

Procedure Type: Blood Collection under Anesthesia Procedure

Title: Blood Collection in Anesthetized Mice Species: Mouse,
Domestic

Pain/Distress Category: D

Anesthetic Regimen: (select all that apply)

Parameters monitored: Response to toe pinch, respiration, and mucosal membrane color

Anesthetic Agents:

	Agent Name	Dosage (in mg/kg if possible)	Route
<input type="checkbox"/>	Isoflurane in oxygen	Induce 3-4%; Maintain 1-2%	Inhalation (IH)
<input type="checkbox"/>	Isoflurane drop jar/nose cone	To effect	Inhalation (IH)
<input type="checkbox"/>	Ketamine + Xylazine	90-120 mg/kg (ket) + 5-10 mg/kg (xyl)	SC or IP

Note: If using Isoflurane drop jar/nose cone, isoflurane should be adequately scavenged.

Procedure Description: (select all that apply)

With the exception of RO and cardiac puncture, these blood collection techniques are typically done in a properly restrained conscious animal; anesthesia should only be used if it is a necessary part of the study protocol (i.e., blood collection during imaging, etc.).

	Site	Procedural Description
<input type="checkbox"/>	Lateral tail vein or tail artery	<p>General blood withdrawal guidelines: Mice have an average circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g mouse = 1.8 ml circulating blood volume for a 25 g adult mouse). 7.5% of the circulating blood volume can be safely removed with a recovery period of 7 days. If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week.</p> <p>Note: Vessels are easier to visualize in light-colored mice.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none">1. Anesthetize mouse per regimen.2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress).3. Moisten site with alcohol.4. Using a 25-27g needle on a 0.5 – 1cc syringe, insert the needle, bevel facing up into vessel. Gently pull back on the plunger to avoid collapsing the blood vessel.5. Alternatively, puncture the blood vessel with the needle and allow the blood to drip into a microcentrifuge tube or collect via capillary action into a blood collection tube.6. Remove needle and apply pressure to puncture site with gauze pad until bleeding stops. <p>Potential Adverse Events: Excessive bleeding, hematoma formation, tissue trauma, or infection.</p>

<input type="checkbox"/>	Facial vein (Submandibular)	<p>General blood withdrawal guidelines: Mice have an average circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g mouse = 1.8 ml circulating blood volume for a 25 g adult mouse). 7.5% of the circulating blood volume can be safely removed with a recovery period of 7 days. If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week.</p> <p>Note: Hemostasis may take longer than other methods of blood collection.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> 1. Anesthetize mouse per regimen above. 2. Puncture facial vein, located slightly behind the mandible, but in front of the ear canal near the bald spot or “dimple”, in a swift, lancing motion with a 4.0-5.5mm lancet or tip of a 19-25g needle; blood will flow immediately if in the correct location. 3. Collect sample into a pipette via capillary action or allow blood to drip into a microcentrifuge or blood collection tube. 4. Apply pressure with a gauze pad until bleeding stops. <p>Potential Adverse Events: Depth of the puncture must be controlled or excessive bleeding, entry into the ear canal, entry into the oral cavity, hematoma formation, trauma to the underlying muscles or infection can occur.</p>
<input type="checkbox"/>	Lateral saphenous	<p>General blood withdrawal guidelines: Mice have an average circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g mouse = 1.8 ml circulating blood volume for a 25 g adult mouse). 7.5% of the circulating blood volume can be safely removed with a recovery period of 7 days. If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> 1. Anesthetize mouse per regimen above. 2. Extend hind limb. Position over top edge of the collection vessel, applying gentle pressure above the knee joint or use a small tourniquet to hold off the vessel. 3. Add sterile ophthalmic ointment to allow the blood to pool at the site, and part hair to visualize vessel. 4. Puncture vessel with 25g needle in a swift, lancing motion; blood will flow from site and pool on the ointment. 5. Collect sample into a pipette via capillary action or allow blood to drop into a microcentrifuge or blood collection tube. 6. Release downward pressure on leg and apply gentle pressure to venipuncture site with a gauze pad until bleeding stops. 7. Removal of the scab will enable serial sampling. <p>Potential Adverse Events: Excessive bleeding, hematoma formation, tissue trauma, or infection.</p>

☐	Cardiac puncture	<p>General blood withdrawal guidelines: For terminal blood draws via cardiac puncture there is no volume limit.</p> <p>Note: Terminal procedure under deep general anesthesia only!</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> 1. Anesthetize mouse per regimen above and check toe pinch reflex before proceeding. 2. Lie animal on its back and insert a 25-30g needle on a 1 ml syringe just behind the xiphoid cartilage, at 10-30° from the horizontal axis of the sternum, slightly lateral to the midline (animal's left side). 3. Withdraw blood slowly. 4. Euthanize mouse following collection per protocol-approved method. <p>Potential Adverse Events: If the mouse wakes up during procedure, they will immediately be euthanized.</p>
---	------------------	---

Post-Procedural Care:

Hemostasis will be verified, and mice will be monitored until they are fully awake (e.g., upright and ambulatory), before returning any animal to their housing room. Mice will be examined immediately following blood collection and weekly thereafter, for general appearance and activity level, as well as potential adverse events based on blood collection method (see above).

Procedure Endpoints:

Blood collection amounts and frequency will not exceed stated guidelines. Mice undergoing cardiac puncture will be euthanized immediately afterwards.

Early Euthanasia Criteria:

If moribund, or if any other abnormal signs are noted, the mouse will be euthanized immediately.

Literature Search for Alternatives:

Key Words	Search Site	Years Covered
Retro orbital, submandibular, saphenous, tail vein, blood collection, cardiac puncture, jugular, mouse, alternatives, refinement	PubMed, SCOPUS	1991-[insert current year here]