Blood Collection

Site Preparation: Certain general procedures and precautions are applicable to methods of blood collection as well as to administration of fluids and anesthetics. When venipuncture is required, hair should be shaved from the site for better visibility. The area of injection should be cleansed with alcohol. Some procedures will require anesthesia; others may be carried out without anesthesia, provided suitable restraint is possible. In order to visualize veins better, one of several methods of dilation may be used. The vessel may be occluded with digital pressure to cause enlargement. Heat will also cause dilation. When using the rabbit ear, or mouse or rat tail, a low watt light bulb may be used for heat. The preferred method of collection of large volumes of blood from the rabbit ear is with the use of a droperidol-fentanyl tranquilizer that promotes arterial dilation and makes blood collection from rabbits simple for even the inexperienced phlebotomist (Drug Dosage Table -Table 1).

Guidelines for blood collection

Equipment needed: Needles of appropriate gauge and length must be selected with care. For the tail vein or artery of rats and mice, small needles (25-30g) should be used. For other vessels in other animals, the suitable size will depend upon the size of the animal and vessel.

Technique: Proper insertion of the needle into the vein or artery is the most tedious part of the procedure. Certain guidelines may be given, but only practice can be expected to provide any proficiency. A precise, careful introduction of the needle is always best. The needle is inserted parallel above the vessel and the tip directed into the lumen along with the longitudinal axis. The intracardiac puncture generally represents the most practical method of blood collection from small rodents when more than a few drops are required. It is also useful in rabbits for exsanguination. Animals must be anesthetized and placed in dorsal recumbency. The point of the strongest heart beat is determined with the forefinger. The needle is inserted through the skin, between the Aab at this site, directly into the heart. Blood should be withdrawn slowly. The cardiac route for blood collection is a terminal procedure.

Submandibular Bleeding (Mice)
A relatively simple way to obtain blood from a mouse is to puncture the area behind the hinges of the jawbones. Veins that drain blood from other parts of the face meet in this area and form the jugular vein. Scruff the mouse and poke a small hole. Various items may be used to puncture the skin including 19, 22, or 23 gauge needles, number 11 scalpel blades, or specially made mouse bleeding lancets. Information on the lancets and a video of this procedure may be seen by going to the following URL:
http://www.medipoint.com
Please contact DLAM (5-2653) to arrange for training.

Saphanous Vein
This method of obtaining blood is often used when a series of small samples is needed. Place the mouse in a conical tube and shave the caudal surface of the thigh. The saphenous vein can be seen in this area. It is advantageous to apply a lubricant to prevent wicking. Place a tourniquet above the knee and poke the vein with a 22 gauge needle. Microhematocrit and microvette tubes work well to collect the blood. This method of blood withdrawal does not require anesthesia, however we find the method of restraint is cumbersome. For detailed instructions and pictures of this procedure please visit
http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html

In the rodent, blood collection by cutting off toes is not permitted. Collection from the tail artery may be increased by warming it in water. Animals should be restrained in restraining device or anesthetized. After cleaning, a small nick is made on the ventral midline of the tail and blood is collected. Digital pressure will stop the blood flow. Withdrawal of blood from the orbital venous plexus of rats and orbital sinus of mice and hamsters is frequently used. When bleeding the mouse, hamster and rat by the retrobulbar technique, the hematocrit capillary tube is directed toward the major venous structures of the orbit. Knowledge of the location of the venous structures and the technique is essential (Figures 10 &
Anesthesia is required for all retrobulbar bleeding procedures. Instruction on all of these blood-collection techniques is available through DLAM.

Figure 11: Retrobulbar Blood Sample Collection in the Mouse

**Maximum Blood Volume for Survival Collection in Lab Animals**
The maximum amount of blood to be collected, as a survival procedure, from the following laboratory animals is 15% of the circulating blood volume. Frequency of collection should not exceed every other week. Hematocrit must be monitored and fluid replacement considered for protocols which require blood collection in larger volumes or at more frequent intervals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Blood Volume</th>
<th>15% Blood Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>58 ml/kg b.w.</td>
<td>8.7 ml/kg b.w.</td>
</tr>
<tr>
<td>Mouse</td>
<td>78 ml/kg b.w.</td>
<td>11.7 ml/kg b.w.</td>
</tr>
</tbody>
</table>