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## IG032: GUIDELINE ON SAFE BLOOD WITHDRAWAL

Blood withdrawal is a routine procedure in many research and clinical animal activities. Typically, restoration of blood volume and its components occur within two weeks of blood withdrawal in healthy animals. Though volume is often replaced within 24 hours, two to four weeks may be required to replace all blood constituents. As a general principle, sample volumes and number of samples should be kept to a minimum.

### BLOOD COLLECTION GUIDANCE\*\*

The **maximum volume of blood that can be collected** may not exceed 1% of the body weight (or 10 ml/kg) in any animal in a two-week period. For example:

- No more than 0.3 ml (300 µl) can be collected from a 30 g mouse, over a two-week period.
- No more than 100 ml can be collected from a 10 kg dog, over a two-week period.
- These volume guidelines are intended for healthy animals. Stressed, sick, or otherwise compromised animals may not tolerate this blood collection criteria. Veterinary staff should be consulted before blood collection is performed in these cases.

If the maximum volume of blood (10 ml/kg) is collected at one time, warm isotonic fluids (e.g., 0.9% saline, lactated Ringer's solution) must be provided.

- For a mouse, 0.5-1.0 ml of warmed, sterile isotonic fluids should be administered subcutaneously (SC) or intraperitoneally (IP) after a 10 ml/kg blood collection.
- For a rat, 2.0-4.0 ml of warmed, sterile isotonic fluids should be administered subcutaneously (SC) or intraperitoneally (IP) after a 10 ml/kg blood collection.
- For larger species (larger than rats) the volume of isotonic fluids replaced should be equal to the volume of blood collected and administered subcutaneously (SC) or intravenously (IV).

Following venipuncture, **complete hemostasis** should be achieved before animal returns to normal housing.

Depending on the procedure, this may be done by using direct (manual) pressure applied to the site, cauterization, styptic powder, silver nitrate sticks (not recommended for rodents) or other methods.

Manual pressure, applied to the site for 1-2 minutes, is the most common method used. In some cases, pressure may need to be applied for several minutes to achieve complete hemostasis.

- Some methods, such as cautery and silver nitrate sticks, should only be used on animals maintained at a surgical plane of anesthesia. Please consult with veterinary staff ([carvets@msu.edu](mailto:carvets@msu.edu)) for the best option.
- **If bleeding cannot be stopped, the CAR veterinary staff ([carvets@msu.edu](mailto:carvets@msu.edu)) must be contacted immediately for emergency assistance.**

Other potential complications of blood withdrawal include hypovolemic shock and anemia.

- If too much blood is withdrawn too rapidly or too frequently without replacement, the animal may experience hypovolemic shock. Signs of shock include fast and thready pulse, pale dry mucous membranes, cold skin and extremities, restlessness, and sub-normal body temperature. Hypovolemic shock is an emergency condition and must be treated promptly.
- Signs of anemia include pale mucous membranes, pale skin, ears, or gums, exercise intolerance, and increased respiratory rate.
- If signs of shock or anemia are observed, contact the veterinary staff.

**Terminal Blood Collection** is only to be performed on animals maintained under a surgical plane of anesthesia, and death of the animal must be verified at the completion of the bleed. As with all euthanasia methods, a secondary confirmatory method (e.g. pneumothorax, cervical dislocation, etc) should also be performed.

\*\*Exemptions to this guideline may be considered by the IACUC if *scientifically justified*.

**Table 1: Summary of Blood Sampling Techniques for Mice and Rats**

Route	General Anesthesia Required	Repeated Sampling	Approximate Volumes Obtainable	Species	Comments
<b>Retro-Orbital</b>	Yes	Yes, should alternate eyes with $\geq 7$ days between collections (14 days for the same eye)	Up to maximum volume	Mouse	Rapid, potential for complications
<b>Mandibular</b>	No	Yes	Up to maximum volume	Mouse	Rapid, easy and repeated samples possible
<b>Submental</b>	No	Yes	Up to maximum volume	Mouse	Rapid, easy and repeated samples possible
<b>Saphenous</b>	No	Yes	Up to maximum volume (mouse) 50-200ul (rat)	Rat, Mouse	Not as rapid as other techniques, low potential for tissue damage
<b>Tail Vein or Artery</b>	No	Yes	100-200ul (mouse) 100-300ul (rat)	Rat, Mouse	Repeatable, simple, variable sample quality
<b>Tail Clip</b>	No	Yes	1-2 drops	Rat, Mouse	Repeatable if scab is gently removed
<b>Jugular</b>	Recommended	Difficult	Up to maximum volume	Rat	Limited application, poor for repeated sampling

**Table 2: Approximate Allowable Blood Collection Volume by Species**

Species (approximate weight)	Allowable Blood Collection Volume (1% of body weight)
<b>Amphibian (3 g)</b>	0.03 ml
<b>Mouse (25 g)</b>	0.25 ml
<b>Rat (250 g)</b>	2.5 ml
<b>Ferret (1 kg)</b>	10 ml
<b>Rabbit (4 kg)</b>	40 ml
<b>Cat (5 kg)</b>	50 ml
<b>Dog (10 kg)</b>	100 ml
<b>Minipig (15 kg)</b>	150 ml
<b>Sheep (23 kg)</b>	230 ml
<b>Cattle (640 kg)</b>	6 L

**For additional guidance on sites and techniques for collection, please contact  
Campus Animal Resources ([carvets@msu.edu](mailto:carvets@msu.edu)) for assistance.**

### **Alternate Formula for Large Animals**

Frequency of blood sampling is dependent upon total volume to be requested. As a general guide, no more than 10% of total circulating blood volume (TBV) should be withdrawn over multiple sampling points within a 2-week period.

- Where blood is sampled repeatedly (e.g. weekly), the suggested limit is typically 15% TBV over 28 days. For repeat bleeds at shorter intervals (e.g. over a few days), a maximum of 1.0% TBV every 24 hours is recommended.
- In healthy animals, the approximate circulating blood volume is 6% of total body weight. As a guideline, a safe amount of blood to collect (in milliliters) can be calculated as:

**Animal's Weight (in kg) x 1000 (g per kg) x 0.06 (6% total body weight) x 0.10 (total blood withdrawal per two weeks)**

- Care should be taken in these calculations, as the percentage of TBV will be lower (~15%) in obese and older animals.

### **References:**

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