

University of Maryland College Park Animal Care and Use Standard

## Distal Phalanx Biopsy (Refined Toe Clipping) in Rodents

**<u>Purpose</u>**: This standard describes the conditions under which toe clipping of rodents may be acceptable and provides specific considerations for conducting the procedure.

**Background:** Per the *Guide for the Care and Use of Laboratory Animals* (the *Guide*), toe clipping of small rodents should be used for identification "…only when no other individual identification method is feasible. It may be appropriate for identifying neonatal mice up to 7 days of age, especially if toe clipping and genotyping can be combined." Studies have shown that the refined distal phalanx biopsy of newborns, if properly performed, does not seem to affect mice more than tail or ear biopsy, in either the short or the long term.

## **Definitions:**

1. *Toe clipping* – refers to removal of a distal portion of a digit (toe), regardless of the extent of biopsy or age of the animal, for purposes of identifying small rodents by using a predetermined numbering code.

2. *Distal phalanx biopsy (refined toe clipping)*– refers to the removal of the distal phalanx of a newborn animal (ideally seven days of age with p0 as day of birth), that is then used as a source of DNA. This method also serves to identify the animal. This is a refinement of toe-clipping procedures.

**Standards:** Toe clipping of rodents must be described in the protocol and approved by the Institutional Animal Care and Use Committee prior to implementation. Refined toe clipping procedures should be performed, i.e., limited biopsy in neonatal, altricial (e.g., mice and rats) rodent pups. When approved for use in animal study protocols, toe clipping in rodents should meet the following conditions:

- At the time of the biopsy, the animals should be approximately seven days old;
- Only the most distal phalanx of only one toe per paw should be removed;
- No further distal phalanx biopsies should be performed;
- Personnel must be trained and proficient in proper technique.

## Methodology:

- 1. Use very sharp scissors (fine pointed tips work best).
- 2. Scissors must be sterilized prior to use
- 3. Scissors must be cleaned and sanitized (or preferably, sterilized) between animals.
  - Ideally, a hot bead sterilizer should be used after cleaning and before the next animal.
  - Alternately, scissors may be sanitized with 70% ethanol or antiseptic solution (e.g., povidone iodine, chlorhexidine). N.B.- Per current standards of veterinary care, use of ETOH is acceptable for this procedure.
- 3. Aseptically prepare the digit before clipping (e.g., wipe with betadine or alcohol).
- 4. Carefully grasp the pup by the scruff of the neck (limbs will typically extend).
- 5. No more than **1 toe per paw** may be clipped.
  - Avoid clipping digits/toes on fore paws if possible.

- DO NOT clip the 1st digit/toe (i.e., hallux/thumb) on either fore paw.
- Only remove the distal (3<sup>rd</sup>) phalanx (i.e., last bone of a digit); that is, amputate at the joint between the 2nd and 3rd bones/phalanges.
- 6. Monitor animals continuously until bleeding has stopped.
  - Bleeding may be stopped using a piece of gauze with gentle pressure applied by fingertip.

7. Contact DLAR veterinary staff promptly if toe does not heal properly or if the animal cannot ambulate normally following the procedure.

## **References:**

1. Institute for Laboratory Animal Research, National Research Council. (2011). Guide for the Care and Use of Laboratory Animals. Pg 75. Washington, DC: National Academy Press. Retrieved from https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratoryanimals.pdf

2. National Institutes of Health, Office of Animal Care and Use, Animal Research Advisory Committee. Guidelines for Toe Clipping of Rodents, Revised 2/23/19 Retrieved from https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b9 toe clipping.pdf on 5/26/20.

3. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. Lab Anim. 2013 Jan;47(1):2-11. doi: 10.1177/002367712473290

4. Bonaparte (Convenor) D, Cinelli P, Douni E, Hérault Y, Maas A, Pakarinen P, Putanen M, Santos Lafuente M, Scavizzi F. Federation of European Laboratory Animal Science Associations Working Group. 2013. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents: a report of the Federation of European Laboratory Animal Science Associations Working Group

Lab Anim 47(3) 134-145.

5. Assistant Laboratory Animal Technician Manual, American Association for Laboratory AnimalScience, 2009, p 74-75.

6. Castelhano-Carlos M, Sousa N, Ohl F, Baumans V. Identification Methods in Newborn C57BL/6 Mice: A Developmental and Behavioral Evaluation. 2010. Lab Anim. p 88-103. DOI:10.1258/1a.2009.009044.

7. Iwaki S, Matsuo A, Kast A. Identification of Newborn Rats by Tattooing. 1989. Lab Anim. p361-364.

8. Paluch L, Lieggi C, Dumont M, Monette S, Riedel E, Lipman N. Developmental and Behavioral Effects of Toe Clipping on Neonatal and Preweanling Mice with and without VapocoolantAnesthesia. 2014. JAALAS. p 132-140.

9. Schaefer D, Asner I, Seifert B, Bürki K, Cinelli P. Analysis of Physiological and Behavioral Parameters in Mice after Toe Clipping as Newborns. 2010. Lab Anim. p 7-13.