

Murine Tail Tip Biopsy Procedure

Policy Number: _____

Date: _____

ACUC Authorized Signature: _____

1.0 Introduction

1.1 A conventional procedure for genotyping transgenic animals entails cutting off the distal portion of the animal's tail (tip biopsy). From this tissue, template DNA may be extracted to ascertain the presence or absence of a specific transgene using PCR or other DNA analysis techniques.

1.2 The PHS Policy¹ requires the minimization of pain and distress, consistent with the aims of the study, whether it is for collection of tissue for DNA analysis or other procedures.

1.2.1 This procedure must be limited to a maximum of two times, since the tail is important in body temperature control and balance in rodents.

1.2.2 The minimum amount of tissue required for analysis should be obtained, with 5 mm (0.5 cm) being the maximum allowed without specific prior approval by the ACUC.

1.2.3 In altricial rodents and animals less than 3weeks (21 days) of age, biopsy of the distal tail may be performed without use of analgesia or anesthesia. However, if there is concern about the survival or health or growth of the animals (extreme circumstances**), the tail biopsy can be performed in animals up to 25 days of age without anesthesia following approval from the IACUC.

1.2.4 Appropriate local analgesics (spray on or topically applied agents) or general anesthetics must be used in rodents weighing 50 grams or more and in animals over 3 weeks (21 days) of age or older, except in extreme circumstances, when up to 25 days of age can be allowed. The IACUC must approve the extreme circumstances prior to performing tail tip biopsy without the use of analgesics or anesthetics.

1.3 The ACUC must approve any research protocol where tail tip biopsy procedures are used.

1.4 Alternatives to tail biopsies should be considered:

1.4.1 Tissue can be obtained by ear punching, which can also serve as identification

1.4.2 Small quantities of blood from distal veins such as the saphenous vein or the periorbital sinus may be used for analysis

1.4.3 PCR analyses using saliva, hair, rectal scrapings, and feces have also been described²⁻⁵

1.5 Recommended age for performing tail tip biopsy in rodents is between 8 to 21 days of age. In rodents, the terminal tail ossifies between 2 and 3 weeks of age. Thus, tail tip sampling is recommended in animals less than 3 weeks of age. The tail of murine rodents contains a variety of tissues, including bone, cartilage, blood vessels and nervous tissues. In an altricial or pre-weanling animal, the tissue near the tail tip is soft, and the bones have not completely mineralized. Therefore, amputation of the tail tip in *altricial and preweanling* animals probably amounts to momentary pain for the animal, similar to that observed when injections are given. As the animal ages, tissue maturation occurs, which includes mineralization of the tail bone, increased vascularity, and maturation of the nervous system. Tail tip amputation performed on *older animals* is likely to involve more than momentary pain and distress, as well as the potential for significant hemorrhage.

It is the purpose of this policy to describe the requirements and restrictions for protocols that request murine tail tip biopsy procedures and provide consistency of ACUC protocol review.

2.0 Requirements

2.1 Murine tail tip biopsies should be conducted using sterile, sharp instruments such as a stainless steel surgical blade or sharp scissors. The instrument should be decontaminated between animals with 70% Isopropyl alcohol, or an appropriate disinfecting agent such as Nolvasan (chlorhexide diacetate). Alternately, instruments can be heat sterilized using a hot bead sterilizer or flame. If a chemical disinfectant is used, the instrument must be rinsed with sterile water or saline between animals. The instrument must be changed when dullness of the cutting edge is noted.

2.2 Since bleeding may occur post-tail tipping, the biopsy site **must** be observed for active bleeding, and hemostasis should be affected post-procedurally if required. Hemostasis can be achieved by digital pressure, cautery, tissue adhesives, coagulation powder or coagulation sticks.

2.3 Appropriate local analgesia or general anesthetics **must** be used on animals greater than 3 weeks of age (21 days) unless justified in writing and approved in advance by the ACUC. In altricial rodents or animals less than 21 days of age, use of analgesics/anesthetics is optional.

2.4 Specific justification to the ACUC and prior approval must be obtained, prior to completing tail tip biopsies on rodents weighing more than 50 grams.

2.4 The recommended size of the tail tip biopsy should be less than 5 mm (0.5 cm). A maximum of two biopsies can be performed on an individual animal. In the event that more than two biopsies or more than 5 mm needs to be removed at one time, the investigator must:

1. Provide justification to the ACUC for review and approval; and
2. Local analgesics or general anesthetics must be used for the second procedure, regardless of the animal's age.

3.0 Restrictions

3.1 No more than 5 mm (0.5 cm) of tail tissue may be removed from any one animal during a biopsy procedure.

3.2 No more than two tail biopsies are permitted per animal.

3.3 Animals should not be returned to their home caging until hemostasis and complete recovery from anesthesia has been obtained. NOTE: Hypothermia should be prevented at all times during the procedure.

4.0 Biopsy Equipment/Procedure

4.1 Equipment Needed:

- Disposable gloves (preferably sterile) and other personnel protective equipment, as needed.
- Sharp scissors or surgical blade
- Appropriate collection vessel
- Isopropyl alcohol or appropriate disinfectant (sterile saline/water for rinsing)
- Gauze
- Hemostasis equipment (digital pressure, cautery, tissue adhesives, coagulation powder or coagulation sticks)

4.2 Collection tubes should be labeled with relevant transgene name, animal number, date of birth and cage number (if applicable). This ensures that if the rack is tipped over, tubes can still be sorted out.

4.3 Procedure:

- Restrain animals (if less than 21 days of age or up to 25 days in extreme circumstances) or use appropriate analgesia/anesthesia
- Expose and disinfect the tail tip
- Clip less than 5 mm off the tip of the tail with sharp instrument
- Apply hemostasis, and recover the animal in a warm environment
- Return to its home cage after full recovery

5.0 Training

Personnel in the Department of Comparative Medicine will be happy to assist and train personnel in the above techniques. Training is available free of charge, and can be arranged by contacting the Comp. Med. Secretary at 448-5656.

****Extreme Circumstances** that would allow tail tip biopsy from 21-25 days without the use of analgesics and/or anesthetics are defined as lines of animals whose offspring exhibit a failure to thrive, have developmental or growth delays, or a genetically induced disorder that causes fragile health. These extreme circumstances must be described in the original protocol or must be approved as an amendment to the protocol. The IACUC must review and approve the extreme circumstances prior to the performance of the tail tip biopsy.

1. Public Health Service Policy on Humane Care and Use of Laboratory Animals, PHS, 1986.
2. Irwin MH, Moffatt RJ, and Pinkert CA: Identification of transgenic mice by PCR analysis of saliva. Nature Biotechnology, 14(9), 1146-48, 1996.
3. Schmitteckert EM, Prokop CM, and Hedrich HJ: DNA detection in hair of transgenic mice – a simple technique minimizing the distress on the animals. Lab Animal 33, 385-89, 1999.

4. Lahm H, Hoeflich A, Rieger N, et al: Identification of transgenic mice by direct PCR analysis of lysates of epithelial cells obtained from the inner surface of the rectum (published erratum appears in *Transgenic Res* 7:495, Nov 1998) *Transgenic Research* 7 (2): 131-34, 1998.
5. Frantzen MA, Silk JB, Ferguson JW, et al: Empirical evaluation of preservation methods for faecal DNA. *Molecular Ecology* 7 (10), 1423-38, 1998.