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# 1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures for individual identification of animals.

# 2.0 SCOPE

2.1 This procedure applies to rats and mice in the MMRI animal care facility.

# 3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the use of each animal is accounted for on an individual basis.

#### 4.0 **RESPONSIBILITIES**

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

#### 5.0 REFERENCES

- 5.1 SOP# ANP016, Anesthesia Procedures
- 5.2 SOP # ANPO06, Animal Handling Restraint and Transfer
- 5.3 JoVE Science Education Database. *Lab Animal Research.* Rodent Identification I. JoVE, Cambridge, MA, (2019). <u>https://www.jove.com/science-education/10189/rodent-identification-i</u>
- 5.4 JoVE Science Education Database. *Lab Animal Research.* Rodent Identification II. JoVE, Cambridge, MA, (2019). <u>https://www.jove.com/science-education/10182/rodent-identification-ii</u>
- 5.5 BMDS DAS-7008/9 Small Smart Reader Manual
- 5.6 BMDS SP-6004/SP-6005 User Manual (Probe for RF Identification)

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# 6.0 MATERIALS AND EQUIPMENT

6.1 Cage cards

6.2 Identification specific materials (e.g. ear tags, ear punch, transponders, etc)

#### 7.0 PROCEDURE

- 7.1 All animals must be individually identified with a unique animal number. The animal's actual identification will be recorded on the animal's cage card.
- 7.2 Generation of Identification Number
  - 7.2.1 Animals procured from vendors and outside laboratories
    - 7.2.1.1 On January first of each year, numbers start at 0001 for each species and continue sequentially. The sequential number is preceded by the last two digits of the current year (i.e., 19 for 2019) and separated by a dash (i.e., 19-0001).
    - 7.2.1.2 When animals are received, they are counted and issued a block of numbers in sequential order. Numbers are recorded on the Animal Log Sheets and on corresponding invoices. The numbers are printed sequentially on a set of cage cards.
    - 7.2.2 Animals bred in-house
      - 7.2.2.1 For each breeding colony, the founders are assigned sequential numbers starting from 0001. For any live births, pups are assigned numbers sequentially in order of birth date, after the founders have been assigned. For example, the 4 founders would be assigned 0001-0004. The first 5 pups born would be assigned 0005-0009. The numbering system continues indefinitely and does not reset on January first.

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#### 7.3.1 Cage Cards – REQUIRED FOR ALL ANIMALS

When group housed animals will be identified by multiple cage cards placed on the cage. When housed individually animals will be identified by single cage cards. In addition to cage cards, animals may also be individually marked with the assigned unique animal number or on-study numbers using identification methods found in this SOP.

# 7.3.2 Ear Tag Identification:

Customized ear tags can be ordered from manufacturers with alphabetical and/or numerical codes, which allows for the differentiation of mouse strains or investigators. Ear tags can be made of metal or plastic.

- 7.3.2.1 Select the ear tags appropriate for the animals to be identified. There are several styles of appliers available from ear tag manufacturers. Be sure to order the applier that is for the specific size and style of ear tags being used.
- 7.3.2.2 Gently remove a tag from the cardboard holder and orient it into the applier so that the end with the hole is positioned over the notched area of the applier. The pointed end of the tag should be opposite the notch.
- 7.3.2.3 Restrain the animal so that the ears are easily accessible. Animals may be less likely to struggle if the hind limbs can rest on a surface, such as a cage top or a counter top.
- 7.3.2.4 Properly position the ear tag for placement.
  - 7.3.2.4.1 Position the point of the ear tag as deeply in the concha of the ear as possible, so the tag numbers face the back of the animal.
  - 7.3.2.4.2 To have the ear tag numbers facing the nose of the animal, place the point of the tag at the base of the pinna on the back of the ear.
- 7.3.2.5 Apply the tag.

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- 7.3.2.5.1 Firmly squeeze the applier closed. The ear tag will pierce the ear and lock together.
- 7.3.2.5.2 Release the applier, and the tagged ear will fall out of the applier.



Figure 1. A mouse with an ear tag that is properly positioned on the pinna.

# 7.3.3 Ear Punch Identification

Ear punch codes have been developed as a quick and inexpensive method to label individual animals. There are three styles of ear punches: the scissor punch, the thumb punch, and the lever punch. The animal's right ear is used for single digits, the left ear for tens, and the middle of the ears for hundreds.

- 7.3.3.1 Restrain the animal so that the ears are easily accessible. Most mice are ear tagged for individual identification at weaning. This requires gentle handling due to their smaller, more fragile size. Some mice are less likely to struggle if the hind limbs can rest on a surface such as a cage top or a counter top.
- 7.3.3.2 Determine the ear punches needed to correspond to the animal's experimental number or to identify it within a cage.

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- 7.3.3.3 Punches will be notches or holes depending on the code used.
- 7.3.3.4 If animals are on a short-term study in stable cage groups, they can be given sequential numbers. This is often done when there are no ear tags present.
- 7.3.3.5 Make the ear punch.
  - 7.3.3.5.1 To make a notch, the punch is placed on the edge of the ear pinna.
  - 7.3.3.5.2 To make a hole, place the ear in the punch so that the hole of the punch is positioned away from the pinna margin in the desired position.
  - 7.3.3.5.3 Apply pressure to the thumb or lever punch or close the scissor punch quickly and firmly to cut through the skin of the ear.
  - 7.3.3.5.4 Gently lift the punch away from the ear when using the thumb or lever punches. Open the scissor punch to remove it from the ear.
  - 7.3.3.5.5 Avoid pulling or twisting the punch to prevent tearing the ear.
  - 7.3.3.5.6 Punches do dull after time. Should a punch fail to cut through the ear, release the punch and select a new ear punch. Reposition the new punch in the same spot.
- 7.3.3.6 Clean the punches after each use.
- 7.3.3.7 All punch types will dull if autoclaved. It is recommended that they be cleaned with a disinfectant and rinsed with alcohol. Dry them thoroughly to prevent rusting.
- 7.3.3.8 Ear punches to be used for PCR are routinely soaked for 3 minutes in a solution to remove residual DNA or RNA. Before use they are rinsed in alcohol and wiped dry.

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Figure 2: Ear Punch, Scissor type

Figure 3: Ear Punch, thumb type



Figure 4. Pattern for ear punch numbering system

# 7.3.4 Tail Tattooing

7.3.4.1 Prepare the tattoo equipment according to the manufacturer's instructions. A small amount of ink is required. Black dye should be used for albino mice or rats, and green dye for pigmented mice or rats.

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7.3.4.2 Prepare the animals for identification.

NOTE: To minimize apprehension or distress, tail tattooing should be performed in a procedure room rather than in an animal housing area. This procedure is easier to perform under general anesthesia. If not using general anesthesia, a local anesthetic on the tail should be applied before tattooing, such as EMLA cream or a local anesthetic spray.

- 7.3.4.3 Appropriately restrain the animal for tattooing so that movement is minimal.
  - 7.3.4.3.1 Weanling rats are restrained using a plasticnotched cup, with the lip of the cup secured under the bolts on the tattoo platform, and the tail extending through the notch.
  - 7.3.4.3.2 Adult mice can be restrained by means of a cup restrainer, with the tail pulled taut from the opening and the flange of the cup held in place via a platform or other device.
- 7.3.4.4 Clean the tail with a small amount of diluted tissue cleaner applied with a cotton-tipped swab. Adult rats generally have a buildup of scale and debris on the tail that must be cleaned prior to applying the tattoo. A soap and water scrub of the tail will remove the majority of the dirt and debris. Dry the tail well before proceeding.
- 7.3.4.5 Apply tissue oil to the skin with a cotton-tipped swab just prior to tattooing. The tissue oil is necessary to minimize tissue damage by softening the skin and lubricating the tattoo needle. In addition, the oil dissolves any dirt not removed by the cleaning process and prevents ink staining of nontattooed skin.
- 7.3.4.6 Apply the tattoo.
  - 7.3.4.6.1 Dip the tip of the needle into the pigment with the machine off.

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- 7.3.4.6.2 Bring the tip of the needle to the tattoo site with the gun activated by the foot pedal.
- 7.3.4.6.3 Make short, even strokes in one direction. Numerals and letters should be tattooed in such a way so that they are constructed in segments with minimal angles and curves (when possible).
- 7.3.4.6.4 Penetrate deeply enough into the dermis with the needle for permanent pigment deposition. A change in the sound of the activated needle can help discern the depth.
- 7.3.4.6.5 Approach the skin with the needle at a 90° angle for adequate deposition of pigment.

#### 7.3.4.7 Post-tattoo cleanup and care

- 7.3.4.7.1 Blot (do not wipe) the completed tattoo of excess pigment with a paper towel.
- 7.3.4.7.2 The presence of blood on the tattoo or the paper towel indicates that the tattoo was made too deep and is therefore unlikely to be permanent.
- 7.3.4.7.3 Reinforce thin areas of tattoo characters with additional pigment. Characters are reinforced with pigment deposited parallel to, but not on top of, already-deposited pigment.
- 7.3.4.7.4 To add additional pigment, dip the tip of the needle into the dye reservoir for every two characters for mice and every one character for rats.
- 7.3.4.7.5 Reasons for fading tattoos include inadequate amounts of pigment, tattoos made too shallow, tattoos made too deeply, an inappropriate angle of the needle to the skin, blunt or hooked needles being used, or the tattoo being made too quickly.

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7.3.4.7.6 Thoroughly clean the tattoo equipment after each use according to the manufacturer's instructions.



Figure 5. Tail tattooing on adult mice



Figure 6. Example numerical tattoo on adult

Figure 7. Toe application numbering

7.3.5 Toe tattooing of neonates

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Experimental protocols can require the genotyping of neonates as early as day one, which makes it essential that these pups be permanently identified. The use of toe tattooing allows the animals to be identified until they are large enough for ear tagging or ear punching.

- 7.3.5.1 Restrain pre weaned pups.
  - 7.3.5.1.1 Cup the neonate in the hand on a gauze pad. Position the foot between the thumb and crook of the index finger to expose the chosen toe, or toes, for tattooing.
  - 7.3.5.1.2 Hold the foot close to the toes so that the toe has a solid surface behind it, preventing it from bending away.
  - 7.3.5.1.3 Do not overtwist the leg when positioning the foot. Pups that are restrained may wiggle, but no vocalization is necessarily heard.
- 7.3.5.2 Prepare the equipment.
  - 7.3.5.2.1 Select a 4.5 mm Goldenrod animal lancet.
  - 7.3.5.2.2 Place a small spot of green tattoo paste on a nonporous surface; aluminum foil works well.
  - 7.3.5.2.3 Dip only the tip of the lancet in the tattoo paste.
  - 7.3.5.2.4 Use only a small amount of paste.
- 7.3.5.3 Apply the tattoo.
  - 7.3.5.3.1 Poke the desired toe with the Goldenrod lancet according to an identification code.
  - 7.3.5.3.2 Puncture the skin to introduce the paste into the skin, leaving a mark.

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- 7.3.5.3.3 Poke the same spot three times to insure proper penetration.
- 7.3.5.3.4 Avoid poking so deeply that the toe bleeds; this can result in a fainter tattoo.
- 7.3.5.4 Post-tattoo cleanup and care
  - 7.3.5.4.1 Gently blot the spot with an absorbent wipe/towel to remove excess paste.
  - 7.3.5.4.2 Do not attempt to clean excess paste from the foot or body.
  - 7.3.5.4.3 Return the animal to its cage.
  - 7.3.5.4.4 When nursing pups are tattooed, it is advisable to also tattoo the dam. This may prevent her from overreacting to the paste on the pups, which could lead to excessive grooming of the tattoo.
  - 7.3.5.4 5 Use a new lancet for each litter.
  - 7.3.5.4.6 The tattoos should be verified on the day after the procedure. Redo the tattoo if the dots are faint or not visible. If the dots are visible the day after tattooing, they should remain visible for the lifetime of the animal.

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**Figure 8.** The proper restraint technique for tattooing a neonate mouse. The lancet has the green dye on the tip



Figure 9. Toe tattoo on neonate

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Figure 9. Toe application numbering

# 7.3.6 Toe tattooing of adult rats and mice

When a litter of neonates is tattooed, it is suggested that the mother also be tattooed so that the paste on her pups is not foreign to her.

7.3.6.1 Restraint of the animal.

- 7.3.6.1.1 Restrain the animal using either a plastic cone or a Plexiglass restrainer that allows access to the hind feet.
- 7.3.6.1.2 Extend the foot from the restraint device.
- 7.3.6.1.3 Position the foot between the thumb and crook of the index finger to expose the chosen toe, or toes, for tattooing

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7.3.6.1.4 Hold the foot close to the toes so that the toe has a solid surface behind it, preventing the toe from bending away.

7.3.6.1.5 Do not over-twist the leg when positioning the foot. Adults that are restrained may try to withdraw the foot.

#### 7.3.6.2 Prepare the equipment.

- 7.3.6.2.1 Select a 5 mm Goldenrod animal lancet.
- 7.3.6.2.2 Place a small spot of green tattoo paste on a nonporous surface; aluminum foil works well.
- 7.3.6.2.3 Dip only the tip of the lancet in the tattoo paste.
- 67.3.6.2.4 Use only a small amount of paste.
- 7.3.6.3 Apply the tattoo.
  - 7.3.6.3.1 Poke the desired toe with the Goldenrod lancet according to an identification code.
  - 7.3.6.3.2 Puncture the skin to introduce the paste into the skin, leaving a mark.
  - 7.3.6.3.3 Poke the same spot three times to insure proper penetration.
  - 7.3.6.3.4 Avoid poking so deeply that the toe bleeds; this can result in a fainter tattoo.
- 7.3.6.4 Post-tattoo cleanup and care
  - 7.3.6.4.1 Gently blot the spot on an absorbent wipe/towel to remove excess paste.
  - 7.3.6.4.2 Do not attempt to clean excess paste from the foot or body.

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- 7.3.6.4.3 Return the animal to its cage.
- 7.3.6.4.4 Use a new lancet for each animal.
- 7.3.6.4.5 The tattoos should be verified on the day after the procedure. Redo the tattoo if the dots are faint or not visible. If the dots are visible the day after tattooing, they should remain visible for the lifetime of the animal.



Figure 10. Toe tattoo on an adult.

# 7.3.7 Temporary identification

Nontoxic dyes and animal markers can be used for temporary identification of mice.

- 7.3.7.1 Apply unique identifier to base of proximal tail
- 7.3.7.2 If the ID number/mark begins to fade, rewrite it so it is legible.

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7.3.7.3 Check the marking periodically if using this method as the sole identifier for more than a few days.

# 7.3.8 RFID or Micro-Chip transponders.

- 7.3.8.1 Subcutaneous Injection (SC) of implantable microchip.
  - 7.3.8.1.1 Hand-restrain animal or place animal into restraint device (e.g., disposable plastic restrainer such as DecapiCone) if necessary.

NOTE: Isoflurane anesthesia may be used.

- 7.3.8.1.2 The hair may be clipped. Wipe intra-scapular area with alcohol.
- 7.3.8.1.3 Gently grasp the skin in the intra-scapular region, and lift to form a pocket (tent/skin fold).
- 7.3.8.1.4 Insert disposable needle containing transponder (microchip) at the base of the skin fold between the muscle and skin (in cranial direction).
- 7.3.8.1.5 Depress plunger to release transponder (microchip) (Figure 11).



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# Figure 11. Transponder Injection

- 7.3.8.1.6 Remove needle and apply pressure to injection site (to ensure bleeding has stopped).
- 7.3.8.1.7 Dispose of needle into sharps container.
- 7.3.8.1.8 Monitor for potential signs of injury and confirm RFID transponder is functioning via scan.
- 7.3.8.1.9 If there are no signs of injury and transponder is functioning, return the animal to its original cage as applicable.

# 7.3.9 IDENTIFICATION TECHNIQUES TRAINING AND ANESTHESIA

Animals may be lightly anesthetized/chemically retrained with isoflurane. Some prefer this to hand restraint and especially when training new individuals in ID techniques.