Procedure Type: Blood Collection Procedure Title: Blood Collection in Conscious Mice Species: Mouse, Domestic Pain/Distress Category: C

Procedure Description: (select all that apply)

| Site | Procedural Description |
|-------------------------------------|---|
| Lateral tail vein or tail artery | 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 If blood must be drawn more frequently, it may be divided into several draws, but the total amount withdrawn should not exceed 7.5% of the circulating blood volume per week. Note: Vessels are easier to visualize in light-colored mice. Procedural Steps: 1. Restrain mouse in rodent restraint apparatus. 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 be collected by capillary action into a blood collection tube. 6. Remove needle if utilized and apply pressure to puncture site with gauze pad until bleeding stops. Potential Adverse Events: Excessive bleeding, hematoma formation, tissue trauma, or infection. |

| Facial vein | General blood withdrawal guidelines: Mice have an average | |
|--------------------|--|--|
| (Submandibular) | circulating blood volume of 72 ml/kg (0.072 ml/g x 25 g | |
| (Submanubular) | 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| | but the total amount withdrawn should not exceed 7.5% of | |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. | |
| | Note: Hemostasis may take longer than other methods of blood collection. | |
| | Procedural Steps: | |
| | 1.Scruff mouse by grasping loose skin over the shoulders | |
| | between thumb and index finger of non-dominant hand. | |
| | 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. | |
| | 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. | |
| Lateral saphenous | General blood withdrawal guidelines: Mice have an average | |
| Lateral sapilenous | 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. | |
| | Procedural Steps: 1 Place mouse head first in restraint tube (a E0 ml plastic | |
| | 1. Place mouse head first in restraint tube (a 50 ml plastic | |
| | conical test tube or syringe casing works well). | |
| | 2. Extend hind limb over top edge of the tube, 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 | |
| | pressure to venipuncture site with a gauze pad until | |

| Lateral saphenous (cont.) | bleeding stops. 7.Removal of the scab will enable serial sampling. Potential Adverse Events: Excessive bleeding, hematoma formation, tissue trauma, or infection. |
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Procedure Endpoints:

Hemostasis will be verified before returning any animal to their home cage. Blood collection amounts and frequency will not exceed stated guidelines.

Early Euthanasia Criteria:

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). 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 |
|---|-------------------|---------------------------------------|
| Submandibular, saphenous, tail vein, blood collection, jugular, mouse, alternatives, refinement | PubMed, SCOPUS | 1991-[insert current year here] |