I. Purpose

The purpose of this guideline is to provide recommended blood sampling volumes and guidance on a variety of acceptable blood collection techniques in the anesthetized and conscious rodent.

II. Scope

This policy applies to all personnel collecting blood samples from laboratory rodents.

III. Guidance

A. General Information

1. Factors to consider when selecting the appropriate blood collection technique for research purposes include, but are not limited to:
   
   a. The species to be bled
   b. The size and age of the animal to be bled and the estimated total blood volume
   c. The type of the sample required (e.g. serum, whole blood cells, etc.)
   d. The quality of the sample required (sterility, tissue fluid contamination, etc.)
   e. The quantity of blood required (taking into account extraneous blood loss due to a selected method)
   f. The frequency of sampling
   g. The health status of the animal being bled
   h. The training and experience of the phlebotomist
   i. The size and type of capillary tube is appropriate
   j. The effect of the site, restraint or anesthesia on the blood parameter measured.

2. The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate. The approximate circulating blood volume of adult rodents varies with species and body weight. For purposes of calculating the maximum blood volume that may be sampled, the following reference values for total blood volume (TBV) are to be used:
   
   a. Mouse 72 ml/kg
   b. Rat 64 ml/kg
   c. Hamster 78 ml/kg
d. Guinea pig 75 ml/kg

3. Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1% every 24 hours.

4. The guidance provided below is for healthy, normal adult animals. Animals that are young, aged, stressed, have cardiac or respiratory disease, or are otherwise compromised may not be able to tolerate recommended amounts of blood removal.

5. If the experimental design requires blood volumes and/or frequency of collection that fall outside the recommendations within this guideline, consult the AV and include justification in your protocol for IACUC consideration and approval.

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight (g)</th>
<th>*CBV (ml)</th>
<th>~1% CBV every 24 hrs. †</th>
<th>~7.5% CBV every 7 days †</th>
<th>~10% CBV every 2 - 4wks †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>20</td>
<td>1.10 - 1.40</td>
<td>11 - 14 µl</td>
<td>90 - 105 µl</td>
<td>110 - 140 µl</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.37 - 1.75</td>
<td>14 - 18 µl</td>
<td>102 - 131 µl</td>
<td>140 - 180 µl</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.65 - 2.10</td>
<td>17 - 21 µl</td>
<td>124 - 158 µl</td>
<td>170 - 210 µl</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>1.93 - 2.45</td>
<td>19 - 25 µl</td>
<td>145 - 184 µl</td>
<td>190 - 250 µl</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.20 - 2.80</td>
<td>22 - 28 µl</td>
<td>165 - 210 µl</td>
<td>220 - 280 µl</td>
</tr>
<tr>
<td>Rat</td>
<td>125</td>
<td>6.88 - 8.75</td>
<td>69 - 88 µl</td>
<td>516 - 656 µl</td>
<td>690 - 880 µl</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>8.25 - 10.50</td>
<td>82 - 105 µl</td>
<td>619 - 788 µl</td>
<td>820 - 1000 µl</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>11.00 - 14.00</td>
<td>110 - 140 µl</td>
<td>825 – 1050 µl</td>
<td>1.1 - 1.4 ml</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>13.75 - 17.50</td>
<td>138 - 175 µl</td>
<td>1.0 – 1.3 ml</td>
<td>1.4 - 1.8 ml</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>16.50 - 21.00</td>
<td>165 - 210 µl</td>
<td>1.2 – 1.6 ml</td>
<td>1.7 - 2.1 ml</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>19.25 - 24.50</td>
<td>193 - 245 µl</td>
<td>1.4 – 1.8 ml</td>
<td>1.9 - 2.5 ml</td>
</tr>
</tbody>
</table>

*Circulating blood volume (1ml = 1000µl) †Maximum sample volume for that sampling frequency

C. Survival blood sampling

1. Procedures that may be performed without anesthesia:
a. Dorsal Pedal/Metatarsal vein  
b. Jugular vein  
c. Lateral tail vein  
d. Saphenous/Medial vein  
e. Submandibular vein  
f. Tail nick  
g. Sublingual vein  

2. Procedures requiring anesthesia:  
   a. Retro-orbital This procedure is strongly discouraged by the IACUC as a primary method of blood collection. Scientific justification and IACUC approval are required for use of this collection technique for primary survival sampling.  
      1) Only one eye may be sampled at any time. If repeated sampling within an 8 hour period is necessary, the retro-orbital sinus may be resampled by disrupting the blood clot without repeated damage to the sinus.  
      2) Alternate between left and right eyes per session.  
      3) No more than one collection performed per 5 days.  
      4) A maximum of 3 procedures may be performed per eye.  

D. Non-survival (terminal) blood sampling  
   1. Terminal blood collection is only to be performed on animals maintained under a surgical plane of anesthesia. Death of the animal must be verified at the completion of the bleed.  
   2. An unlimited amount of blood may be withdrawn (i.e., exsanguination) regardless of route. Animal must be immediately euthanized following blood collection.  
   3. Terminal blood collection may be performed from the following:  
      a. Abdominal vena cava  
      b. Renal Vein  
      c. Brachial plexus  
      d. Cardiac puncture  

E. References


