

Procedure Type: Blood Collection under Anesthesia
 Procedure Title: Blood Collection in Anesthetized Rats
 Species: Rat, 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 mg/kg (ket) + 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	Procedure Description
<input type="checkbox"/>	Tail vein or artery	<p>General blood withdrawal guidelines: Rats have an average circulating blood volume of 64 ml/kg (0.064 ml/g x 200 g rat = 12.8 ml circulating blood volume for a 200 g rat). 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 If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week.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> <p>Syringe Method -</p> <ol style="list-style-type: none"> 1. Anesthetize rat per regimen above. 2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress). 3. Moisten venipuncture site with alcohol. 4. Using a 23g or smaller needle on a 1 cc syringe, line up the needle, bevel facing up, with the vessel, and gently insert into lumen of vessel. 5. Gently pull back on the plunger and let blood slowly fill the syringe. 6. Remove needle and apply pressure to site with gauze pad until bleeding stops. <p>Tail Nick Method –</p> <ol style="list-style-type: none"> 1. Anesthetize rat per regimen. 2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress) 3. Moisten venipuncture site with alcohol. 4. Puncture the vessel with a 21 g needle. 5. Collect sample into a pipette via capillary action or allow blood to drop

	Tail vein or artery (cont.)	<p>into a microcentrifuge or blood collection tube.</p> <p>6. Apply gentle pressure to puncture site with a gauze pad until bleeding stops.</p> <p>Potential Adverse Events: Excessive bleeding, hematoma formation, tissue trauma, or infection.</p>
☐	Jugular	<p>General blood withdrawal guidelines: Rats have an average circulating blood volume of 64 ml/kg (0.064 ml/g x 200 g rat = 12.8 ml circulating blood volume for a 200 g rat). 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: This technique generally requires two people, one to position the rat, and the other to collect the blood. Care must be taken not to impede breathing.</p> <p><u>Procedural Steps:</u></p> <ol style="list-style-type: none"> 1. Anesthetize rat per regimen above and scruff by firmly grasping the loose skin over the neck/shoulder region with your thumb and index finger; gently turn your hand so that the rat is lying comfortably in the palm of your hand; use your free hand to hold the front legs back with your thumb, and gently extend the head back between the index and middle finger, with your index finger resting on the rat's chin. 2. A second person clips the ventral aspect of the neck and moistens the venipuncture site with alcohol. 3. The restrainer applies light pressure to the vein with their thumb, just proximal to the venipuncture site, to make the vessel easier to visualize. 4. Using a 21 g, 1" needle on a 1-3 cc syringe, the syringe is inserted into the jugular vein at a 30 degree angle, with the syringe directed towards the hind end of the rat. 5. Gently pull back on the plunger and let blood slowly fill the syringe. 6. Remove needle and apply pressure to site with gauze pad until bleeding stops. <p>Potential Adverse Events: Excessive bleeding, difficulty breathing, hematoma formation, tissue trauma, or infection.</p>

<input type="checkbox"/>	Lateral saphenous	<p>General blood withdrawal guidelines: Rats have an average circulating blood volume of 64 ml/kg (0.064 ml/g x 200 g rat = 12.8 ml circulating blood volume for a 200 g rat). 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 rat per regimen above. 2. Place the rat on its side and extend the uppermost hind leg, applying gentle pressure above the knee joint so that the lateral saphenous vessel is visible. 3. Moisten the venipuncture site with alcohol, followed by sterile ophthalmic ointment, and part the hair to visualize vessel. 4. Puncture vessel with 25-27g needle in a swift, lancing motion; blood will flow from site. 5. Collect sample into a pipette via capillary action or allow blood to drop into a microcentrifuge or blood collection tube. 6. Release 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>
<input type="checkbox"/>	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 rat per regimen above and check toe pinch reflex before proceeding. 2. Lie animal on its back and insert a 21-25g, 1" needle on a 1-5 ml syringe just behind the xiphoid cartilage, at 20-30° from the horizontal axis of the sternum, slightly lateral to the midline (animal's left side). 3. Withdraw blood slowly. 4. Euthanize rat following collection per protocol-approved method. <p>Potential Adverse Events: If the rat wakes up during procedure, it must be euthanized immediately.</p>

Post-Procedural Care:

Hemostasis will be verified, and rats will be monitored until they are fully awake (e.g., upright and ambulatory), before returning any animal to their housing room. Rats 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. Rats undergoing cardiac puncture will be euthanized immediately afterwards.

Early Euthanasia Criteria:

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

Literature Search for Alternatives:

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