GUIDELINES FOR MAXIMUM BLOOD WITHDRAWAL VOLUMES

These guidelines are meant to assist investigators in the safe, humane collection of blood samples from laboratory animals. The PI must ensure that all personnel involved in these procedures are appropriately trained. Veterinary consultation and pilot studies are recommended when there is doubt about the effects of blood withdrawal on animal health.

The volume of blood removed from an animal will normally be determined by the scientific protocol, which in turn will depend on aspects such as the sensitivity of assays to be used. General guidelines would include withdrawal of the minimum amount necessary to achieve accurate results while minimizing stress, discomfort or adverse physiologic changes to the animal.

Removal of about 10% of an animal’s total circulating blood volume will initiate homeostatic mechanisms. If 15-20% volume is removed, cardiac output and blood pressure will be reduced. Removal of 25% or more can induce shock and mortality. The following adverse effects can be expected from a single, acute blood withdrawal that is too large or removed too rapidly:

- **Hypovolemic Shock** (25-30% of the total blood volume). Signs include:
  - Fast and thready pulse
  - Pale dry mucous membranes
  - Cold skin and extremities
  - Restlessness
  - Hyperventilation
  - Sub-normal body temperature

- **Mortality** (30% or more of the total blood volume)

A. **Estimating the Total Blood Volume (TBV)**

Determinations of the maximum blood volumes that can be withdraw from a given animal species are typically based on an estimate of the total circulating blood volume. For most species, the TBV is typically 6-8% of the body weight in grams. The average of this range can be considered an estimate of the TBV.

<table>
<thead>
<tr>
<th>Species</th>
<th>Range of TBV (ml/kg)</th>
<th>Average TBV (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>62-80</td>
<td>71</td>
</tr>
<tr>
<td>Rat</td>
<td>58-70</td>
<td>64</td>
</tr>
<tr>
<td>Rabbit</td>
<td>44-70</td>
<td>57</td>
</tr>
<tr>
<td>Swine</td>
<td>51-69</td>
<td>60</td>
</tr>
</tbody>
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As a rough guideline, up to 10% of the circulating total blood volume of an animal can be taken on a single occasion from normal adult mature animals on an adequate plane of nutrition. For physiologic data collection, if blood withdrawal exceeds 7% of the total blood volume, normal animal physiology may be affected. If less than the maximum amount of blood is withdrawn (see below), an animal will replace approximately 1 ml/kg/day after blood withdrawal.
The following are examples, based on percent of animal body weight in grams, for the preferred method of calculating maximum blood sample collection volumes:

**B. Survival Blood Removal and Rest Time**

1. **Acute (single withdrawal):**
   A maximum of ~1% of the animal’s body weight in grams can be removed at one time with a minimum 2-week rest period without requiring supplemental fluids.
   
   **Calculation Examples:**
   
   - 15 g mouse = 0.01 (1% body weight) x 15 g = 0.15 ml.
   - 20 kg pig (20,000 g body weight) = 0.01 x 20,000 g = 200 mls.

2. **Multiple samples within 24-48 hrs:**
   A maximum of ~1.4% of the animal’s body weight in grams can be removed during closely spaced serial samples within a 24-48 hr. period, with a minimum of 2-week rest period.
   
   **Calculation Examples:**
   
   - 15 g mouse = 0.014 (1.4% body weight) x 15 g = 0.21 ml total amount removed during the 24-48 hours
   - 20 kg pig (20,000 g body weight) = 0.014 x 20000 g = 280 ml total amount removed during the 24-48 hours

3. **Multiple samples spanning several weeks:**
   A maximum of ~0.75% of the animal’s body weight in grams can be removed as a weekly limit for repetitive blood sampling involving consecutive week collections. The veterinary staff will work with investigators to assess hematocrit and/or hemoglobin levels to ensure that chronic repeat blood sampling does not lead to anemia. Iron may be offered to animals to address chronic blood sampling, per veterinary recommendations.
   
   **Calculation Examples:**
   
   - 250 g rat = 0.0075 (0.75% body weight) x 250 g = 1.9 ml weekly total blood collection
   - 20 kg pig (20,000 g body weight) = 0.0075 x 20,000 g = 150 ml weekly total blood collection volume

**C. Terminal Blood Withdrawals (Exsanguination)**

- Animals must be anesthetized prior to exsanguinations. The total amount of blood obtained can be substantially increased if the heart is beating during blood collection. Up to 50% of the TBV can usually be obtained.
- Giving fluids during blood withdrawal to maintain the animal’s blood pressure can increase the total volume of blood obtained.

**D. Scientific Justification**

Anything outside these guidelines for normal collection volume or rest period needs scientific justification in the IACUC protocol. Fluid replacement, monitoring of hematocrit/packed cell volume (PCV), and endpoints described for cessation of collection should be addressed.
E. Fluid Replacement

If the volume of blood removed from an animal exceeds the recommended maximums (i.e. > 1% body weight every 14 days), intravenous replacement of the removed volume with warm (30-35°C) isotonic fluids (e.g., 0.9% saline, lactated Ringer's solution) constitutes accepted veterinary practice. Withdrawal of blood volumes larger than recommended must be done at a slow, steady rate, and the volume of replacement fluids should be administered similarly.

References