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Direction des services vétérinaires

Standard Operating Procedure

Re: Blood collection in rats	Number: P-1
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 rats	Version 2

General considerations

- Before starting, verify the animal's identification and observe its general condition. Note any anomalies.
- Consult *SOP P-16 General procedure for blood collection / 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 the caps on.
- 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 rat in a restraint device or hold it against your body and immobilize a 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 an 18G to 23G needle.
- Collect the blood drop(s) with a previously opened collection tube or capillary.
- Apply pressure to stop the bleeding.

Jugular vein

- Hold the rat with its forelegs drawn toward the back and lift its head by grasping the neck skin with your index finger.
- Apply some alcohol to visualize the sampling site.
- Insert a 23G or 25G needle, bevel up, into the vein.
- Withdraw the desired blood volume and remove the needle.
- Apply pressure to stop the bleeding.

Tail vein

- Place the animal in a restraint device designed for intravenous injections.
- 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). 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.
- Prick the vein with a 23G needle perpendicular to the skin.
- Collect the blood drop(s) with a previously opened collection tube or capillary.
- Apply pressure to stop the bleeding. Be careful not to hold the tail tightly if the rat moves because the tail skin could pull off.

Terminal techniques

Cardiac puncture

- Anesthetize the rat with isoflurane according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents / A-1 Anesthésie et analgésie des rongeurs*.
- Place the animal in dorsal recumbency.
- Check the depth of anesthesia.
- Palpate the tip of the sternum and the depression to its left (left side of the rat).
- Use a 21G 1" or 23G 3/4" needle and a 10 to 20 ml syringe depending on the rat's size.
- Fully insert the needle into the depression, bevel up, at an angle between 30° and 45°.
- 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 rat with isoflurane according to the procedure in *SOP A-1 Analgesia and anesthesia in rodents*
- Place the animal in dorsal recumbency.
- Check the depth of anesthesia.
- Keep a haemostat handy to stop accidental bleeding.
- Open the abdominal cavity in a "V" shape.
- Move the intestines to the left.
- Locate and isolate the abdominal aorta.
- Use a 23G or 25G needle, depending on the rat's size.
- 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.
- Carry out a second method of anesthesia 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	100 to 400 μ l
Tail vein	50 to 200 μ l
Jugular vein	100 μ l to 1 ml
Cardiac puncture	5 to 15 ml
Abdominal aorta	5 to 15 ml

References

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

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SOP Revision History		
Version 2	March 8, 2016	Added clarifications on techniques (non-terminal and terminal)