



Subject: Administration and injection of substances in mice	Number: AD-2
Scope: A directive from the Direction des services vétérinaires to users and staff of Université Laval animal facilities (campus and affiliated research centres).	
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Revised by Anne-Marie Catudal <i>Clinical Veterinarian, Direction des services vétérinaires</i>	Date: June 13 th , 2022
Purpose: Describe the procedures for administering and injecting substances in mice.	Version 8

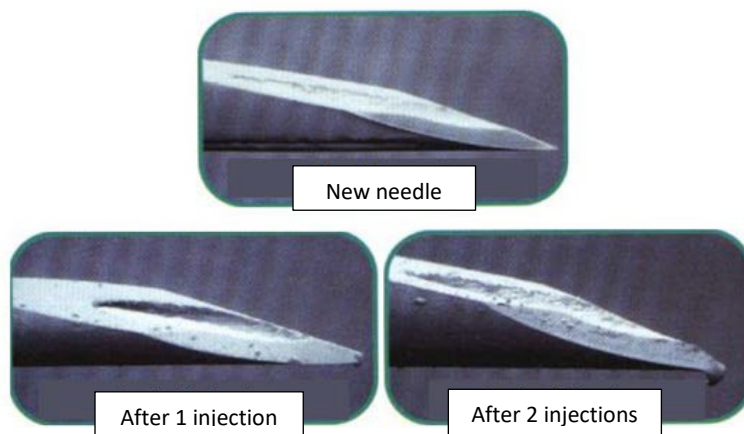
General Considerations

- Before starting, check the animal's identification and observe its general condition. Note any anomalies.
- Weigh the animal so that an adequate dose can be calculated before the injection. This way, each animal receives an equivalent dose (mg/kg), which facilitates the analysis of the results and ensures an overdose or underdose is not administered.
- Any substance administered parenterally must be sterile and should be isotonic with a physiological pH. If not sterile, it should be filtered through a 0.22 µm filter.
- It is recommended that you warm substances on a heating mat or in a heating water bath to avoid lowering the body temperature during administration. This is especially true when injecting large volumes. Bring substances to at least room temperature before administering them.
- Limit the number of subcutaneous injections per day to three.
- Limit the number of injections per day to two for the intramuscular, intravenous, intraperitoneal, intradermal, and intranasal routes.
- Always clean with alcohol the tip of the container in which the product to be administered is stored before drawing the injection dose.
- Fill the syringe to the correct volume and remove any air bubbles.
- Limit the number of failed attempts to three, after which you must seek help from someone with experience.

Note: Limit the number of failed attempts to two for intraosseous injection.

- If the administered product leaks from the site during or after administration, note the loss so as to ensure it is taken into consideration when analyzing the results.

- If you inject into the wrong site (e.g., during an IV injection, an amount is injected SC), note this for consideration when analyzing the results.
- Immediately dispose of syringes and needles, without putting on the caps, into a biohazard sharps container.
- Avoid the intramuscular route in mice due to the heightened risk of pain and necrosis given their small muscle mass. This route must be justified before use.
- Change the needle between each animal to avoid cross-contamination. In addition, small gauge needles wear out very quickly (after a single insertion into the skin) and thus become less effective at piercing the skin without the risk of injury to the animal (see the image below).



Source: http://www.caninediabetes.org/pdoraq/bd_needle.htm

- In rare cases, the same needle may be used exclusively for subcutaneous injections administered to several animals housed in the same cage. Note that zero dead space syringes and needles exist and can be used.

Definitions

- **Gavage**: introducing a substance into the stomach through a feeding tube
- **ID**: intradermal
- **IM**: intramuscular
- **IN**: intranasal
- **IO**: intraosseous
- **IP**: intraperitoneal
- Isotonic: a solution containing the same concentration of solutes as blood
- **IT**: intratracheal
- **ITh**: intrathecal
- **IV**: intravenous
- Parenteral: administering substances outside the gastrointestinal tract
- **PO**: per os - voluntary oral administration
- **SC**: subcutaneous
- **SO**: retro-orbital sinus
- **TOP**: topical

Procedures

- Select the appropriate needle size (G) for the injection site:

Gavage	ID	IM	IP	IO	IV	SC	SO	ITh
18-22	29-30	27	25-27	28-30	25-27	25-27	27-30	27-30

- Follow the volume limits in the table below when administering a substance to an adult animal:

	Gavage (ml)	ID (ml/site)	IM (ml/site)	IN (µl)	IP (ml)	IO (µl) ^a	IV bolus (ml) ^b	IV slow injection (ml) ^c	SC (ml) ^d	SO (µl/eye)	Ith (µl)	IT (ul) ^a
Ideal volume	0.25	0.05	0.05	35-50	0.50	10	0.12	0.62	0.25	150	10	50

^a This is the maximum injection volume, even if the mouse weighs more than 25 g.

^b Product is administered rapidly, within 30 seconds to 1 minute.

^c Product is administered over at least a 5-minute period.

^d The volume of fluid injected subcutaneously during an anesthesia procedure or following a blood sampling differs. Fluids will then be administered following SOP A-1 during anesthesia and following SOP P-16 during blood sampling.

Gavage

- Select the appropriate size gavage needle (stainless steel or plastic) for the animal's size. Determine the length required by measuring from the animal's mouth to the furthest ribs.
- Hold the mouse so that its head is immobile during the procedure.
- Introduce the needle near the incisors at a 45° angle, inserting it by carefully advancing along the palate. Slowly descend without forcing.
- Administer the volume and carefully withdraw the needle.



Proper restraint for gavage

Intradermal injection (ID)

- Anesthetize the animal according to SOP A-1.
- Shave the injection site.
- Clean the site with 0.05% chlorhexidine.
- Insert the needle parallel to the skin, bevel up.
- Only insert the bevel. You will feel resistance when the needle is inserted.
- Inject the required volume (resistance will be felt on the plunger) and withdraw the needle. A small bump will appear where the liquid was injected.

Intramuscular injection (IM)

* Since this method involves risks to the animal's health, it should not be used if other practical methods are available for the experiment and the species. Its use must be justified to the animal protection committee.

- Immobilize the left or right hind leg by pinching the skin in front of the knee (a restraint device can be used if needed).

- Insert a needle at a 30° angle caudally, bevel up, into the thigh muscle. Be careful not to touch the sciatic nerve.
- Aspirate and check for blood (if blood appears, remove the needle and repeat).
- Inject the required volume.
- Release the plunger and withdraw the needle.

Intranasal administration (IN)

- Use a micropipette to draw the exact volume of product to administer.
- Holding the animal's head, bring the tip of the micropipette to one nostril.
- Carefully deliver the dose onto one nostril (not inside, to avoid injuring the animal).

Note: If the administration is performed under sedation, place the animal in a clean induction chamber connected to the anesthesia machine. Adjust the isoflurane to 3–4% and the oxygen flow rate to 0.8–1.5L/min. Administer once the sedation level adequate. Repeat the procedure if the animal regains consciousness before you finish administering.

Intraosseous injection (IO)

- Weigh the animal.
- Since intraosseous injection into the femur is painful, administer buprenorphine SR (0.75 mg/kg) SC at least 30 minutes prior to the procedure.
- Anesthetize the mouse by isoflurane inhalation following SOP A-1 Analgesia and Anesthesia for Rodents and apply ophthalmic ointment. Have a heat source ready.
- Place the animal in the supine position.
- Shave or pluck the knee area and remove the hair with dry gauze.
- Check that there is no withdrawal reflex.
- Alternating, apply 70% isopropyl alcohol/0.5% chlorhexidine three times to the shaved area, always finishing with chlorhexidine. If using Baxedin Preop® (a mixture of 70% isopropyl alcohol and 0.5% chlorhexidine), apply it only three times. Using one gauze or cotton swab per application, apply from the center of the site to the periphery. (See SOP C-1 Aseptic Surgery in Rodents.)
- Immobilize the leg by folding it (keep it folded with your index finger on the femur and your thumb on the leg). Slightly tip the knee towards you.

- Locate the patellar tendon. Insert a 27G x 1/2" needle into the head of the femur, which sits slightly above the ligament.
- Using rotational movements, insert the entire needle into the femur.
- Check that the needle is positioned correctly. When rotating the needle, the leg should follow the movement. When the leg is folded and unfolded, the needle should follow the movement of the femur.
- Gently withdraw the needle, blotting any bleeding as you go with sterile cotton swabs. As you withdraw the needle completely, keep an eye on the insertion site created.
- Insert a smaller gauge needle mounted on the syringe into the insertion site.
- Check for correct positioning by folding and unfolding the leg.
- Slightly withdraw the needle to create space; unfold the leg and inject the required volume.
- Monitor the animal in the cage until it is fully awake.

Intraperitoneal injection (IP)

- Hold the animal with its head down (so that gravity makes the organs descend).
- Insert a needle at a 45° angle, bevel up, into the lower right quadrant of the abdomen.
- Aspirate and check for blood or urine (if blood or urine appears, remove the needle and repeat).
- Inject the required volume.
- Release the plunger and withdraw the needle.



Intraperitoneal injection

Intrathecal injection (ITh)

- Anesthetize the mouse by isoflurane inhalation following SOP A-1 Analgesia and Anesthesia for Rodents and apply ophthalmic ointment. Have a heat source ready.
- Shave the back of the animal from the tail base to the first ribs. Remove hair with a chlorhexidine 0.05% humid gauze.

Note: The intrathecal injection is done between lumbar vertebrae 5 (L5) and 6 (L6) to minimize the risks of damage to the spine.

- Place the animal in the prone position and tuck its hind limbs under the abdomen to form an arch with the back. The use of a tube (e.g. Falcon 15ml tube) placed under the animal can facilitate positioning.
- Alternating, apply 70% isopropyl alcohol/0.5% chlorhexidine three times to the shaved area, always ending with chlorhexidine. If using Baxedin Preop® (a mixture of 70% isopropyl alcohol and 0.5% chlorhexidine), apply only three times. Using one gauze pad or cotton swab per application, apply from the center of the site to the periphery
- Put on a sterile glove and locate the spinous process of the L6 and L5 vertebrae with the tip of a finger or nail.
- Locate the L6 vertebra, which should be the most prominent. Hold the spine in place with gentle pressure.

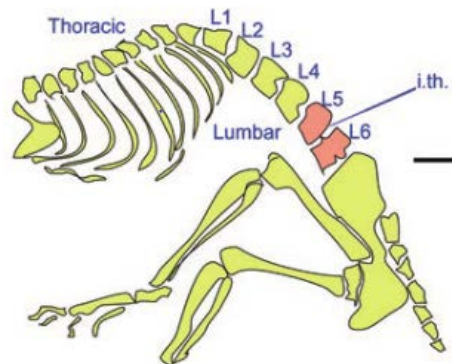


Image tirée de: Huang, Nan-Xing & Shen, Yun-An & Mei, Feng. (2018). Modeling CNS Myelination Using Micropillar Arrays: Methods and Protocols. 10.1007/978-1-4939-7862-5_13

- At a 90° angle, gently insert the needle between the blade of the L6 and L5 vertebrae. Observe a tail flick and resistance to confirm the correct placement of the needle.
- Secure the position of the syringe with one hand, gently pull the plunger with the other hand to check for blood and inject gently. Wait a few seconds before taking the needle out to avoid backflow.
- Wake the animal following SOP A-1.

Intratracheal injection (IT)

* The oxygenation of the animal must be checked throughout the procedure (respiratory rate and color of the mucous membranes). If necessary, the catheter should be temporarily removed.

Notes: Intratracheal injection should always be slow to avoid reflux of the product into the esophagus.

To ensure better oxygenation after the injection, it is recommended to administer air to the lungs via the catheter before its removal at a volume of 100ul for a mouse of 8 weeks and older. For mice less than 8 weeks old, follow the veterinarian's instructions.

- To optimize the diffusion of the product in both lungs, to avoid reflux towards the esophagus and to reduce the risks for the animal, place the mouse in supine position at an angle of 45°.
- Anesthetize the animal by inhaling isoflurane according to SOP A-1 Analgesia and anesthesia of rodents and apply ophthalmic ointment.

Direct injection (standard syringe)

- Fill the syringe and remove air bubbles.
- Intubate the animal with a catheter of appropriate length and caliber and check the correct positioning according to SOP A-1.
- Connect the syringe to the catheter and inject the required amount slowly.
- Empty the contents remaining in the catheter by injecting air with another syringe prepared for this purpose.
- Remove the catheter from the trachea.

Spray injection (Micro Sprayer type syringe)

- Fill the Micro Sprayer syringe and remove air bubbles.
- Intubate the animal with a catheter of appropriate length and caliber and check the correct positioning according to PNF A-1.
- Insert the Micro Sprayer needle into the catheter and inject the product.
- Remove the needle and catheter from the trachea.

Passive Injection

- Prepare a catheter of appropriate length and caliber for intubation.
- Inject the quantity of product to be administered into the tip of the catheter. Connect an empty syringe to the catheter to prevent product loss.
- Intubate the animal with the catheter mounted on the syringe, according to SOP A-1.
- Remove the syringe from the catheter. Correct positioning will be confirmed by the

descent of the product into the lungs via the animal's breathing rate.

- Remove the catheter from the trachea.

Awakening

- Provide oxygen to the animal via an anesthesia mask until it shows signs of awakening (voluntary movements).
- Wake the animal in accordance with SOP A-1.

Intravenous injection (IV)

- Use the right or left tail vein
- Place the animal in a restraining device designed for intravenous injections.
- Clean the tail with 0.05% chlorhexidine.
- If necessary, warm the animal with a heat lamp or a heating mat or warm the tail with warm water to induce vasodilation. The temperature of the system you use must be continuously monitored (maximum 40°C). Pay close attention to the animal at all times to avoid hyperthermia or burns.
- Start as close to the tail tip as possible so that in the event of failure, you can try again higher up.
- Insert the needle at a 30° angle, bevel up. To reach the vein, keep the needle parallel to the tail once the skin is pierced.
- Aspirate and check for blood. If no blood appears, try to reinsert the needle carefully and aspirate again.
- Inject the required volume, making sure there is no swelling or resistance.
- Keep the plunger down and withdraw the needle.
- Stop any bleeding by applying gentle pressure to the injection site.

Retro-orbital sinus injection (SO)

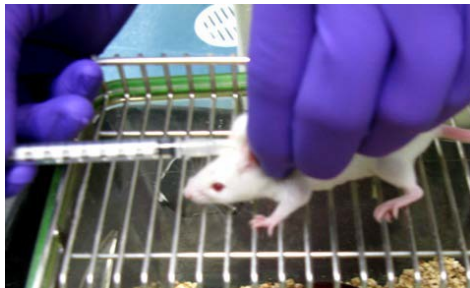
* Since this method involves risks to the animal's health, it should not be used if other practical methods are available for the experiment and for the species. Its use must be justified to the animal protection committee.

- Anesthetize the mouse by isoflurane inhalation following SOP A-1 Analgesia and Anesthesia for Rodents. Have a heat source ready.
- Apply one drop of 0.5% Alcaine to the eye you will use. If you are not using the other eye, apply ophthalmic ointment.
- Using your thumb and index finger, open the eye as much as possible.
- Insert the needle, bevel down to avoid damaging the eye, into the medial canthus at a 45° angle to the snout.

- Inject and withdraw the needle.
- Using gauze, close the eye and gently apply pressure to prevent bleeding.
- Do not give more than 2 injections into the retro-orbital sinus of each eye during the course of an animal's life.
- Space injections into the same eye 24 hours apart.

Subcutaneous injection (SC)

- When possible, administer subcutaneous injections between the shoulder blades.
- Pinch and lift the skin at the injection site to form a tent.
- Insert a needle, bevel up, at the base of the tent, parallel to the animal.
- Aspirate and check for blood (if blood appears, remove the needle and repeat).
- Inject the required volume.
- Release the plunger and withdraw the needle.
- Injection sites can be rotated when subcutaneous injections are administered regularly.



Subcutaneous injection

Topical application (TOP)

- Shave the area where the application will be made, if necessary. Shave with the blade parallel to the skin, against the direction of the hair. Do not use the tip of the blade's teeth. Remove hair with a dry gauze. Shave the smallest area possible.
- Depilatory cream is recommended for sites where shaving is difficult or dangerous. Apply the cream with a cotton swab and use circular motions on the site to be depilated for up to 35 seconds, taking care to avoid the eyes and genitals. Carefully remove all cream with wet gauze and, if necessary, repeat. Prolonged contact time or failure to remove all traces of the cream may severely burn the skin.
- Apply the required amount of topical product with a clean cotton swab or micropipette.

Voluntary oral administration (PO)

Substance administered through the water bottle

- Know the water intake of the mice you will treat before adding product to the water.

Note: Water intake can vary, especially by life stage and strain. If this information is unavailable (e.g. [Forty mouse strain survey of water and sodium intake](#)), obtain it before starting the protocol.

- Ensure the product's solubility and stability before starting the protocol.
- Dilute the product, taking into consideration the mice's daily water intake to be sure the dose is sufficient.
- If necessary, use an opaque bottle to protect the substance from light.
- Monitor the animals' hydration status during administration to ensure the substance does not adversely affect taste.
- Replace the water once a week or more often, depending on the product's stability.

Substance administered via food (wet, mash, gel, treat)

- Use certified commercially available food.
- Ensure the product's stability before beginning the protocol.
- Blend the product into the food, taking into consideration the mice's daily food intake to ensure the dose is sufficient.
- When administering substances that supplement the diet provided, note any amounts not consumed.
- Monitor the animals' weight during administration to ensure the substance does not adversely affect taste.

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SOP Revision History		
Version 2	March 6, 2013	Removed the maximum injection volumes. The ideal volume is now the maximum volume. Nuanced the guidelines regarding multiple uses of the same needle for subcutaneous injections. Clarified bolus and slow IV injections.
Version 3	June 10, 2013	Clarified the ideal subcutaneous volume.
Version 4	October 2, 2015	Modified the measurements used (ml instead of ml/kg).
Version 5	November 20, 2015	Clarified IV injection speeds.
Version 6	June 14, 2019	Added ideal volume for intradermal injections. Added injection into the retro-orbital sinus.
Version 7	March 24, 2020	Changed the requirement to reheat substances to a recommendation. Added details about IN administration under sedation with isoflurane. Added the PO and intraosseous administration routes.
Version 8	June 13, 2022	Added the IT and ITh injections and topical application.

