colony and individual identification and states: “As a method of identification of small rodents, toe clipping should be used only when no other individual identification method is feasible. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age, especially if toe clipping and genotyping can be combined. Under all circumstances, aseptic preparation should be followed. Use of anesthesia or analgesia should be commensurate with the age of the animals.” Similarly, NIH guidelines limit toe clipping to altricial rodents, preferably mice 5-7 days old, and stipulates to minimize pain and distress by clipping only one digit per animal, use hind paws when possible, never clip the hallux, and ensure proper training.² The FDA discourages the use of toe clipping as a means of animal identification.³

Procedures, Guidelines, and Exceptions:

1. Principal investigators should use the following table for reference:

Method	Advantages	Disadvantages	Anesthesia Required
Fur clipping	<ul style="list-style-type: none">▪ Simple and rapid▪ Patterns may be used to distinguish individuals	<ul style="list-style-type: none">▪ Equipment (clippers) required▪ Temporary	No
Hair dyes or Permanent Marker	<ul style="list-style-type: none">▪ Simple and rapid▪ May be used on fur or tail	<ul style="list-style-type: none">▪ Temporary	No
Ear tag	<ul style="list-style-type: none">▪ Simple and rapid▪ Relatively permanent	<ul style="list-style-type: none">▪ May be traumatically removed▪ May cause slight head tilt▪ May be associated with infection or local hyper-reactivity	No
Ear notch or punch	<ul style="list-style-type: none">▪ Simple and rapid	<ul style="list-style-type: none">▪ Local trauma may render	

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	<ul style="list-style-type: none"> ▪ Sample may be used for genotyping ▪ Standard template available 	<ul style="list-style-type: none"> unreadable ▪ Tools dull and must be replaced regularly 	No
Tail tattoo	<ul style="list-style-type: none"> ▪ Permanent 	<ul style="list-style-type: none"> ▪ Some skill required ▪ Specialized equipment required 	Recommended
Toe tattoo	<ul style="list-style-type: none"> ▪ Simple ▪ Permanent ▪ Standard template available (same as toe clipping) ▪ Can be performed at any age 	<ul style="list-style-type: none"> ▪ Some skill required 	No
Subcutaneous transponders (microchips)	<ul style="list-style-type: none"> ▪ Permanent ▪ Models measuring physiological indices available ▪ May be read without handling animals 	<ul style="list-style-type: none"> ▪ Specialized equipment required 	No

2. Evidence suggests that toe-clipping (amputation of distal phalangeal bone according to a standardized numbering code) is painful and may affect behavior, alter gait or weight-bearing, and reduce grasping ability. Therefore, toe-clipping may be considered only under the following conditions:
- a. The investigator must provide compelling scientific justification as to the necessity of toe clipping, including written consideration of why all alternative methods are not feasible or suitable. The justification should specifically identify the alternative methods considered and why they are not feasible.
 - b. Toe clipping must be limited to altricial pre-weanling rodents (*e.g.*, mice and rats) up to 7 days of age. Toe clipping rodents older than 7 days of age requires additional scientific justification and the use of anesthesia and/or analgesia depending upon age.
 - c. Clipping must be limited to one digit per animal, preferably on a hind paw, and must not involve the hallux (“dew claw” or “thumb”). Only the most distal phalanx (P3) may be removed.
 - d. Sterile sharp scissors, a scalpel, or razor blade must be used. Instruments must be disinfected between animals if reused (*e.g.* with a bead sterilizer). Hemostasis must be ensured (*e.g.*, digital pressure with styptic powder or silver nitrate if necessary).
 - e. Personnel must be appropriately trained and the Principal Investigator is responsible for ensuring a project participant’s proficiency prior to using this technique.
 - f. Aseptic technique must be followed. Recommendations prior to clipping include cleaning the digit with alcohol or a surgical disinfectant (povidone iodine or chlorhexidine) and administering a local anesthetic (*e.g.*, cetacaine, ethyl chloride, lidocaine, bupivacaine). Local vapocoolant anesthesia is not recommended.^{2,4}

References:

1. Institute of Laboratory Animal Resources, National Research Council. 2011. The Guide for Care and Use of Laboratory Animals. National Academy Press, Washington, DC, p. 75.
2. Animal Research Advisory Committee, National Institutes of Health. 2019. Guidelines for Toe Clipping of Rodents

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3. Food and Drug Administration. 1989. *Federal Register* **54**: 75, 1-3.
4. Paluch *et al.* (2014). Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. *JAALAS* 53(2): 132-140.

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