Background:

Guidelines for safe blood withdrawal for laboratory animals takes into account the species specific blood volume to body weight ratio measured in milliliters of blood to kilogram of body weight. These guidelines are for normal, healthy adult animals. Animals that are young, aged, stressed, have undergone experimental manipulations, or are suffering from cardiac or respiratory disease may not tolerate this amount of blood loss. Blood collection techniques must be described and approved by the Institutional Animal Care and Use Committee (IACUC).

Policy:

Restraint during blood draw: Appropriate instruction for all species can be obtained from the Department of Comparative Medicine (DCM-C), 503-494-8425 or the Division of Comparative Medicine (DCM-W), 503-690-5213.

- Dogs, Cats, and Sheep: usually require only physical restraint to collect blood.
- Swine: may require only physical restraint if they have been trained to the procedure. Otherwise, chemical restraint or sedation is recommended.
- Rabbits, Mice and Rats: may be placed in appropriate restraining devices, manually restrained, and/or chemically restrained with anesthetics or sedatives.
- Birds: usually require only physical restraint.
- Non-human Primates (NHP): Blood collection in a sample acquisition tower should be done according to the DCM-W SOP for restraining NHPs (West campus SOP AC 037). Animals may also be trained to present a limb for sampling. NHPs on infections disease studies must be anesthetized prior to collection of blood.

Anesthesia is required to perform blood collection from the retro-orbital sinus in rodents or by cardiac puncture. With appropriate justification, permission may be granted by the IACUC to collect blood from the retro-orbital sinus in rodents without anesthesia.

Blood collection volumes (general guidelines):

- 1 percent of body weight = maximum volume per collection every two weeks.
- 3 percent of body weight = amount expected from exsanguination.

Blood Collection Volumes for Nonhuman Primates

Up to 12.5% total blood volume (TBV) within a 21 day period (up to 7 ml of blood/kg of body weight for rhesus macaques; up to 8 ml of blood/kg of body weight for cynomolgus macaques). Up to 20% TBV (11 ml/kg for
rhesus; 13 ml/kg for cynos) can be withdrawn within a 21 day period with prior approval of the veterinarian and the IACUC. If an overdraw occurs, it must be reported to the veterinarian immediately for evaluation of the animal’s condition. See also West campus SOP RS-001.

**Bone Marrow Aspirate for Nonhuman Primates**
Bone marrow aspiration is considered equivalent to blood collection, and aspiration volume is included in the sum total of blood volume collected over a given time period.

**Single blood draw:** A volume of blood equivalent to a maximum of 1% of the animal’s body weight may be removed as a single blood draw. For example:
- 0.15 ml from a 15 gram mouse
- 50 ml from a 5 kg cat
- 400 ml from a 40 kg dog
- 12.5% TBV or 20 TBV with specific approval from Attending Veterinarian and IACUC from NHP with reconstitution period of 21 days (SOP RS-001)

Approximately 14 days are needed for the average healthy adult animal to completely recover from this blood loss (21 days for NHPs). Although the blood volume is restored within 24 hours, two weeks (3 weeks for NHPs) are needed for all blood constituents to return to normal. As a rule, an animal will replace blood constituents at a rate of 1 ml/kg/day.

**Serial and Multiple Blood Draws:** A volume of blood equivalent to a maximum of 1% of the animal’s body weight may be removed as a series of blood draws on a single day, once a day over multiple days, or a combination thereof. The maximum blood volume may be divided by the number of blood collections needed in a given two-week time period in order to determine the blood volume per collection point. For example, 0.25 ml (250µl) from a 25 gram mouse may be collected as:
- Five 50µ serial blood draws in one day
- Five 25µ serial blood draws per day over two consecutive days
- Five 25µ serial blood draws on a given day, once a week for six weeks (no more than 0.25 ml is drawn in any 2 week period)
- One 125µ blood draw per week over the course of 2 weeks

For serial blood draws, multiple sites for blood collection should be employed. If possible, each blood collection site should be utilized no more than once every 3 days unless blood can be collected from that site using the pre-existing puncture.

In mice, collection via medial saphenous vein is preferable to orbital sinus bleeding. Collecting blood by lacerating ear or tail vessels is prohibited in all species. These procedures are more painful than needle puncture and there is greater risk of lacerating an artery with subsequent hemorrhage.

Regardless of the method of collection used, an animal may not be returned to its cage until complete hemostasis at the collection site has been achieved. Hemostasis may be achieved using gauze and direct pressure. Up to several minutes of pressure may be required following arterial puncture.

**Monitoring:** By monitoring the hematocrit (Hct or packed cell volume – PCV) and/or hemoglobin of the animal, is possible to evaluate whether the animal has sufficiently recovered from a single or multiple blood draws. After a sudden or acute blood loss, it takes up to 24 hours for the hematocrit and hemoglobin to reflect this loss. In general, if the animal’s hematocrit is less than 35 percent or hemoglobin concentration is less than 10 g/dl it is not safe to remove the volume of blood listed above.
Terminal blood draws: Terminal bleeds are only allowed on animals under general anesthesia and the animal’s death must be verified at the end of the bleed. A secondary euthanasia method is recommended after the blood withdrawal.

Generally, an animal’s blood volume is 10 percent of its body weight, and only about half of that can be recovered when the animal is bled out. Therefore, as a terminal bleed, 5-6 percent of an animal’s body weight is a reasonable amount of blood (ml) that may be collected.

Rabbit Bleeding for Polyclonal Antibody Assays: Blood volume limits need to be considered in blood collection because over bleeding can leave the animal hypovolemic, anemic, weak and susceptible to disease and death.

Total blood volume is approximately 6% of body weight. 10% blood volume taken within two weeks is recommended.

<table>
<thead>
<tr>
<th>Species</th>
<th>Typical Sites of Collection (1)</th>
<th>Permitted Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse(2)</td>
<td>Cardiac (3), orbital sinus (anesthetized required), tail vein, saphenous vein, (submandibular) facial vein.</td>
<td></td>
</tr>
<tr>
<td>Rat(2)</td>
<td>As with mouse, plus jugular (anesthesia required), tail veins and artery, dorsal metatarsal vein.</td>
<td>Anesthesia is recommended for submandibular blood collection in rats</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>Cardiac (3), jugular vein (anesthesia required), lateral saphenous.</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Cardiac (3), marginal ear vein, jugular (no anesthesia required).</td>
<td>It is often helpful to apply a vasodilator on the ears of rabbits prior to collecting blood, such as oil of wintergreen (menthylsalicylate).</td>
</tr>
<tr>
<td>Dog &amp; Cat</td>
<td>Cephalic, saphenous veins, femoral and jugular veins</td>
<td></td>
</tr>
<tr>
<td>Ruminants</td>
<td>Jugular Vein</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>Jugular Vein, anterior vena cava, ear veins</td>
<td></td>
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<tr>
<td>Chicken</td>
<td>Brachial vein, right jugular vein, cardiac (3)</td>
<td></td>
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<tr>
<td>Non-human Primate</td>
<td>Femoral, saphenous, cephalic veins</td>
<td></td>
</tr>
</tbody>
</table>

(1) Other access routes can be approved by IACUC. (2) Tail cutting in rodents: Lancing blood vessels in the tail and cutting the distal tip of the tail are not acceptable methods of blood collection in rodents. IACUC approval is required prior to performing these procedures in rodents. (3) Cardiac puncture for blood collection must be performed as a terminal event, and the animal must be under general anesthesia.