

Shock Center Protocol

Protocol: Animal Identification

Date: 2/20/17

Originator: J Neal

Note:

Following is the SOP for Ear Notching.

1. PURPOSE AND SCOPE

Describes the procedure for identifying mice using ear tags (with and without bar codes), ear notches, micro-chip implants and toe clips; applies to all trained personnel identifying mice using these methods.

2. MATERIALS

- 1-2mm punch
- 4 × 4 gauze squares
- 70% ethanol
- Bar coded ear tags(s) and applicator
- 3M™ Precise™ Skin Tape Remover
- Chlorhexidine gluconate
- Ear tag(s) and applicator
- Microchip(s) and applicator
- Sharp micro-dissecting scissors
- Sterile swab
- Tissue adhesive

3. DEFINITIONS AND ACRONYMS

- **Ear notch:** A permanently placed notch or circle placed on a mouse's ear to identify it.
- **Ear tag:** A permanently placed earring placed on a mouse's ear to identify it.
- **IACUC:** Institutional Animal Care and Use Committee
- **PPE:** Personal Protective Equipment
- **Segregating colonies:** Colonies with segregating alleles that require genotyping for breeder identification and confirmation, and identification of specific genotype(s) in the deliverables.
- **Subcutaneous microchip implant:** A subcutaneous microchip implant is a biocompatible microchip placed under the skin that permits the wireless transmission of an identifying number to a remote scanner. Microchip implant(s) are commercially available as a sterile, individually wrapped chip and applicator unit.
- **Toe clip:** A minor surgical procedure that permanently impairs the mouse's ability to grip surfaces and groom itself.

4. ANIMAL WELFARE KEY POINTS

- 4.1 Ear notchers must open and close easily, have room between the punch and the hole for the ear and be rust free.
- 4.2 Disinfect ear notchers with 70% ethanol and dried off between animals.
- 4.3 Disinfect the ear tag (both bar coded and traditional) with 70% ethanol after proper placement in the applicator during ear tag procedures.
- 4.4 Mice must be 21 days or older to be implanted with a microchip.
- 4.5 Use anesthesia during microchip implantation if the gauge of the needle is larger than 18g.

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- 4.6 Separate toes if toe clipping mice 4 days or younger to ensure that only the desired toes are removed.
- 4.7 Use micro-scissors to toe clip mice that are 7 days or younger.
- 4.8 Special permission from the IACUC is required, as well as anesthesia, to toe clip mice that are 8 days or older.

5. SAFETY KEY POINTS

- 5.1 Use caution when handling animals. A mouse appearing docile may still bite if given the opportunity.
- 5.2 Appropriate PPE per work area requirements must be worn at all times.
- 5.3 Allow scissors sterilized in a glass bead sterilizer to cool for 90 seconds prior to use.
- 5.4 Dispose of bar coded ear tags in a sharps container.

6. QUALITY KEY POINTS

- 6.1 Ensure that the identification is clear. If there is any ambiguity, document on cage card accordingly.
- 6.2 Stainless steel instruments are preferred.
- 6.3 Refer to [Table 1](#) for mouse identification methods and standards.
- 6.4 Ear Notching

METHOD	STANDARD
Ear notch	Not to be used on mice < 14 days of age
Ear tag	Not to be used on mice < 14 days of age
Micro-chip implant	Not to be used on mice < 21 days of age
Toe clip*	Used in neonates ≤ 7 days of age. Justification required by IACUC.
* Female mice from wild-derived strains or females with first litters from inbred strains may abandon or cannibalize pups if neonatal litters are handled.	

Table 1

- 6.4.1 Test ear notchers prior to its use by making sure the ear notcher opens and closes freely. **NOTE:** Discard notchers that are hard to close or do not open immediately when pressure is released.
- 6.4.2 Discard ear notchers that become rusty from alcohol disinfection.
- 6.4.3 A small 1-2mm punch is used to produce a small hole or notch near the end of the mouse's ear(s). Refer to _____ and _____ for commonly used numerical codes.
- 6.5 Subcutaneous Microchip Implant:
 - 6.5.1 Handle the microchip implant applicator aseptically. Refer to _____
 - 6.5.2 Mice may need to be anesthetized. For mice 21 days or older, anesthesia is required if the needle is larger than 18g. **NOTE:** Microchip implants are not for mice under 21 days of age.
 - 6.5.3 Use each microchip implant applicator once.
 - 6.5.4 Scan the microchip to ensure functionality after implantation.
- 6.6 Toe Clip:
 - 6.6.1 Toe clipping mice older than 7 days is not recommended and must be justified on the animal protocol form.
 - 6.6.2 Anesthesia is required to toe clip mice who are 8 days and older.

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- 6.6.3 Place adult mice into a clean cage. Once toe clipped and bleeding is controlled, place the pups into the clean cage with the adult mice.
- 6.6.4 Neonatal mice one week of age (7 days or younger) can be toe clipped without anesthesia.
- 6.6.5 To reduce the risk of dam abandoning or cannibalizing the litter:
 - Transfer dam to another cage while pups are handled
 - Control bleeding
- 6.7 Ear Tag (Bar Code):
 - 6.7.1 Dip the tip of the applicator and remover in 70% ethanol and allow to dry prior to use and between mice.
 - 6.7.2 No more than two attempts should be made to place an ear tag in a mouse.
 - 6.7.3 Post procedure: The pinna may appear slightly red for 3-4 days where the prongs pass through the pinna and over time the tissue surrounding the prongs may become thickened. Consult with the veterinary staff if you see any changes other than these.
 - 6.7.4 Individuals must be trained by a Comparative Medicine & Quality approved trainer before applying or removing ear tags.
 - 6.7.5 Many ear tagged mice for breeder confirmation may still be breeding or have litters. Remove ear tags only from male breeders or female breeders that are not obviously pregnant or nursing a litter.
 - 6.7.6 Immunosuppressed mice: Decontaminate the ear prior to and after the insertion of an ear tag

7. PROCEDURE

7.1 Ear Notching:

- 7.1.1 Soak a 4x4 gauze pad with 70% ethanol. Rub the gauze pad on the ear notcher to disinfect it and let dry. **NOTE:** Placing the ear notcher in a holder that contains 70% ethanol is also acceptable.
- 7.1.2 Restrain the mouse per
- 7.1.3 Using a small (1-2mm) punch, create circle(s) or notches(s) in the appropriate ear (refer to or for examples). Place a circle 1-2mm from the margin of the ear of post weanling mice (over 4 weeks of age), 1mm from margin for younger animals. **NOTE:** Circles placed too close to the margin of the ear may tear and become notches.
- 7.1.4 Return the mouse to its cage.

7.2 Ear Tag:

- 7.2.1 Restrain the mouse per
- 7.2.2 Insert the ear tag into the applicator.
- 7.2.3 Dip the tip of the applicator and ear tag in 70% ethanol and let dry, or dry with a paper towel.
- 7.2.4 Restrain the mouse per
- 7.2.5 Position the applicator so that the pointed end of ear tag is within the area shown by the dashed oval (see [Figure 1](#)) and the curved end of tag is on lower end of the ear.

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- 7.2.6 Position the ear tag applicator so any visible blood vessels are avoided and so that the tag will be far enough into the ear so it doesn't dangle. **NOTE:** The tag should not be so tight to the ear that there isn't space for growth of the ear (see [Figure 1](#) for proper placement).
 - 7.2.7 Squeeze the ear tag applicator so that the tag is pushed far enough through the ear to bend the tip of the tag to secure it. Tag should appear "U" shaped, not "V" shaped when properly applied.
 - 7.2.8 Return the mouse to its cage.
- 7.3 **Ear Tag (Bar Code):**
- 7.3.1 Application procedure:
 - 7.3.1-A Immunosuppressed mice only:
 - 7.3.1-A1 Dip a sterile mini swab into a container of 2% chlorhexidine gluconate.
 - 7.3.1-A2 Restrain the mouse per _____ then swab the inside of the ear where the prongs of the ear tag will pierce the ear.
 - 7.3.1-A3 Discard the swab.
 - 7.3.1-B Load the sterilized cassette containing the bar code into the applicator.
 - 7.3.1-C Restrain the mouse per _____ then slide the ear into the guide on the applicator.
 - 7.3.1-D Position the applicator so the two prongs are placed through the cartilage near the periphery of the ear at the apex of the pinna. **NOTE:** At least one prong must be inserted completely through the thicker cartilage and both prongs must be folded over on the back of the ear and touching (similar to a staple in paper). Refer to [Figure 2](#) for proper placement.
 - 7.3.1-E Carefully slide the applicator away from the ear and check the tag to ensure proper placement of the tag. If the tag is improperly placed (e.g., fold of tissue trapped beneath the tag), remove the ear tag and replace it with a new one. No more than two attempts should be made to place an ear tag in a mouse. Return the mouse to its cage.
 - 7.3.1-F Check mice daily for 3-4 days. Contact veterinary staff if any concerns are noted.
 - 7.3.2 Removal:
 - 7.3.2-A Immunosuppressed mice:
 - 7.3.2-A1 Dip a sterile mini swab into a container of 2% chlorhexidine gluconate.
 - 7.3.2-A2 Restrain the mouse per _____ then swab the inside and outside of the ear where the prongs of the ear tag have pierced the ear.
 - 7.3.2-A3 Discard the swab.
 - 7.3.2-B Dip the tip of the remover in 70% ethanol and allow it to dry.

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- 7.3.2-C Manually restrain the mouse and position the remover such that the lower jaw (has two teeth) of the remover is placed between the back of the ear tag and the ear and the upper jaw (has one tooth) is placed on the face of the ear tag.
- 7.3.2-D Squeeze the handles of the remover to slightly bend the ear tag. This opens the prongs of the ear tag on the back of the ear. Do not excessively bend the face of the tag as this over extends the prongs and results in injury to the ear upon removal.
- 7.3.2-E Once the prongs of the tag are open, if necessary, continue to grasp the ear tag remover and rock the remover to one side to pull one prong of the tag out of the ear and then rock the remover to the opposite side to remove the second prong from the ear.
- 7.3.2-F Discard the tag in a sharps container.
- 7.3.2-G Examine the mouse to see if the remover affected the ear. Compile observations in the "Ear Tag" folder on the "Production Projects" shared drive.
- 7.3.2-H Return the untagged mouse to the holding box. Observe mice with untagged ears daily (up to a week) to ensure each ear sustained no obvious damage or infection. Initially, the untagged ear may appear slightly reddened. During this observation period, contact Clinical Medicine so they can observe the untagged animals. **NOTE:** Contact Clinical Medicine if a mouse's ear appears infected, seriously damaged or if the mouse appears to be in distress.
- 7.3.2-I After a week's observation, mice with unaffected ears are considered normal and will continue in breeding or be discarded as retired breeders.
- 7.3.2-J Once ear tag removal has been completed for the day, submerge the remover in 70% ethanol and allow it to air dry. Discard any remover that malfunctions.

7.4 Subcutaneous Microchip Implant:

- 7.4.1 Anesthetize the mouse if the needle is larger than 18g or if mouse is younger than 21 days of age. Refer to
- 7.4.2 Pinch skin along the midline of the mouse, tenting the skin
- 7.4.3 Insert the microchip applicator needle under the skin directed cranially on the dorsal midline 1/2" - 1" caudal to the shoulder blades. **NOTE:** The microchip implant may be placed in another location with permission from veterinary staff.
- 7.4.4 Compress the plunger to release the microchip then remove the needle from the mouse.
- 7.4.5 Seal the puncture wound with a drop of tissue adhesive (optional).
- 7.4.6 Dispose of the applicator in an approved sharps container.
- 7.4.7 Scan the microchip to ensure functionality and then return the mouse to its cage.

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7.5 Toe Clip:

- 7.5.1 Sterilize the sharp micro-dissecting scissors in a glass bead sterilizer. Micro-dissecting scissors must be sterilized between litters and at the start of work. **NOTE:** 70% ethanol is not a sterilant.
- 7.5.2 Restrain the mouse by cradling the pup in hand (non-dominant) and extending the paw to index finger to hold in place during the procedure.
- 7.5.3 Soak a 4×4 gauze pad with 70% ethanol. Rub the gauze pad on the toes to be amputated. **NOTE:** If the toes are still fused, use the micro-dissecting scissors to separate the toes to ensure correct excision of the digit.
- 7.5.4 Excise the third phalanx of the appropriate toes using the sterile scissors.
- 7.5.5 Re-sterilize the tips of the scissors in a glass bead sterilizer or wipe them with 70% ethanol (for pups of the same litter only). **NOTE:** Place the scissors on a sterile surface if being used on more than one mouse.
- 7.5.6 Stop the bleeding with a sterile swab and direct pressure as necessary.
- 7.5.7 Return the mouse to its cage.

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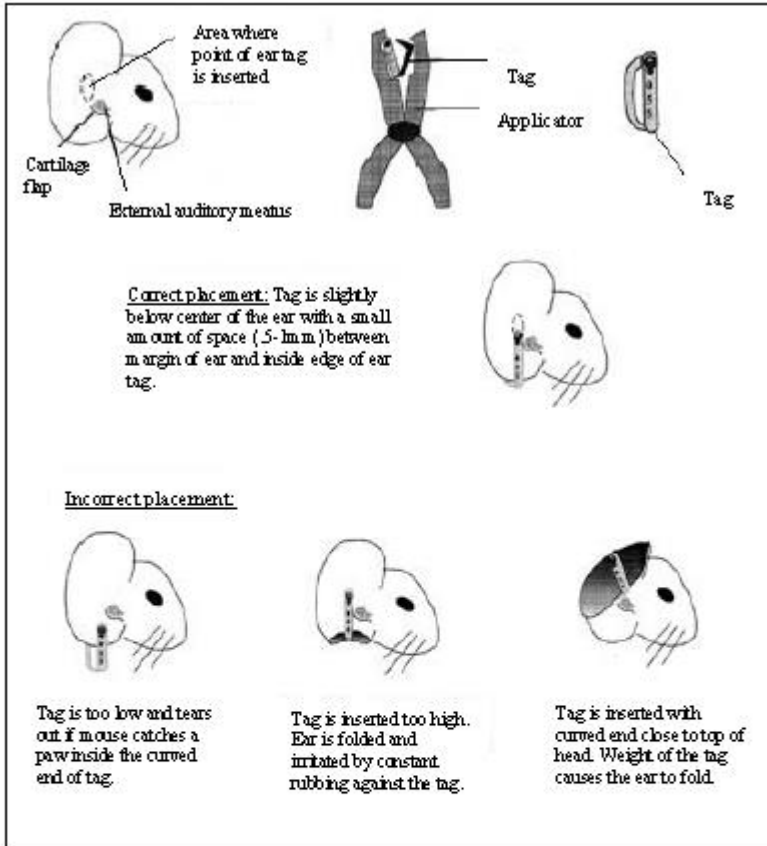


Figure 1

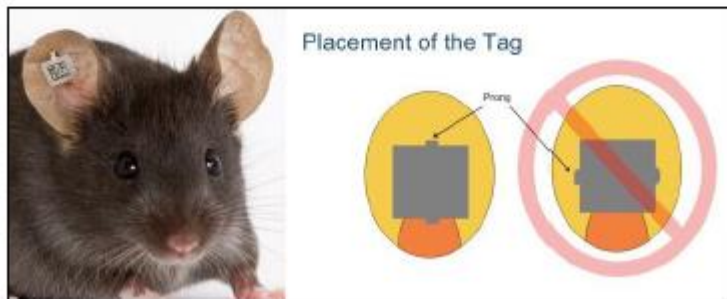


Figure 2