

## **CULATR approved standard procedures for blood collection in laboratory animals**

### Saphenous Vein (Lateral or Medial) Blood Collection (Mouse, Rat, Guinea Pig, Rabbit)

1. Animal is restrained in a suitable restraint device (such as falcon tube for mice), with the hind leg accessible.
2. The lateral/medial side of the hindleg is prepared by clipping the fur followed by wiping with antiseptic or alcohol.
3. A thin film of bland ointment such as Vaseline® will then be applied to the skin to aid the formation of a blood drop.
4. With the animal properly restrained, the leg is gently manipulated to occlude the saphenous vein.
5. The exposed vein is punctured with a needle or lancet.
6. Blood droplets is collected via a collection tube or capillary/microhematocrit tube.
7. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
8. When sample has been collected, gentle pressure is applied on the puncture site until bleeding stops.

### Tail vein blood collection (Mouse, Rat)

1. Animal is restrained in a restraint device with the tail accessible.
2. The collection site is prepared by wiping with alcohol or other antiseptic solution.
3. For better vein visibility, vasodilation may be initiated by warming up either the whole mouse in a ICU (for no longer than 10 minutes), or just its tail with a heating lamp (for no longer than 3 minutes, at a distance of no closer than 12 inches).
4. The lateral tail vein is accessed by immobilizing the tail with the non-dominant hand while inserting needle into the vein, or pricking vein with lancet using the other hand.
5. The blood will drip from the needle hub and into a collection tube. Alternatively, the needle can also be removed from the puncture site and droplets of blood can be collected into the collection tube.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
8. When sample has been collected, gentle pressure is applied on the puncture site until bleeding stops.

### Marginal ear vein/artery blood collection (Rabbit)

1. Animal is restrained using a restraint device with the ears accessible.

2. The ear surface is disinfected with alcohol, and topical local anaesthesia cream is applied 20-30 minutes prior to venepuncture.
3. The vessel is occluded by holding the ear flap at a distant from the collection site with the non-dominant hand (or by an assistant) while inserting the needle or catheter into the vein with the other hand. The use of butterfly catheter with syringe or vacutainer collection tube is recommended.
4. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
5. When sample has been collected, gentle pressure is applied on the puncture site until bleeding stops.

#### Tarsal Vein Blood Collection (Guinea Pig)

1. Animal is held or restrained using a restraint device with one or both hindlegs accessible.
2. Collection site is prepared by clipping the surface fur to view the tarsal veins and topical local anaesthesia cream is applied 20 to 30 minutes prior to venepuncture.
3. Blood vessel is occluded to collect blood sample. Blood will be collected by insertion of needle connected to syringe into the vessel, or the vessel will be punctured by a lancet.
4. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
5. When sample is collected, gentle pressure is applied on the puncture site until bleeding stops.

#### Dorsal Pedal Vein Blood Collection (Mouse, Rat)

1. Animal is restrained using a restraint device with one or both hindlegs accessible.
2. Collection site is prepared by disinfecting with alcohol.
3. A thin layer of petroleum jelly or ophthalmic ointment is applied to help in visualizing the the blood vessel for blood collection.
4. The ankle is held with one hand to locate the medial dorsal vein at the top of the foot and the other hand is puncturing the vein with a needle or lancet.
5. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
6. When sample is collected, gentle pressure is applied on the puncture site until bleeding stops.

#### Facial/Maxillary Vein Blood Collection (Mouse)

1. The Mouse is restrained by scruffing the skin along the back

2. Visualise the landmarks used to determine location for sample collection (a small bald spot or whorl of hair along the curve of the mandible – the vein is just below this point, behind the eye, and below the ear).
4. Apply firm pressure at the identified point with a lancet. The lancet should be inserted perpendicular to the bleed site, to avoid puncturing the ear canal
5. Place a blood collection tube below the puncture site to collect the drops of blood.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
7. When sample is collected, gentle pressure is applied on the puncture site until bleeding stops.

#### Jugular Vein Blood Collection (Rabbit, Goat, Pig)

1. Animal is restrained using an appropriate restraint method to allow the neck to be placed in a hyperextended position.
2. The collection site is prepared by clipping fur at the ventrolateral region of the neck.
3. The clipped portion is disinfected with alcohol and topical anesthetic cream is applied 20-30 minutes prior to venepuncture.
4. Visualise the jugular vein and insert the needle. Blood is withdrawn slowly with a syringe.
5. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
6. When sample is collected, gentle pressure is applied at the puncture site until bleeding stops.

#### Tail Snip (Mice <17 days)

1. This method is used after carefully considering that no other blood sampling route is possible, and is the option of last resort.
2. Animal is restrained using an appropriate restraint device.
3. Topical local anaesthesia cream is applied to the tail 20-30 minutes prior to sampling procedure.
4. The tail tip is disinfected with alcohol.
5. 1-2 mm of the tail tip is snipped off using a sterile scalpel blade. The distance will be accurately measured against a ruler.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
7. The blood drops from the tail will be collected.
8. When sample is collected, gentle pressure is applied at the amputation site until bleeding stops. A drop of tissue glue will may be necessary to control the bleeding.
9. Post-procedure analgesia (such as meloxicam) will be administered.

10. During the following 24 hours, the scab/clot from the original cut at the end of the tail can be disrupted to collect further blood (after local anaesthesia has been applied to the tail for 20-30 minutes).
11. The tail can only be snipped again after a 14 day recovery period, and no more than 3 times in total.

#### Cephalic Vein (Ferret, Rabbit)

1. Animal is firmly and securely restrained with the aid of an assistant.
2. The collection site is prepared by clipping fur along the dorsal aspect of the forelimb.
3. The clipped portion is disinfected with alcohol. Topical anaesthetic cream may or may not be applied 20-30 minutes prior to venepuncture.
4. The blood vessel is occluded (by methods such as quick release tourniquet, or by digital pressure from the assistant).
5. The needle connected to syringe is inserted into the vessel, and blood is withdrawn into the syringe.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
7. When sample is collected, the tourniquet is removed (if used), and gentle pressure is applied at the puncture site until bleeding stops.

#### **Blood Collection Requiring General Anesthesia**

##### Tail Snip (Mice >17 days)

1. This method is used after carefully considering that no other blood sampling route is possible and is the option of last resort.
2. Animal is anaesthetised.
3. Topical local anaesthesia cream is applied to the tail 20-30 minutes prior to sampling procedure.
4. The tail tip is disinfected with alcohol.
5. 1-2 mm of the tail tip is snipped off using a sterile scalpel blade. The distance will be accurately measured against a ruler.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
7. The blood drops from the tail will be collected.
8. When sample is collected, gentle pressure is applied at the amputation site until bleeding stops. A drop of tissue glue may be necessary to control the bleeding.
9. Post-procedure analgesia (such as meloxicam) will be administered.
10. During the following 24 hours, the scab/clot from the original cut at the end of the tail can be disrupted to collect further blood (without anaesthetizing the animal, but after local anaesthesia has been applied to the tail for 20-30 minutes).

11. The tail can only be snipped again after a 14 day recovery period, and no more than 3 times in total.

#### Cranial Vena Cava (Guinea Pig, Ferret)

1. The animal is anaesthetised, and the collection site may be prepared by clipping fur at the ventral region of the neck, above the sternum.
2. The animal is placed lying on its back (in dorsal recumbency), and the collection site is disinfected with alcohol.
3. A needle attached to syringe is inserted lateral to the manubrium and cranial to the first rib.
4. The needle is inserted at a 45 degree angle, and directed towards the opposite hind limb.
5. Gentle suction is applied to the syringe and blood withdrawn. If blood is not initially obtained, then the needle is slowly withdrawn while the gentle suction is maintained.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)

#### Jugular Vein Blood Collection (Mouse, Rat, Guinea Pig, Hamster, Ferret)

1. The animal is anaesthetised, and the collection site is prepared by clipping fur at the ventrolateral region of the neck.
2. The clipped portion is disinfected with alcohol.
3. The head is tilted back in a hyperextend position to expose the neck.
4. The jugular vein is visualised and the needle is inserted. Blood is withdrawn slowly with a syringe.
5. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
6. When sample is collected, gentle pressure is applied at the puncture site until bleeding stops.

#### Orbital Venous Sinus Blood Collection (Mouse, Hamster, Rat)

1. As a recovery procedure, this method is used after carefully considering that no other blood sampling route is possible and is the option of last resort.
2. The animal is firstly anaesthetised. Ophthalmic lubricant is applied to the surface of the eye.
3. The head will then be secured using the thumb and forefinger.
4. A capillary tube or pipette is inserted at the inner corner of the eye socket (medial canthus), and directed towards the back of the eye socket.
5. While the tube is gently advanced, it is carefully rotated at the same time to puncture the venous sinus to collect the blood.
6. The blood volume collected (in ml) will be no more than either:
  - a. 1% of total body weight (g) over a 4 week period, or
  - b. 0.7% of total body weight (g) removed as one single bleed, or multiple smaller bleeds within 24 hours (but still not exceeding 0.7% over the 24 hours, or 1% over a 4 week period)
  - c. More blood can be taken if it is a terminal procedure.

7. When sample is collected, the eyelid should be held closed for a short period to allow the punctured sinus to clot.
8. Animal is closely monitored until full anesthetic recovery.

#### Cardiac Blood Collection (Terminal Procedure, all species)

1. Animal is firstly anaesthetized, and confirmed to be in a surgical plane of anaesthetic depth.
2. The heartbeat can be palpated to aid with determining puncture site.
3. The animal may be placed in lateral recumbency (with its right side downward), and the needle inserted between the ribs towards the heart, or the animal may be placed in dorsal recumbency (lying on its back), with the needle directed from the sternum and punctured upward through the diaphragm towards the heart.
4. Blood is withdrawn slowly to avoid collapse of the heart chambers.
4. When sample is collected, a secondary method of euthanasia is necessary to confirm death (such as thoracotomy or cervical dislocation)

#### Blood Collection from Abdominal/Thoracic Blood Vessel (Terminal Procedure, all species)

1. Animal is firstly anaesthetized, and confirmed to be in a surgical plane of anaesthetic depth.
2. Incision is made into the abdomen or thoracic cavity.
3. The abdominal aorta or vena cava or the aortic arch is located.
4. Once identified, a needle with syringe is inserted into the vessel. Blood is slowly withdrawn from the vessel.
5. When sample is collected, if using abdominal vessel, a secondary method of euthanasia is necessary to confirm death (such as thoracotomy or cervical dislocation).