

 BROWN	Institutional Animal Care and Use Committee (IACUC): Policy on Blood Collection in Laboratory Animals	POL 10.10.20
		Effective Date: October 4, 2016

1.0 Policy Purpose

The purpose of this policy is to provide direction to researchers on appropriate blood collection methods for common laboratory animal species.

2.0 To Whom the Policy Applies

This policy applies to all individuals who will be utilizing blood collection methods in laboratory animals at Brown University under an IACUC-approved animal use protocol.

3.0 Policy Statement

Many experimental animal use protocols require the collection of blood from laboratory animals for a variety of purposes. It is important for the researcher to consider the serious side effects of repeated blood collection and how to prevent them while planning an acceptable blood collection regimen. Collection of blood above the recommended volumes can lead to physiologic stress, anemia, hypovolemic shock, and death.

3.1 Circulating Blood Volume and Other Factors to Consider

When determining the amount of blood that can be safely collected, the phlebotomist must know the approximate circulating blood volume of the particular species. The circulating blood volume (CBV) is species-specific and is generally estimated as 5.5-7% (55-70 ml/kg) of total body weight. This can be used to determine the total blood volume (TBV) of an animal. However, in sick, pregnant, obese, and geriatric animals, the percent of total blood volume may be as much as 15% lower as compared to a healthy animal. In addition, care should be taken when handling pregnant females, since stress may induce premature birth. See Table 1 for circulating blood volumes and withdrawal volume examples for commonly used species. See Table 2 for recommended blood collection sites by species.

It has been shown that the method of blood collection may significantly affect blood values, most notably the white blood cell count. For example, warming of mice prior to blood collection via the tail vein may alter the Complete Blood Count (CBC) values as compared to non-warmed mice. Therefore, it is important that the same blood collection methods be used consistently throughout the research project.

Other factors that the researcher must consider prior to blood collection are:

- the method that causes the least amount of pain and distress to the animal;
- the volume of blood needed to fulfill the requirements of the experiment;
- the frequency of blood collection; and
- whether or not anesthesia is required.

Considering these factors will help to preserve the health status of the animal and maintain the validity of experimental results. In all cases, non-terminal blood collection without replacement fluids is limited to 10% of the total circulating blood volume of a healthy animal during a two-week period, unless otherwise approved in the IACUC protocol. If an animal shows signs of shock (*e.g.*, increased pulse, pale mucus membranes, cold skin/extremities, hyperventilation) following blood collection, Center for Animal Resources and Education (CARE) veterinary staff must be contacted.

3.2 Determining Total Blood Volume (TBV)

In order to determine the allowable blood volume that can be collected, the total blood volume (TBV) estimate for the animal must be calculated.

TBV must be calculated by the following formula:

Large Animals: (Body Weight in (Kg)) x (0.6) = Total Blood Volume (liters)

Rodents: (Body Weight in (g)) x (.06) = Total Blood Volume (ml)

3.3 Repeat Blood Collection

If repeat blood collection is necessary, the researcher must monitor the hematocrit (packed cell volume [PCV]) and/or hemoglobin in order to evaluate if the animal has sufficiently recovered from single or multiple blood draws. It is important to note that when acute blood loss has occurred, it may take up to 24 hours for the hematocrit (PCV) and hemoglobin to reflect accurate values.

If the animal is anemic (*i.e.*, below a species' normal PCV range as listed in Table 1), or the hemoglobin concentration is less than 10 gm/dL, then the researcher may not withdraw blood until the animal has had sufficient time to recover.

3.4 Training

Only trained personnel may collect blood from a research animal. Training in blood collection techniques is available through scheduled group training classes given by the CARE veterinary staff, or by request. For species not listed in Table 2, a CARE veterinarian must be contacted for guidance on blood collection volumes and phlebotomy sites.

4.0 Definitions: N/A

5.0 Responsibilities

All individuals to whom this policy applies are responsible for becoming familiar with and following this policy. University supervisors are responsible for promoting the understanding of this policy and for taking appropriate steps to help ensure compliance with it.

6.0 Consequences for Violating this Policy

Violation of this policy may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violations are a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

Failure to comply with this and related policies is subject to disciplinary action, up to and including suspension without pay, or termination of employment or association with the University, in accordance with applicable (e.g., staff, faculty, student) disciplinary procedures.

7.0 Related Information

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, **Brown's Anonymous Reporting Hotline** allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this policy and is not an all-inclusive list of policies, procedures, laws and requirements.

7.1 **Related Policies:** N/A

7.2 **Related Procedures:** N/A

7.3 **Related Forms:** N/A

7.4 **Frequently Asked Questions (FAQs):** N/A

7.5 **Other Related Information:** References:

- Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, deVorstenbosch CV. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl. Toxicol. 21:15-23 (2001).
- Guidelines for survival bleeding of mice and rats; NIH: http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf
- Hoggatt, J, Hoggatt, A, Tate, T, Fortman, J, Pelus, L. Bleeding the Laboratory Mouse: Not All Methods are Equal. J Exp Hematology. 44(2):132-137(2015)
- The Jackson Laboratory <https://www.jax.org/news-and-insights/2005/october/how-much-blood-can-i-take-from-a-mouse-without-endangering-its-health>
- University of Minnesota Guidelines for Collection of Blood from Experimental Animals

8.0 Policy Owner and Contact

8.1 **Policy Owners:** Vice President for Research

8.2 **Policy Approved by:** Vice President for Research

8.3 Subject Matter Contact: Brown University Animal Research Compliance

- Telephone: 401-863-3050
- Email: IACUC@Brown.edu

9.0 Policy History

9.1 Policy Effective Date: October 4, 2016

9.2 Policy Last Reviewed: January 21, 2021

9.3 Policy Update/Review Summary: This policy is not new; it was converted to the University's new policy template and re-reviewed by the IACUC at its convened meeting October 2, 2020. Previous policy version in old format superseded by this policy:

- IACUC Policy on Blood Collection in Laboratory Animals. Date of IACUC review and approval: October 4, 2019

Table 1. Circulating Blood Volumes and & Withdrawal Volume Examples for Common Species

Species (Weight)	Circulating blood volume (ml/kg)	Total Blood Volume (ml)	Normal PCV	1% CBV (ml) every 24 hrs	7.5% CBV (ml) every 7 days	10% CBV (ml) every 2 weeks	15% CBV (ml) every 4 weeks
Mouse (25g)	79	1.9	39-49%	0.02	0.14	0.19	0.29
Rat (250g)	64	16	36-54%	0.16	1.2	1.6	2.4
Rabbit (4kg)	56	224	30-50%	2.2	16.5	22.4	33.6
Pig (90kg)	65	5850	35-40%	58	440	585	878
Minipig (45kg)	65	2925	32-50%	29	219	292	438
Sheep (55kg)	60	3300	24-45%	33	247.5	330	495
Marmoset (230g)	70	16	23-52%	0.16	1.2	1.6	2.4
NHP rhesus (15kg)	67	1005	26-48%	10	75	100	150

Table 2. Recommended blood collection sites by species

<u>Mouse</u>	<u>Rat</u>	<u>Rabbit</u>	<u>Pig</u>	<u>NHP</u>	<u>Sheep/Ruminant</u>
Submandibular Vein	Submandibular Vein	Marginal Ear Vein	Marginal Ear Vein	Femoral Vein	Jugular Vein
Anesthesia: No	Anesthesia: No	Sedation: Yes	Sedation: Yes	Anesthesia: Yes	Anesthesia: No
Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes
Expected volume: 100-200 uL	Expected volume: 100-200 uL	Expected volume: 1-3 ml			
Lateral Tail vein	Lateral Tail Vein	Lateral Saphenous Vein	Right Jugular Vein	Cephalic Vein	Cephalic Vein
Anesthesia: No	Anesthesia: No	Sedation: Yes	Sedation: Yes	Anesthesia: required	Anesthesia: No
Repeat bleeds: Yes	Repeat Bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes
Expected volume: 50-100uL	Expected volume: 0.5-1 ml				
Saphenous Vein	Saphenous Vein	Cephalic Vein	Cephalic Vein	Saphenous Vein	Saphenous Vein
Anesthesia: No	Anesthesia: No	Sedation: Yes	Sedation: Yes	Anesthesia: Yes	Anesthesia: No
Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes
Expected volume 100-200 uL	Expected volume: 300-400 uL				
Retro-Orbital Sinus	Retro-orbital Plexus	Jugular Vein	Anterior Vena Cava	Brachial Vein	
Anesthesia: required	Anesthesia: Required	Anesthesia: Yes	Anesthesia: Yes	Anesthesia: required	
>7d between subsequent blood collections, alternate eyes between bleeds	>7d between subsequent blood collections, alternate eyes between bleeds	Repeat bleeds: Yes	Repeat bleeds: Yes	Repeat bleeds: Yes	
Expected volume: ~200uL	Expected volume: 0.5 – 1 ml				
Distal tail transection	Jugular Vein	Cardiac puncture: terminal			
(1-2 mm) No more than 4 mm total to be removed	Anesthesia: Required	Anesthesia: Required			
Anesthesia: if >21 days old	Repeat bleeds: Yes	Repeat bleeds: Terminal			
Repeat bleeds: Limited	Expected volume: 0.5-1 ml				
Cardiac puncture: terminal	Cardiac puncture: terminal				
Anesthesia: Required	Anesthesia: Required				
Repeat bleeds: Terminal	Repeat bleeds: Terminal				
Expected volume: 1 ml	Expected volume: 8-10 ml				