



Re: Blood collection in mice	Number: P-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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Purpose: Describe the procedures for blood collection in mice	Version 3

General considerations

- Before starting, verify the animal's identification and observe its general condition. Note any anomalies.
- Consult *SOP P-16 General procedure for collecting blood / PNF P-16, Procédure générale pour les prélèvements sanguins* for maximum blood volumes, recovery periods, and clinical signs to watch for when collecting blood.
- Choose a sampling site appropriate for the blood volume required (see Table 1).
- Regarding punctures, if you do not succeed after three attempts, seek help from someone with experience.
- Immediately dispose of syringes and needles in a biohazard sharps container without putting on the caps.
- Make sure bleeding has stopped before returning the animal to its cage.
- After collecting blood, check the animal's condition before leaving the room.

Procedures

Non-terminal techniques

Lateral saphenous vein

- Place the mouse in a restraint cone, with one hind limb outside the cone and immobilize the hind leg by pinching the skin at the knee.
- Shave the back of the leg.
- Apply a small amount of ophthalmic ointment or petroleum jelly with a cotton swab to prevent the blood from spreading.
- Prick the vein perpendicular to the skin with a 23G or 25G needle.
- Collect the drop(s) of blood with a previously opened collection tube or capillary.
- Apply pressure to stop the bleeding.

Medial saphenous vein

- Place the mouse in a restraint cone, with one hind limb outside the cone and immobilize the hind leg by pinching the skin at the knee or restrain the mouse with one hand and press on the groin with your middle finger to immobilize the hind leg.
- Apply a small amount of ophthalmic ointment or petroleum jelly with a cotton swab to prevent the blood from spreading.
- Prick the vein perpendicular to the skin with a 23G or 25G needle.
- Collect the drop(s) of blood with a previously opened collection tube or capillary.
- Apply pressure to stop the bleeding.

Submental vein

- Hold the animal so that the head is straight. If the animal can move its head or bring it back towards its body, blood circulation can be compromised.
- Locate the sampling sites that are to the left and right of the ventral aspect of the mandible.



- Use a 25G to 27G needle depending on the size of the mouse and the amount of blood desired or a lancet of the appropriate size. Puncture the submental vein perpendicularly.
- Collect the drop(s) of blood with a previously opened collection tube or capillary.
- Place the mouse in a container with a paper towel at the bottom. If drops of blood continue to flow, pick up the mouse and apply gentle pressure to stop the bleeding.

Mandibular vein

This method involves risks to the animal's health and 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.

- Use a basic technique to restrain the animal, ensuring its head is properly immobilized.
- On one cheek, locate the small hairless spot. The mandibular vein is situated above this spot, slightly to the rear. The exact site may vary depending on the mouse strain used.
- Prick the mandibular vein perpendicular to the skin with a 20G to 23G needle.
- Collect the drop(s) of blood with a previously opened collection tube or capillary.
- Apply gentle pressure to stop the bleeding.

Jugular vein

- Use a basic technique to restrain the animal.
- Hold the mouse with your thumb and middle finger and raise its head by grasping the skin of the neck with your index finger. Alternatively, pass a string behind the incisors and pull the head up, holding the ends of the string with the restraining hand.
- Draw the right or left forelimb to the animal's back and maintain it in position with the restraining hand. To avoid injuring the animal, be careful to follow the normal movements of the joint.
- Apply alcohol to visualize the vein and the clavicle.
- Insert a 25G needle mounted on a 1cc syringe or less bevel up into the vein.
- Withdraw the desired blood volume and remove the needle. To prevent collapse of the vein, never pull too hard or fast on the plunger.
- Apply pressure to stop the bleeding.

Tail vein

Option 1:

- Place the animal in a restraint device designed for intravenous injections.
- Use the right or left tail vein.
- Clean the tail with 0.05% chlorhexidine, as needed.
- 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 for the methods discussed above (maximum 40°C). Always pay close attention to the animal to avoid hyperthermia or burns.
- Prick the vein with a 25G needle or make a 3–4 mm-long superficial incision with a scalpel blade.
- Collect the blood drop(s) with a previously opened collection tube or capillary.
- Apply pressure to stop the bleeding.

*The scab can be removed for repeated sampling if the scalpel blade incision technique was used.

Option 2:

- Wrap the mouse's body in a small towel with the tail protruding.
- Be sure not to exert pressure on the mouse that might restrict its breathing.
- Place gauze under the tail tip.
- Immobilize the tail tip with a finger and identify either the right or left tail vein.
- Use a 20G needle to pierce, in one go, the two veins in the last 10 mm of the tail (the needle must exit via the vein underneath).
- Gently rub the tail from the base to the tip to stimulate blood flow and collect the drops formed on either side. Be careful not to irritate the skin with excessive rubbing.
- Blot the blood and return the animal to its cage.
- For a subsequent sample (up to 60 min later), gently rub the tail from base to tip to restart the bleeding.

Terminal techniques

Cardiac puncture

- Anesthetize the mouse according to the approved procedure.
- Check the depth of anesthesia.
- Place the animal in dorsal recumbency and palpate the tip of the sternum and the depression to its left (left side of the mouse). Apply alcohol to view the site properly.
- Use a 25G 5/8" needle and a 1 to 3 ml syringe, depending on the size of the mouse.
- Gently insert the needle into the depression, bevel up, at an angle between 30° and 45° while exerting negative pressure on the syringe plunger.
- Draw as much blood as possible.
- If blood does not flow, redirect the needle position (deeper or shallower, change of angle, etc.) while applying negative pressure on the syringe plunger.
- Perform a second method of euthanasia and verify the animal is dead before disposing of the carcass (refer to *SOP EU-1 Rodent euthanasia / PNF EU-1, Euthanasie des rongeurs*).

Abdominal aorta

- Anesthetize the mouse according to the approved procedure.
- Place the animal in dorsal recumbency.
- Check the depth of anesthesia.
- Keep a haemostat on hand to stop accidental bleeding.
- Open the abdominal cavity in a "V" shape.
- Move the animal's intestines to the left.
- Locate and isolate the abdominal aorta.
- Use a 25G needle and a 1 to 3 ml syringe, depending on the size of the mouse.
- Insert the needle, BEVEL DOWN, at the base of the aorta to avoid getting blood in your face due to the high blood pressure.
- Once you insert the needle, draw as much blood as possible.
- Perform a second method of euthanasia and verify the animal is dead before disposing of the carcass (refer to *SOP EU-1 Rodent euthanasia*).

Table 1: Approximate blood volumes that can be obtained from different puncture sites

Site	Approximate volume
Lateral saphenous vein	25 to 100 µl
Medial saphenous vein	25 to 100 µl
Tail vein	25 to 100 µl
Jugular vein	100 to 250 µl
Mandibular and submental vein	100 to 200 µl
Cardiac puncture	0.5 to 1.0 ml
Abdominal aorta	0.5 to 1.0 ml

References

Hoff, Janet, *Methods of Blood Collection in the Mouse*, 2000.

National Centre for the Replacement, Refinement and Reduction of Animals in Research, *Blood sampling microsite*, site consulted in January 2013.

Janis Ott Joslin, *Blood Collection Techniques in Exotic Small Mammals*, 2009.

SOP Revision History		
Version 2	March 21, 2019	Added option 2 for tail vein sampling.
Version 3	March 29, 2022	Added submental vein technique