The University of Texas at Austin
Office of Research Support and Compliance
Office of the Vice President for Research

Guidelines for Blood Collection

The University of Texas at Austin Institutional Animal Care and Use Committee

These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.

This document provides information to be used when planning and performing blood collection procedures in vertebrate animals used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into two sections:

Section A – Background Information
Section B – Restraint During Blood Collection
Section C – Blood Collection Guidelines: General Volumes, Frequency and Other Considerations
Section D – Blood Collection Sites and Volumes by Species
Section E – Acknowledgements and References

Section A – Background Information

There are several factors to consider when determining the appropriate blood collection volume and technique. These include:

- The species to be sampled;
- The size, age, and health of the animal to be sampled;
- The minimum volume required for analysis;
- The frequency of sampling necessary;
- The training and experience of the personnel performing the collection; and
- The suitability of sedation and/or anesthesia.

The sample volume selected should always be the minimum volume of blood that satisfies experimental needs. Appropriate restraint (physical or chemical) should be employed to minimize risk of injury to the animal and personnel.

Approximate volumes for animals commonly used in the laboratory:

- 5-10 percent of body mass = total blood volume
- 3-6 percent of body mass = expected yield of terminal exsanguination
- 1-2 percent of body mass = volume that can be safely collected with minimal physiological disruption

Section B – Restraint During Blood Collection

Restraint is necessary to prevent movement that may result in laceration of a blood vessel or other organ and serious complications. Restraint can be physical or chemical in nature, and should be chosen based on the species

and the site being used for blood collection.

For some species, blood collection may be adequately performed while the animal is awake using the appropriate restraint. Other times, chemical restraint may be required to collect blood from some animals/species. See the Animal Resource Center's anesthesia guidance for more information about species-specific anesthesia guidelines: https://research.utexas.edu/arc/arc-guidance/.

Section C – Blood Collection Guidelines: General Volumes, Frequency and Other Considerations

1) ***Single Blood Draw or Repeated Sampling at Intervals of Two Weeks or More***

The volume equivalent of 1% of the animal's body mass may be routinely removed. See Section D: Blood Collection Sites and Volumes by Species of this guideline for specifics per species.

This is the maximum blood volume to be routinely removed during a single survival bleed. It can be repeated at two-week intervals. It may be appropriate to sample a higher volume than this if the bleeding will NOT be repeated (for example, a single sampling of 1.5%) but this must be specifically documented and justified in the approved IACUC protocol.

If the blood volume removed from an animal exceeds the recommended 1.5% of body weight blood collection volumes but is approved in the IACUC protocol, fluid replacement should be considered in the IACUC protocol. The best fluid replacement options are Lactated Ringer's Solution (LRS) or 0.9% saline warmed to (30-35ºC).

- For mice: administered 1 ml of warmed LRS IP or SC.
- For rats: administer 5 -10 ml via SC administration.

2) ***Repeated Sampling at Intervals of Less Than Two Weeks***

If more frequent bleeding is needed, this volume should be proportionally reduced (i.e. only 50% of this volume or 0.5% of body mass sampled if weekly bleeds are to be performed). If more blood than this recommended maximum volume is needed, it must be specifically documented and justified in the approved IACUC protocol. In addition to addressing short-term volume replacement, the investigator may be required to coordinate with the veterinary group to arrange for animal observation and possible hematocrit monitoring.

An animal may go into hypovolemic shock if too much blood is withdrawn too rapidly or too frequently without replacement (approximately 2% of the animal's body weight at one time). If signs of shock are observed (e.g., increased pulse, pale mucus membranes, cold skin/extremities, hyperventilation), immediately contact ARC veterinary staff.

3) ***Terminal Blood Withdrawal***

It is not possible to extract all of the blood from an animal, but with some methods, up to 50-75% of total blood volume (equivalent to 4-5% of body weight) can be obtained by terminal exsanguination. Exsanguination by the removal of more than the maximum blood volumes listed in (1) or (2) above is allowed only if the animal has already been euthanized using an approved euthanasia method, or is anesthetized properly to eliminate distress during the terminal procedure. The animal's death must be verified at the end of the bleeding. Exsanguination under anesthesia is considered an appropriate euthanasia method in most cases, but this must be specifically described in the approved IACUC protocol.

4) ***Other Considerations***

Site Preparation: For blood collection, appropriate site preparation must be considered. Collection of sterile blood samples requires aseptic collection technique, including clipping or shaving of fur followed by aseptic preparation of the skin at the site prior to needle puncture of the vessel.

Blood Collection via Surgically Implanted Cannulas: The use of implanted cannula(s) allows for the removal of blood samples with minimal disturbance to the animal. If a cannula is exteriorized, it is important to ensure that the cannula is protected from trauma that may be caused by the animal or its cage mates. Proper flushing and maintenance is important to prolong catheter/cannula patency and ensure that a non-contaminated blood sample is obtained.

Blood Collection via Vascular Access Port (VAP): Subcutaneous vascular access ports (VAP) provide easy access to vessels and are a recommended alternative when serial sampling is required over days/weeks. Since the catheter does not exit through the skin, the risks of infection, tissue damage, and morbidity are reduced. Consideration should be given to establishing limits on the frequency and/or total number of times a subcutaneously implanted access port can be punctured over time. Repeated puncturing of the skin over an access port may cause necrosis of the skin and subsequent exposure of the access port through the skin with associated risk of infection. It is also important to ensure aseptic preparation of the skin over the port to minimize risk of infection and ensure use of appropriate non-coring needles to maintain the integrity and useful life of the VAP.

Section D – Blood Collection Sites and Volumes by Species

Blood collection sites and volumes for common laboratory animal species are described in this section. **Sites are listed from most common/desirable to least common/desirable based on ease of collection**. **For animal species not listed below, please contact the ARC veterinarians for more information.**

Key considerations:

- For smaller species, the approximate volume of blood attainable for each site is listed. Volumes are an estimate and will also depend on the size, health, and hydration status of the animal as well as the experience and skill level of the person collecting the sample.
- Certain sites may be preferable based on study goals and requirements. Additionally, publications indicate that blood analysis results (especially cellular indices) may vary based on blood collection site; consult the literature for more information.
- Cardiac puncture may be used to obtain a single, large volume of blood from heavily anesthetized (terminal procedure only) or euthanized animals.
- Alternatives to collecting blood by lacerating an ear or tail vessel must be considered and will be addressed during review and approval by the IACUC. These procedures may be more painful than needle punctures, can lead to excessive hemorrhage, will result in a prolonged time for wound healing and may be more susceptible to infection and other complications.
- An animal may not be returned to its cage until complete hemostasis has been achieved (i.e., no more blood is coming from the collection site), regardless of the collection method. Achieve hemostasis using gauze and direct pressure. Up to several minutes of pressure may be required following arterial puncture.

MICE

Total blood volume of a mouse is 75 ml/kg or 7.5% of total body weight or 0.75 ml/10 g body weight (BW). As

a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 20 g mouse mass volume= 200 ul; 25 g mouse mass volume= 250 ul; 30 g mouse mass volume= 300 ul

Site	Anesthesia	Repeated bleeds	Expected volume
Saphenous vein	No	Yes	100-200 ul
Submandibular vein	No	Yes	200-500 ul
Distal tail snip (1-2 mm)	Recommended*	Yes – limited	1-2 drops
Retro-orbital sinus	Required	Yes – limited	200 ul
Lateral tail vein	No	Yes	50-100 ul
Cardiac puncture (terminal only)	Required	Terminal only	~ 1 ml

* Depending upon the methods used, distal tail transection may require the use of general anesthesia and preemptive analgesia (e.g. NSAIDs, opioids) unless scientifically justified and approved in the IACUC protocol. Serial/repeated collections are possible using this technique by gently removing the clot/scab at the transection site. This technique is not appropriate for animals that have had a tail transection for genotyping.

RATS

Total blood volume of a rat is 64 ml/kg or 6.4 % of total body weight (BW). As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 200 g rat= 2 ml; 500 g rat= 5 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Saphenous vein	No	Yes	300-400 ul
Lateral tail vein	No	Yes	200-400 ul
Distal tail snip (1-2 mm)	Recommended*	Yes – limited	1-2 drops
Sublingual vein	Required	Yes	0.5-1.0 ml
Retro-orbital plexus	Required	Yes – limited	0.5-1.0 ml
Cardiac puncture (terminal only)	Required	Terminal only	~3 ml

* Depending upon the methods used, distal tail transection may require the use of general anesthesia and preemptive analgesia (e.g. NSAIDs, opioids) unless scientifically justified and approved in the IACUC protocol. Serial/repeated collections are possible using this technique by gently removing the clot/scab at the transection site. This technique is not appropriate for animals that have had a tail transection for genotyping.

GERBILS

Total blood volume of a gerbil is 72 ml/kg or 7.2% of total body weight or 0.72 ml/10 g body weight (BW). As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 50 g gerbil volume=500 ul; 100 g gerbil volume=1 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Lateral tail vein	No	Yes	200 - 400 ul
Retro-orbital sinus	Required	Yes - limited	100 - 200 ul
Cardiac puncture (terminal only)	Required	Terminal	~3 ml

HAMSTERS

Total blood volume of a hamster is 75 ml/kg or 7.5% of total body weight or 0.700 ml/10 g body weight (BW).

As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 100 g hamster=1 ml; 150 g hamster=1.5 ml; 200 g hamster=2 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Lateral tarsal vein	No	Yes	100 - 200 ul
Retro-orbital plexus	Required	Yes – limited	100 - 200 ul
Cardiac puncture (terminal only)	Required	Terminal only	~3 ml

GUINEA PIGS

Total blood volume of a guinea pig is 70 ml/kg or 7.0% of total body weight or 7 ml/100 g body weight (BW).
As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 700 g guinea pig= 7 ml; 900 g= 9 ml; 1100 g guinea pig= 11 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Auricular vein	No	Yes	50 - 100 ul
Cephalic vein	No	Yes	50 - 100 ul
Saphenous vein	No	Yes	400 - 500 ul
Cranial vena cava	Required	Yes	2 - 3 ml
Cardiac puncture (terminal only)	Required	Terminal only	

RABBITS

Total blood volume of a rabbit is 60 ml/kg or 6.0 % of total body weight (BW). As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 1 kg rabbit= 10 ml; 4 kg rabbit= 40 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Marginal ear vein/central ear artery	Local anesthesia recommended	Yes	0.5-1ml from vein; Up to max allowable from artery
Lateral saphenous vein	No	Yes	< 1ml
Cephalic vein	No	Yes	< 1ml
Jugular vein	Recommended	Yes	Up to max allowable
Cardiac puncture (terminal only)	Required	Terminal only	

NON-HUMAN PRIMATES (MACAQUES)

Total blood volume of a macaque is 55 ml/kg or 5.5 % of total body weight (BW). As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 5 kg macaque: 50 ml; 8 kg macaque=80 ml; 10 kg macaque=100 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Femoral vein	Required	Yes	Up to max allowable
Saphenous vein	Required	Yes	Small volume
Cephalic vein	Required	Yes	Small volume
Brachial vein	Required	Yes	Small volume

NON-HUMAN PRIMATES (MARMOSETS)

Total blood volume of a marmoset is 60 ml/kg or 6.0 % of total body weight (BW). As a general rule, 20 drops = 1 ml (i.e. 5 drops = 250 ul).
1% BW single draw volumes: 300 g marmoset= 3 ml; 400 g marmoset= 5 ml; 500 g marmoset= 5 ml

Site	Anesthesia	Repeated bleeds	Expected volume
Femoral vein	Recommended	Yes	Up to max allowable
Tail vein	Recommended	Yes	Small volumes

BIRDS

Total blood volume and body weight vary by species.

Site	Anesthesia	Repeated bleeds	Expected volume
Jugular vein (right side)	No		
Brachial vein	No	Yes	
Femoral vein	No		
Medial metatarsal vein (not recommended for small birds)	Recommended (general or local)		
Cardiac puncture (terminal only)	Required	Terminal only	

FROGS

While the literature suggests a range of blood that can be safely removed in healthy frogs is 50% of the blood at one time (about 5% of the body mass), it is recommended that collection be limited to 60-80ml/kg body weight.

Site	Anesthesia	Repeated bleeds	Expected volume
Ventral abdominal	No	Yes	
Femoral vein	No	Yes	
Lingual	No	Yes	
Cardiac puncture (terminal only)	Required	Terminal only	

FISH

Total blood volume and body weight vary by species.

Site	Anesthesia	Repeated bleeds	Expected volume
Caudal vein	Recommended		

Section E – Acknowledgements and References

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05/10/2021	• Section A – background information updated • Section B – restraint considerations added • Section C – details about fluid replacement, post-procedural complications, and other considerations added • Sections D and E added