



CM SOP #210 – Rodent Injection Technique

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I. Purpose & Scope

The purpose of this Standard Operating Procedure (SOP) is to establish standardized methods for the administration of injectable substances to mice and rats housed in Comparative Medicine (CM) facilities. This SOP provides guidance on safe and humane injection techniques using intraperitoneal (IP), subcutaneous (SC), and intravenous (IV) routes.

These procedures are intended to:

- Ensure humane handling and administration of injectable substances
- Minimize pain, distress, and tissue trauma
- Reduce risk of infection or injection-related complications
- Promote consistency in technique across personnel and campuses
- Support compliance with institutional animal care and use policies

This SOP applies to all personnel administering injections to rodents in CM-managed facilities under approved IACUC protocols.

II. Roles & Responsibilities

Comparative Medicine Staff

- Performing injections according to approved protocols and this SOP
- Using appropriate restraint and aseptic technique
- Monitoring animals following injection
- Documenting procedures and observations accurately
- Reporting complications or adverse events immediately
- *Note: Veterinary staff serve as technical resources for injection technique, troubleshooting, and animal welfare concerns.*

Research Staff, Students, Visitors

- Performing injections only after documented training and competency
- Following approved IACUC protocol procedures
- Using appropriate volumes, needle sizes, and techniques
- Monitoring animals after injection
- Maintaining accurate experimental records
- Research personnel must consult veterinary staff before:
 - Administering unusually large volumes
 - Using unfamiliar compounds
 - Performing technically difficult injections
 - Deviating from standard technique

CM Management

- Ensuring personnel receive appropriate training and competency assessment
- Maintaining documentation of training records



- Ensuring injection equipment and supplies are available
- Monitoring compliance with this SOP

CM Director and AV

- Providing veterinary oversight of injection practices
- Establishing acceptable injection volumes and techniques
- Serving as a resource for injection-related questions
- Determining appropriate response to complications or adverse events
- Approving exceptions to standard injection parameters

III. General Notes & Definitions

- All injectable substances must be:
 - Sterile
 - Prepared using aseptic technique
 - Administered using sterile equipment
- Maximum injection volumes and needle sizes must be followed to minimize tissue trauma and discomfort.
- If multiple drugs are combined in a single injection, compatibility must be verified prior to administration.
- Anesthesia is recommended when administering hazardous substances or when procedures may cause significant distress.
- Proper restraint is essential to:
 - Prevent injury to personnel
 - Prevent injury to animals
 - Ensure accurate delivery of substances

Table 1: Maximum injection volume

| Route | Mouse | | Rat | |
|----------------------|-------------|-------------|-------------|-------------|
| | Volume (ml) | Needle size | Volume (ml) | Needle size |
| Intraperitoneal (IP) | 2.0 | ≤25G | 10ml/kg | 26G |
| Subcutaneous (SC) | 2.0 | ≤25G | 5ml/kg | 26G |
| Intravenous (IV) | 0.2* | ≤25G | 5 | 23G |

*Volumes exceeding these recommendations must be approved by the veterinarian prior to administration.

IV. Materials & Equipment

- Sterile syringes
- Sterile needles (appropriate gauge)
- Sterile injectable solutions
- Alcohol swabs (70%)
- Gauze
- Animal restraint device (as needed)
- Heating device or warm water for tail vein dilation
- Sharps container
- Personal protective equipment
- Experimental health monitoring sheets
- Laboratory recordbook or electronic documentation system



V. Procedure

A. General Injection Preparation

1. Verify that the procedure is approved in the IACUC protocol.
2. Weigh the animal when required to determine appropriate dosing volume.
3. Calculate the correct dose and confirm calculation accuracy.
4. Prepare the syringe and needle using aseptic technique.
5. Remove all air bubbles from the syringe.
6. Confirm correct drug and concentration before administration.
7. Assemble all required supplies before restraining the animal.
8. Ensure the animal is properly restrained according to FAUSOP 209.

B. Intraperitoneal (IP) Injection

1. Weigh the animal and confirm maximum allowable injection volume.
2. Prepare the syringe with the calculated dose and expel any bubbles.
3. Restrain the animal by grasping the loose skin at the scruff of the neck. Pick the animal up and position the animal so that the abdomen is facing up, supporting the back and hind end.
4. Wipe the lower right or left abdominal quadrant with 70% alcohol and allow the site to dry.
5. Tilt the animal slightly so the head is angled downward.
6. Insert the needle bevel up at approximately a 30-degree angle into the lower abdominal quadrant.
7. Avoid the cecum, which is commonly located in the upper portion of the lower left quadrant.
8. Gently retract the plunger to check for aspiration. If fluid or blood is aspirated, withdraw the needle and prepare a new sterile syringe.
9. Slowly inject the solution.
10. Remove the needle carefully and place entire syringe and needle directly into a sharps container without recapping the needle.
11. Return the animal to its cage.
12. Observe the animal for signs of distress or complications.



C. Subcutaneous (SC) Injection

1. Prepare the syringe with the calculated dose.



2. Restrain the animal by grasping the loose skin at the scruff of the neck with the thumb and forefinger to immobilize the head and torso. Place the animal on a flat surface to stabilize the body.
3. Lift the skin over the injection site to create a tent.
4. Common injection sites include:
 - a) Nape of the neck
 - b) Flank
 - c) Rump
5. Insert the needle bevel up into the skin tent.
6. Gently retract the plunger. If blood or fluid is observed, remove the needle and reposition.
7. Slowly inject the solution.
8. Remove the needle carefully and place entire syringe and needle directly into a sharps container without recapping the needle.
9. If administering large volumes, distribute the volume across at least two injection sites.
 - a) Mouse volume approaches 2 mL
 - b) Rat volume approaches 5 mL
10. Return the animal to its cage.
11. Monitor the animal for signs of leakage, swelling, or distress.



D. Intravenous (IV) Injection — Tail Vein

1. Prepare the syringe with the calculated dose.
2. Warm the tail to dilate blood vessels using one of the following methods:
 - a) Place animals under a heat lamp for no more than five minutes
 - b) Rub the tail gently using gauze soaked in warm water
 - c) Use warming devices integrated into restraint equipment
 - d) Place rat tails briefly in warm water
3. Place the animal into an appropriately sized restraint device.
4. Extend the tail securely.
5. Wipe the tail with 70% alcohol and allow to dry.
6. Identify a lateral tail vein.
7. Insert the needle bevel up at a shallow angle near the distal end of the tail.
8. Advance the needle approximately 1 millimeter and slowly depress the plunger. If resistance is encountered:
 - a) Stop injection
 - b) Remove the needle
9. Apply gentle pressure to the site
10. Reattempt injection slightly proximal to the previous site



11. After injection, remove the needle and place entire syringe and needle directly into a sharps container without recapping the needle.
12. Apply gentle pressure to the injection site for approximately 10 seconds.
13. Confirm bleeding has stopped.
14. Return the animal to its cage.



E. Post-Procedural Monitoring

1. Observe animals following injection and monitor for:
 - a) Bleeding from injection site
 - b) Labored breathing
 - c) Discharge from nose or mouth
 - d) Ruffled fur
 - e) Hunched posture
 - f) Lethargy
 - g) Minimal responsiveness
 - h) Excessive licking or biting at injection site
2. Animals receiving large injection volumes may appear sluggish for up to 20 minutes
3. Continuous observation is required during this period.
4. If abnormal signs persist beyond approximately 30 minutes, veterinary staff must be contacted immediately.
5. Emergency euthanasia may be required based on veterinary guidance.

F. Documentation

1. Personnel must record:
 - a) Animal identification
 - b) Injection route
 - c) Drug name
 - d) Volume administered
 - e) Date and time
 - f) Observations following injection
2. Documentation must be entered into:



- a) Experimental logbooks
 - b) Progress notes
 - c) Health monitoring records
 - d) Diagnostic records
3. Syringes prepared in advance must be clearly labeled to prevent dosing errors.

VI. Health & Safety

- Personnel handling rodents may be exposed to:
 - Animal bites or scratches
 - Sharps injuries
 - Chemical hazards
 - Biological materials
- To reduce risk, personnel must:
 - Wear appropriate personal protective equipment as required by *CM SOP 101: Protective Clothing and Personal Hygiene*
 - Use proper restraint techniques
 - Dispose of sharps immediately after use
 - Avoid overfilling sharps containers
 - Wash hands before and after handling animals
- In the event of a bite or scratch:
 - Wash the wound immediately with soap and water.
 - Apply pressure to control bleeding.
 - Notify the supervisor.
 - Follow procedures in:
 - *CM SOP 008: Reporting Accidents, Injuries, and Illnesses.*

VII. References & Attachments

- Rodent Handling and Restraint — FAUSOP 209
- AALAS Laboratory Animal Technician Training Manual
- Turner PV et al. Administration of Substances to Laboratory Animals. *JAALAS*.
- Hedrich H. Routes of Administration — *The Laboratory Mouse*
- *Guide for the Care and Use of Laboratory Animals*

VIII. Revision History

| Revision Date | Revision Number | Summary of Changes |
|---------------|-----------------|-----------------------------------------------------------------------------------------------------------------------------|
| 03/17/2024 | 2 | Clarification of bevel up; right or left lower quadrant for IP injections; adding anesthesia and to monitor post injection. |
| 03/27/2026 | 3 | Updated format, made ADA compliant, streamlined language |
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