

 <p>University at Buffalo The State University of New York Lab Animal Facilities</p>	<p>STANDARD OPERATING PROCEDURE</p> <p>Footpad Injections in Mice and Rats</p>	<p>Quality Form</p>
		<p>SOP # 2.A.19 Revision: 01 Last Reviewed: 5/7/2025</p>

1.0 **PURPOSE:**

To describe a standardized procedure for footpad injections in mice and rats.

2.0 **SCOPE:**

This applies to all personnel (LAF and Research Staff) involved with footpad injections in mice and rats.

3.0 **DEFINITIONS:**

3.1 **Footpads:** Areas over the plantar aspect (bottom) of the front and hind paws (feet) of mice and rats designed for cushioning of the feet.

3.2 **Footpad injections:** Combined intradermal and subcutaneous injections into footpads used for scientifically justified experimental purposes such as immunization or models of inflammation, arthritis or pain in rats or mice.

4.0 **PROCEDURES:**

4.1 **IACUC Approval and Scientific Justification:**

4.1.1 Footpad injections must be included as a **substance administration procedure** in the approved IACUC protocol. In general, the procedure must include the following details:

- a. General anesthesia is highly recommended, and the details should be given in the procedure.
- b. If manual restraint alone is proposed, provide details of restraint and the rationale for not using general anesthesia while giving injections.
- c. Substance administered, dose (mg/kg), volume (uL), needle size and frequency and duration of injections (how often, for how long).
 - i. Maximum injection volume for **adult mice: 50 uL**
 - ii. Maximum injection volume for **adult rats: 100 uL**
 - iii. Recommended needle size for **mice: 29-30 g**
 - iv. Recommended needle size for **rats: 25 g or smaller**
- d. Only a **single hind paw** should be used for injection.
 - i. Front paws are required to access and manipulate food. **Front paws cannot be injected!**
 - ii. Hind feet are the major weight bearing structures, so **ONLY ONE hind paw may be injected (never both)!**
- e. Details of how rodents will be **monitored** after the injection (how often, for what duration and by what parameters) should be explained.
- f. **Systemic analgesic** (pain relieving drug, route, dose and duration) to be given for at least one day. Scientific justification must be provided if analgesic is not given.
- g. **Humane endpoints** must be included (ulcerations, bleeding feet, self-mutilation at injection site, impaired movement).

- 4.1.2 Per IACUC Policy, animals undergoing footpad injections should be assigned a **pain category of D or E**, depending on expectations and proposed alleviation of pain and distress.
- 4.1.3 An **alternatives search** must be properly conducted.
 - a. When used for immunization purposes, the rationale for the use of footpad injections instead of hock injections must be explained, as lymphatic drainage from the hock enters the same nodes as drainage from the footpads. Hindlimb injections into the hock (lateral tarsal region) are less painful and distressing and must be considered.

4.2 Procedure for Footpad Injections:

- 4.2.1 Prepare injectate material, syringes, needles, anesthesia materials and other necessary drugs or supplies and work area prior to working with animals.
- 4.2.2 Anesthetize the mouse or rat as per protocol description.
- 4.2.3 If (instead of general anesthesia), manual restraint of unanesthetized rodents is used, restrain as per protocol. Be aware that the foot will retract during injection making it more technically challenging.
- 4.2.4 Briefly wipe the plantar aspect of the hind foot to be injected with 70% alcohol to remove debris prior to injection.
- 4.2.5 Pick up the prepared syringe and needle. With the bevel facing up, push the needle into the skin past the bevel before injecting into the foot pad of one hind paw. After injection, withdraw the needle slowly while keeping pressure on the injection site. Continue firm pressure for several seconds following the injection, then check for leakage of injected material. If leakage occurs reapply pressure until it stops.
- 4.2.6 If anesthesia has been used place the animal in a recovery cage with a heat source until the animal can right itself. When the animal is mobile and alert it can be returned to its home cage.
- 4.2.7 Ensure systemic analgesic is administered and documented as per protocol.

4.3 Post Injection Monitoring and Care:

- 4.3.1 **Rodent Anesthesia &/Or Surgery Reports** must be submitted following footpad injections, even if anesthesia is not used. This cues LAF Veterinary Technicians to monitor the animal for two days.
- 4.3.2 Animals should be housed with **deep, soft bedding** following injections. Corn cob bedding is not soft, so shavings, sani-chip or other soft bedding should be used.
- 4.3.3 Providing **wet or dry chow in a dish on the cage floor** makes food more accessible for animals that are not standing comfortably on their feet and should be considered.
- 4.3.4 Animals should be **monitored daily** by Research Staff until all lesions have healed and there is no sign of pain or discomfort.

References:

1. Kamala, T. (2007). “Hock immunization: a humane alternative to mouse footpad injections.” *Journal of Immunological Methods*, 328(1-2), 204–214.
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2464360/>
2. NIH Animal Research Advisory Committee (ARAC) (2022). “Guidelines for the Use of Adjuvants in Research.” <https://oacu.oir.nih.gov/animal-research-advisory-committee-arac-guidelines>
3. [Video: Compound Administration in Rodents- Specialized Injections](#)

Approvals:

Name	Title	Date of Approval
Jennifer Peirick, DVM	LAF Director/Attending Veterinarian	5/7/2025
Jolie McCutcheon, DVM	LAF Clinical Veterinarian	
Amy Snyder, DVM	LAF Clinical Veterinarian	
Institutional Animal Care and Use Committee (IACUC)		

Change History:

Revision #	Description of Change	Effective Date
01	Reformatted and separated IACUC approval/required scientific justification from procedural details. Added information on required pain category classification and alternative search requirements. Added references including JOVE video.	5/7/2025