

University of Ottawa Animal Care Committee (ACC) Policy

ACC-03 BLOOD COLLECTION GUIDELINES

1. PURPOSE

The purpose of this policy is to provide general guidance in determining the volumes of blood to be withdrawn from different laboratory animal species, in chronic and acute situations, as well as accepted methods for blood collection. The guidelines are consistent with the Ontario Animals for Research Act, R.S.O. 1990, c.A22 and Canadian Council on Animal Care standards and policies.

2. GENERAL

When designing a study protocol, the following guidelines must be considered to determine the volume, frequency and methods used for blood collection (please refer to Table 1, 2 and 3 below). This information needs to be defined within the relevant animal use protocol submission to the Animal Care Committee.

For any questions about blood collection or for species or guidelines not listed, an ACVS Veterinarian can assist with the selection of appropriate procedures. Larger volumes of blood can be collected when performed under general anesthesia for terminal purposes.

It is critical, when choosing a technique for blood sampling, that the researcher carefully consider possible adverse side effects associated with the procedure (and with other procedures performed on the same animal) and consult an ACVS Veterinarian as needed.

If the volume of blood required and/or number and frequency of punctures exceeds this guideline, justification will be required prior to ACC review and approval.

3. DEFINITIONS

- A. **Puncture:** Refers to the number of needle/capillary punctures for one site, with or without successful blood withdrawal.
- B. Site: A blood vessel (in this case a vein) will be considered as one site.

| Table 1: Circulating blood volumes in laboratory species | | | |
|--|--|--|--|
| Species | Mean ¹ * Blood Volume (ml/kg) | | |
| Mouse | 72 | | |
| Rat | 64 | | |
| Guinea pig | 70 | | |
| Rabbit | 56 | | |

| Table 1: Circulating blood volumes in laboratory species | | | |
|--|----|--|--|
| Pig | 67 | | |
| Fish | 50 | | |

4. RECOMMENDATIONS FOR BLOOD SAMPLING PROCEDURES VERSUS VOLUME WITHDRAWN

The recommended percentage of blood sampling detailed in the table below should be followed. However, higher percentages of blood withdrawal may be evaluated and approved by the Animal Care Committee under certain conditions.

| Table 2: Limit volumes and recovery periods ¹ | | | | | |
|--|-----------------------------|---|-----------------------------|--|--|
| Single sampling | | Multiple sampling | | | |
| (e.g. toxicity study) | | (e.g. toxicokinetic study) | | | |
| % circulatory blood volume removed | Approximate recovery period | % circulatory blood volume removed in 24 h | Approximate recovery period | | |
| 7.5% | 1 week | 7.5% | 1 week | | |
| 10% | 2 weeks | 10-15% | 2 weeks | | |
| 15% | 4 weeks | 20%² | 3 weeks | | |

¹ This table does not include a terminal sample (taken when the animal is terminally anesthetized).

5. RECOMMENDATIONS FOR MAXIMUM DAILY PUNCTURES/ SAMPLING

For needle puncture, a maximum of five punctures/site/day is suggested. If, due to unforeseen circumstances, the number of punctures exceeds this recommendation, the condition of the site should be evaluated and monitored. A vessel must not be used for subsequent puncture if it shows signs of severe inflammation or hematoma.

Cannulation is an important technique for repeated bleeding. Butterfly needles may be used for the short term (day); however, for long-term use, surgical implantation of a cannula should be considered. This method of cannulation allows for repeated blood drawing with minimal discomfort and distress for the animal. For long-term use, a subcutaneous vascular access port is preferred. Long-term cannulation may lead to thrombosis of the vessel and other medical complications.

| Table 3: Recommended sites for repeated blood sampling | | | | |
|--|--|--|--|--|
| Species | Recommended sites (veins unless stated otherwise) ³ | | | |
| Mouse | Saphenous, lateral tail | | | |
| Rat | Saphenous, lateral tail, sub-lingual | | | |
| Rabbit | Marginal ear, central ear artery, jugular | | | |
| Pig | Cranial vena cava (blind stick) | | | |
| Fish | Caudal, dorsal aorta, caudal artery, cardiocentesis ⁴ | | | |

³ Additional sites might be appropriate and will be evaluated by the Animal Care Committee on a case-by-case basis.

² The higher volume (20%) is intended to facilitate serial blood sampling for toxic- or pharmacokinetic purposes where multiple, small samples are usually required.

⁴ This method does impose greater potential risk and the operator must be very familiar with the species anatomy.

| Table 4: Summary of the advantages and disadvantages of the various methods of blood sampling | | | | |
|---|------------------------|---------------|---------------|--------|
| Route/Vein | Anesthesia requirement | Tissue damage | Repeat bleeds | Volume |
| Jugular | No | Low | Yes | +++ |
| Saphenous/Lateral tarsal | No | Low | Yes | ++(+) |
| Marginal ear | Local | Low | Yes | +(+) |
| Sub-lingual Sub-lingual | General | Low | Yes | +++ |
| Lateral tail | No | Low | Yes | ++(+) |
| Central ear artery | Local | Low | Yes | +++ |
| Central vena cava | No | Low | Yes | +++ |
| Tail tip amputation (<1-3 mm) | General | Moderate | Limited | + |
| Cardiocentesis | General | Moderate | No | +++ |

POLICY HISTORY

| DATE | NEW VERSION |
|---------------|---|
| December 2013 | Policy creation (v1) |
| November 2019 | Policy revised (v2, e.g. format and code updated) |

| Appendix 1: Mouse blood volumes based on body weight | | | | | |
|--|---------------------------------------|-------|------|------|------|
| 2 | Total Volume of Volume per collection | | | | |
| Body Weight (g) | Blood (72 mL/kg) | 7.50% | 10% | 15% | 20% |
| 15 | 1.08 | 0.08 | 0.11 | 0.16 | 0.22 |
| 16 | 1.15 | 0.09 | 0.12 | 0.17 | 0.23 |
| 17 | 1.22 | 0.09 | 0.12 | 0.18 | 0.24 |
| 18 | 1.30 | 0.10 | 0.13 | 0.19 | 0.26 |
| 19 | 1.37 | 0.10 | 0.14 | 0.21 | 0.27 |
| 20 | 1.44 | 0.11 | 0.14 | 0.22 | 0.29 |
| 21 | 1.51 | 0.11 | 0.15 | 0.23 | 0.30 |
| 22 | 1.58 | 0.12 | 0.16 | 0.24 | 0.32 |
| 23 | 1.66 | 0.12 | 0.17 | 0.25 | 0.33 |
| 24 | 1.73 | 0.13 | 0.17 | 0.26 | 0.35 |
| 25 | 1.80 | 0.14 | 0.18 | 0.27 | 0.36 |
| 26 | 1.87 | 0.14 | 0.19 | 0.28 | 0.37 |
| 27 | 1.94 | 0.15 | 0.19 | 0.29 | 0.39 |
| 28 | 2.02 | 0.15 | 0.20 | 0.30 | 0.40 |
| 29 | 2.09 | 0.16 | 0.21 | 0.31 | 0.42 |
| 30 | 2.16 | 0.16 | 0.22 | 0.32 | 0.43 |
| 31 | 2.23 | 0.17 | 0.22 | 0.33 | 0.45 |
| 32 | 2.30 | 0.17 | 0.23 | 0.35 | 0.46 |
| 33 | 2.38 | 0.18 | 0.24 | 0.36 | 0.48 |
| 34 | 2.45 | 0.18 | 0.24 | 0.37 | 0.49 |
| 35 | 2.52 | 0.19 | 0.25 | 0.38 | 0.50 |
| 36 | 2.59 | 0.19 | 0.26 | 0.39 | 0.52 |
| 37 | 2.66 | 0.20 | 0.27 | 0.40 | 0.53 |
| 38 | 2.74 | 0.21 | 0.27 | 0.41 | 0.55 |
| 39 | 2.81 | 0.21 | 0.28 | 0.42 | 0.56 |
| 40 | 2.88 | 0.22 | 0.29 | 0.43 | 0.58 |