The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has developed the following guideline to help research investigators understand the best practices for blood collection, or venipuncture, in animals. Blood collection is a common procedure performed in animal research studies which requires IACUC review, training, and veterinary guidance as mandated by the Animal Welfare Regulations (AWR) and the Public Health Service Policy (PHS). The purpose of this guideline is to minimize pain and distress for the animals by providing information on common (acceptable) sites and volumes of blood collection in a variety of species.

IACUC AND TRAINING REQUIREMENTS

Regulatory agencies require that:

- Procedures involving the collection of blood from living animals are described in the protocol and reviewed by the IACUC (AWR 2.31 (c) (6)).
- All personnel involved in the care and use of animals used for blood collection must be qualified and appropriately trained to perform blood collection procedures (PHS Policy IV.C.1.f).

Blood collection procedures must be described in your animal care and use protocol. If the protocol involves more than one species, these procedures must be described for each species. The description should include each of the points listed below in detail.

- The purpose for blood collection procedure (why is the blood being collected?)
- All potential collection sites
- All potential methods used
- The amount(s) to be withdrawn (at each blood collection procedure)
- The type of restraint needed
- The frequency with which each sampling will be performed

Example of a procedure “description” for blood collection:

Blood will be collected for measurements of _____ (can use general terms, i.e. immunologic parameters, or blood chemistry values) from the _____ (experimental/control/animals in experiments 1, 2 and 3, etc…; be specific) study groups. Measurement of ____ will aid in ____ (purpose of the blood collection procedure). Blood will be collected under [sedation/anesthesia/physical restraint]. Following blood collection hemostasis will be confirmed by ____ (describe method).

Please note that some methods of hemostasis may not be suitable for all blood collection techniques or sites, e.g. styptic powder or silver nitrate may not be used for hemostasis for retro-orbital bleeds, instead manual pressure is advised. Please consult your ULAR veterinarian for additional guidance.

In ARIES protocols: the volume, frequency, and other information requested in the procedure “secondary questions” section need to only be provided in that section, not duplicated in the procedure “description” above.

Veterinary oversight and training:

- Investigators are urged to consult with the ULAR veterinary and training groups for additional guidance and training for blood collection techniques.
- Certain techniques, e.g. submandibular bleeding, may require proof of proficiency before individuals are approved to perform these techniques unsupervised.
To contact a ULAR veterinarian or the ULAR Training Division, visit the “Who Do I Contact For...” page on the ULAR website.

**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 three-week period. For example:

- No more than 0.3 ml (300 µl) can be collected from a 30 g mouse, over a three week period.
- No more than 100 ml can be collected from a 10 kg dog, over a three week period.

If the maximum volume of blood (10 ml/kg) is collected at one time, it is *mandatory* that replacement fluid therapy be provided.

- For a mouse, 0.5-1.0 ml of 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).

After venipuncture, an animal cannot be returned to its cage until bleeding has stopped; that is, *complete hemostasis* is achieved. 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 in order 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 a ULAR veterinarian for the best option.
- If bleeding cannot be stopped, the ULAR veterinary team must be contacted immediately for emergency assistance.

Some protocols may require the use of blood for transfusions or other study related procedures. The expected use of these products should be described in the protocol, if applicable. Blood and/or blood products can be purchased from companies and stored for later use. Please consult with a ULAR veterinarian regarding the purchase of these products. Possible vendor options include:


The use of colony animals to serve as *blood donors* must be approved by the IACUC and described in the animal use protocol. If you require blood donors on your protocol, please be aware of the following items:

- You must provide justification as to why you need a blood donor and why you cannot obtain blood/blood products from another source.
- The protocol should specify if the animal is being used solely as a donor or if it may be used in other procedures described in the protocol.
- The animals should be screened prior to use to ensure they are clinically normal, not harboring disease and, if applicable, are compatible with the recipient(s) (e.g. cross matching).

**UPDATED 5/2013**
Blood donors should be given a “rest period” between samplings and/or other procedures to allow for recovery from significant blood draws. They should also be treated with replacement fluid therapy (see relevant section above). If the maximum amount of blood permitted for sampling is indeed removed, animals must be rested at least 3 weeks before additional blood may be collected again. For frequent transfusion procedures, it may be necessary to maintain more than one donor animal.

The protocol should clearly state who will be performing the blood draws, how and where the blood will be stored, and when the donor blood is expected to be used.

The protocol should clearly state the dose and type of anti-coagulant the blood will be collected into, and list this agent (in a blood transfusion procedure) as a drug used in the recipient animals.

The protocol should clearly state humane endpoints for the donor animals and how long they can serve as possible blood donors within the colony. In addition, disposition of these animals at the endpoints should be described (i.e. adoption, euthanasia or transfer to another protocol).

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 described.

Any exemptions to this guideline may be considered by the IACUC if scientifically justified. Please review Common Sites for Blood Collection on the IACUC website for specific guidance on possible blood collection sites for commonly used species.

Blood collection resources: