IACUC Guidance:	**TAMU-G-017**	Title:	**IACUC Guidelines on Blood Collection**

Location	Effective Date	Review By
College Station/Dallas/Galveston/Kingsville	01/01/2023	12/31/2025
Houston	02/01/2023	12/31/2025

1. PURPOSE

1.1. This document provides direction and guidance on appropriate blood collection methods and volumes for animals used in research, teaching, testing or other purposes at Texas A&M University. These guidelines are intended for use by qualified personnel performing blood collection as described on an IACUC-approved Animal Protocol.

2. SCOPE

2.1. Does not apply to blood collected post-euthanasia of animals.
2.2. Does not include an exhaustive list of all methods of venipuncture.

3. RESPONSIBILITY

3.1. The **AV** (or designee) is responsible for evaluation of large sample volumes or collection frequencies greater than outlined in this document.
3.2. The **IACUC**, **AV** (or designee) together with PI are responsible for minimizing potential adverse effects, including replacement fluids or other methods.
3.3. The **PI** is responsible for:
3.3.1. Providing complete descriptions of proposed animal procedures in the protocol.
3.3.2. Consulting with the AV, especially regarding long-term repeated access to blood vessels.
3.3.3. Providing training opportunities and resources to new personnel learning approved procedures (See TAMU-G-029).
3.3.4. Ensuring AUP personnel have demonstrated proficiency in blood collection procedures <u>before</u> performing these animal activities independently (See TAMU-G-029).
3.4. **AUP Participants** are responsible for seeking assistance from experienced individuals when learning a new method of blood collection.

4. DEFINITIONS AND/OR ACRONYMS

4.1. **AUP**: Animal Use Protocol. Document submitted by the PI indicating the housing and procedures involving animals.
4.2. **AV**: Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
4.3. **CBV**: Circulating Blood Volume. The total amount of fluid circulating within the arteries, capillaries, veins, venules, and chambers of the heart at any time.
4.4. **Crocodilian**: Crocodiles, alligators, and gharial.
4.5. **Hypovolemia/Hypovolemia Shock**: Occurs when there is a significant blood volume deficit (approximately 15-25%).
4.6. **IACUC**: Institutional Animal Care and Use Committee. Institutional body responsible for ensuring adherence to federal regulation and institutional policy relating to the care and use of animals in teaching, testing and research. Appointed by the Institutional Official.
4.7. **PI**: Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
4.8. **Refinement**: A change in some aspect of the experiment that results in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience.
4.9. **Urodele**: An amphibian of the order *Urodela*; a newt or salamander.

4.10. **VVC:** Veterinary Verification and Consultation. Process by which the AV or designee confirms adherence to approved IACUC SOPs or Guidance documents. Does not apply to Houston animal program.

5. GUIDELINES OR PROCEDURE

5.1. There are several factors to consider when determining the appropriate blood collection volume and technique. These include:
- 5.1.1. The species to be sampled
- 5.1.2. The size of the animal to be sampled and the estimated total blood volume
  - 5.1.2.1. When calculating blood volumes based on body weights, remember that body weights in kilograms (kg) will convert to blood volumes in liters, and weights in grams (g) will convert to volumes in milliliters (ml).
- 5.1.3. The age and health of the animal to be sampled
- 5.1.4. The minimum volume required for analysis
- 5.1.5. The type of sample required (whole blood, serum, etc.)
- 5.1.6. The frequency of sampling necessary
- 5.1.7. The training and experience of the personnel performing the collection
- 5.1.8. The suitability of sedation and/or anesthesia

5.2. The sample volume selected should always be the minimum volume of blood which satisfies experimental needs.

5.3. Appropriate restraint (physical or chemical) should be employed to minimize risk of injury to the animal and personnel.

5.4. The maximum permitted volume of blood collected includes any blood lost during sampling.

5.5. **Toenail clipping** for blood collection is generally discouraged where not specifically described within this document.

5.6. Fluid Replacement
- 5.6.1. Consult with the AV, or designee to determine the need for, type, amount, and route of fluid replacement.
- 5.6.2. In general, replacement fluids (e.g. Lactated Ringer's Solution) should be warmed and given subcutaneously, or as directed by the AV, or designee.
- 5.6.3. Protects from hypovolemia.

5.7. Serial Sampling
- 5.7.1. Increases the risk of hypovolemia, pain and distress.
- 5.7.2. If the study involves repeated blood sample collection, the samples can be withdrawn through a temporary cannula, as appropriate per species. This may reduce pain and stress in the experimental animals and is an example of refinement.
  - 5.7.2.1. Consult with the AV, or designee regarding the appropriateness of vascular catheters and opportunities for technique training.

5.8. Hemostasis
- 5.8.1. In most cases, hemostasis can be achieved by applying gentle pressure to the collection site with gauze.
- 5.8.2. If the retro-orbital sinus/plexus is used, gauze should be applied only after closing the lids to protect the cornea.
- 5.8.3. If bleeding does not stop within five minutes, the AV (or designee) must be immediately contacted for guidance.

5.9. Acceptable Quantity and Frequency of Blood Sampling in Rodents
- 5.9.1. Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1% every 24 hours.
- 5.9.2. 1mL=1000µL
- 5.9.3. For estimation, 20 drops = 1 mL (i.e. 5 drops = 250 uL)
- 5.9.4. Approximate Blood Sample Volume Ranges Guide for Mice and Rats

Body Weight (g)	CBV (mL)	1% CBV (mL) every 24 hrs	7.5% CBV (mL) every 7 days	10% CBV (mL) every 2-4 weeks
20	1.1-1.4	0.011-0.014	0.082-0.105	0.11-0.14
25	1.37-1.75	0.014-0.018	0.1-0.13	0.14-0.18
30	1.65-2.1	0.017-0.021	0.12-0.16	0.17-0.21
35	1.93-2.45	0.019-0.025	0.14-0.18	0.19-0.25
40	2.2-2.8	0.022-0.028	0.16-0.21	0.22-0.28
125	6.88-8.75	0.069-0.088	0.52-0.66	0.69-0.88
150	8.25-10.5	0.082-0.105	0.62-0.79	0.82-1.0
200	11.0-14.0	0.11-0.14	0.82-1.05	1.1-1.4
250	13.75-17.5	0.14-0.18	1.0-1.3	1.4-1.8
300	16.5-21.0	0.17-0.21	1.2-1.6	1.7-2.1
350	19.25-24.5	0.19-0.25	1.4-1.8	1.9-2.5

5.10. Distal Tail Amputation (Mice/Rats)

5.10.1. Performed as early as possible to minimize potential pain.
5.10.2. Clean collection site with alcohol.
5.10.3. Excised length is 1-2mm.
5.10.4. For preweanling animals (<21 days of age), the use of anesthesia is suggested.
5.10.5. For mice 21 days of age or older, the use of anesthesia is required unless justified in the AUP and approved by the IACUC.
5.10.6. For rats 21-35 days of age, the use of local or general anesthesia is required unless justified in the AUP and approved by the IACUC.
5.10.7. For rats (>35 days of age) general anesthesia is required.
5.10.8. Post-procedural analgesia should be considered.
5.10.9. For repeat blood collection, it may be possible to remove the scab at the collection site. If not, an alternate, protocol-approved method of blood collection should be employed.
5.2.2.10. Consultation with CMP/ARU/PAR/PRF is recommended for direction in the appropriate choice of anesthetics/analgesics, which must be described in the approved AUP.

5.11. Retro-orbital Blood Collection in Rodents

5.11.1. Retro-orbital blood collection can provide moderate to large amounts of blood when performed by well-trained personnel. However, severe injuries may occur to the animal if this procedure is not done properly. Appropriate training and proficiency in the technique are required.
5.11.2. If retro-orbital blood collection is performed, the following guidelines apply to rats and mice:
5.11.2.1. The use of retro-orbital bleeding must be described in the protocol and approved by the IACUC.
5.11.2.2. Only one eye may be sampled at any time.
5.11.2.3. An alternative site of venipuncture must be described and approved in the protocol for unsuccessful retro-orbital collections, rather than reattempting retro-orbital collection from the same or opposite eye.
5.11.2.3.1. Changes related to blood collection (e.g., frequency, volume, vessel of access) can potentially be made through VVC, provided the changes, in the judgment of the VVC veterinarian, do not unduly impact animal welfare.
5.11.2.4. Veterinary staff should be consulted for demonstration and training of proper technique to reduce risk of trauma.
5.11.2.5. General anesthesia is required. Exceptions require scientific justification, IACUC approval, demonstration of proficiency and pre-procedural application of topical ophthalmic anesthetic (i.e. proparacaine).

5.11.2.5.1. Note: Topical ophthalmic anesthetic should also be considered for rodents under an anesthetic event during retro-orbital blood collection for analgesia.

5.11.2.6. Blood collection via this method is to occur no more frequently than every 10 days in the same orbit.

5.11.2.6.1. Exception: If repeated sampling within 8 hours is necessary and approved in the AUP, the retro-orbital sinus (mouse) may be re-sampled by disrupting the blood clot (from the original collection site) without repeated damage to the sinus, provided the 24 hour maximum blood collection limits are not exceeded.

5.11.2.7. If bleeding does not stop within 5 minutes, or if injury and/or rupture of the eye or surrounding tissues occurs, the animal must be immediately euthanized (per protocol) or the AV, or designee must be immediately contacted for guidance.

5.11.2.8. Following the procedure, monitoring should be increased to note any signs of ocular trauma: squinted eye, ocular discharge, shrunken globe, evidence of (peri-ocular) self-trauma, corneal opacities.

5.11.2.8.1. Early Removal Criteria should address these clinical signs (See TAMU-G-001)

5.11.2.9. Training or competency certification

5.11.2.9.1. See TAMU-G-029 for Guidelines on Animal Protocol Participation

5.12. Guidelines for Blood Collection of Poultry

5.12.1. Poultry have a relatively small percentage of blood volume by body weight, approximately 6–7.5%.

5.12.2. No more than 1% of the body weight equivalent of blood should be taken in a single collection, and the chicken should be allowed at least 14 days to recover before more blood is withdrawn, unless otherwise approved in the animal use protocol.

5.12.3. When collecting from the jugular vein, the right jugular vein is usually chosen over the left as it is typically larger than the left in avian species.

5.13. Guidelines for Blood Collection of Wildlife Species

5.13.1. The limit of blood that can be collected is ≤1% of an animal's body mass.

5.13.2. Sample collections greater than 1% will be carefully evaluated by the IACUC for the appropriateness of the species and situation.

5.13.3. Bats

5.13.3.1. Blood volume of bats comprises ~10% of the total body weight (9.0–11.0 ml per 100g).

5.13.3.2. Consider feeding bats before and after venipuncture as they require energy to compensate for heat loss (especially during anesthesia).

5.13.4. Common Blood Collection Sites in Wild Small Rodents:

5.13.4.1. Facial vein

5.13.4.2. Caudal Vein

5.13.4.2.1. Obtaining from the vein, puncturing the vein, or excising the distal 1-2mm of tail

5.13.4.3. Retro-orbital sinus

5.13.4.3.1. When alternate methods are unsuitable

5.13.4.3.2. Potential sequelae minimized via training of personnel and the use of short-acting anesthesia.

5.14. Guidelines for Blood Collection of Fish

5.14.1. The volume of blood per unit of body weight is less in bony fish (compared to mammals).

5.14.2. During a single collection from a non-compromised, healthy fish, blood can be collected in a volume less than or equal to 0.5 % of body weight. No more than 1% of the fish's body weight (i.e. 10mL/kg) should be removed from a fish that will be recovered from blood collection.

5.14.3. Three main techniques have been devised for collecting blood from fishes: cardiac puncture, venous puncture, and caudal bleeding. The tail is the preferred site for blood sampling. The vessels running beneath the vertebrae of the fish can be sampled by using a lateral or ventral approach.

5.14.4. For adult zebrafish, the volume for repeated blood sampling at intervals should be ≤0.4% of body weight every week or ≤1% every 2 weeks (7.1.20.)

5.15. Guidelines for Blood Collection of Amphibians and Reptiles

5.15.1. During a single collection from a non-compromised, healthy animal, blood can be collected in a volume less than or equal to 0.7 % of body weight.

5.15.2. Common Routes of Blood Collection in Reptiles

Species	Route(s) of Blood Collection
Urodeles	heart, abdominal vein and ventral tail vein
Frogs	lingual venous plexus, ventral abdominal vein, and tail vein
Turtles and Tortoises	jugular vein, brachial vein*, brachial artery*, ventral coccygeal vein, and trimmed toenails; scapular vein*
Crocodilians	supravertebral vessel, heart (via cardiocentesis) and ventral coccygeal vein
Lizards	ventral tail vein (size dependent), toenails, orbital sinus
Snakes	palatine veins, ventral tail vein, and via cardiocentesis**

*vessels associated with limbs can rarely be visualized and sampling is usually blind; samples may be hemodiluted with lymph

**snakes over 300g

5.16. Frequent Calculations (mouse and rat volumes outlined in section 5.8)

Species	**Mean Circulating Blood Volume (mL/kg)**
Mouse	72
Rat	64
Gerbil	72
Guinea Pig	70
Hamster	72
Rabbit	56
Ferret	70
Dog	70-110 (huge breed variation)
Cat	56
Pig	67
Cattle	60
Sheep	60
Horse	75
Goat	70
Snake	50-80

Species (Weight)	**Total Blood Volume**	**Maximum Volume at weekly intervals (7.5% CBV)**	**Maximum Volume at intervals of every 2-4 weeks (10% CBV)**
Gerbil (50-60g)	3.6mL	0.3mL	0.4mL
Guinea Pig(900g)	62mL	4.7mL	6.2mL
Hamster(100g)	7.2mL	0.5mL	0.7mL
Rabbit(4kg)	224mL	17mL	22mL
Ferret(1kg)	70mL	5.2mL	7mL
Dog(10kg)	850mL	64mL	85mL
Snake(100g)	7.2mL	0.5mL	0.7mL
Pig(100kg)	6.7L	500mL	670mL
Cattle (500kg)	30L	2.3L	3L
Sheep (45kg)	2.7L	202mL	270mL

Horse (430kg)	32L	2.4L	3.2L
Goat (40kg)	2.8L	210mL	280mL

5.17. Common Routes of Blood Collection

Species	Common Blood Collection Route(s)	Sedation Recommended	Anesthesia Required	Expected Volume
Mouse	Facial vein [(cheek bleed) limited to adults)**		No	~100 µl
	Tail vein		No	50-100 µl
	Distal tail amputation 1-2mm		Age-Dependent	<100 µl
	Tail nick		No	50-100 µl
	Saphenous vein**		No	100-200µl
	Retro-orbital sinus* (see 5.10)		Required	~200 µl
	Cardiac or Abdominal Aorta (non-survival)		Required	~1mL
Rat	Tail vein		No	200-400 µl
	Distal tail amputation		Age-Dependent	~100 µl
	Tail nick			100-200 µl
	Saphenous vein**		No	300-400 µl
	Jugular vein**		Recommended	0.5-2.0mL
	Retro-orbital plexus* (see 5.10)		Required	0.5-1.0mL
	(Sub)Lingual vein		Required	0.5-1.0 mL
	Cardiac or Abdominal Aorta (non-survival)		Required	~3mL
Fish	Tail Vein		Recommended	
Zebrafish	Dorsal Aorta/Posterior Cardinal Vein		Required	
	Cardiac (non-survival)^		Required	1.0-10 µl
Ferret	Cephalic vein**		No	0.5-1.0 mL
	Saphenous vein**			0.5-1.0 mL
	Jugular vein**	Yes	Recommended	2.0-3.0 mL
	Cranial/Anterior vena cava	Yes	Recommended	2.0-3.0 mL
	Cardiac (non-survival)		Required	~5.0 mL
Rabbit	Marginal ear vein**		Local Recommended	1-3mL
	Central auricular artery**		Local Recommended	1-3mL
	Cephalic vein**		No	0.25-0.5 mL
	Saphenous vein**	Yes	No	0.25-0.5 mL
	Cardiac (non-survival)		Required	20-70 mL (body weight dependent)
Hamster	Saphenous vein**			100-200 µl
	Jugular vein**	Yes		200-300 µl
	Cranial vena cava	Yes		200-300 µl
	Cardiac (non-survival)		Required	1-2 mL
Guinea Pigs	Ear vein**		No	droplet
	Saphenous vein**		No	400-500 µl
	Jugular vein**		Recommended	2-3mL
	Cranial vena cava**	Yes	Recommended	2-3mL

	Cardiac (non-survival)		Required	3-5 mL (body weight dependent)
Gerbils	Lateral saphenous vein**			50-100 µl
	Cranial vena cava**	Yes		100-200 µl
	Cardiac (non-survival)		Required	1-2 mL
Xenopus	Dorsal tarsal vein**		Required	
	Cardiac (survival)		Required	
	Cardiac (non-survival) (also tadpoles)		Required	
Bats	Cephalic Vein*		May be required if phlebotomist is alone	~90 µl (adults)
	Saphenous (Interfemoral Vein)*			
	Cardiac (non-survival)		Yes	
Birds/Poultry	Brachial wing vein**			
	Jugular vein**			
	Medial metatarsal (caudal tibial) vein**			
	Cardiac (non-survival)		Yes	
Dog, Cat	Cephalic vein**		No	1-3 mL
	Saphenous vein (lateral-dog; medial-cat)**		No	1-3 mL
	Jugular vein**		No	5-10 mL
Pig	Ear vein**	Yes	No	1-2 mL
	Cephalic vein**		No	1-2 mL
	Cranial/Anterior vena cava**	As needed	Recommended	5-10 mL (body weight dependent)
	Jugular vein**	As needed		5-10 mL (body weight dependent)
Horses	Jugular vein**		No	>10 mL (body weight dependent)
	Cephalic vein**			
	Transverse facial vein/sinus*			
Ruminants	Jugular vein**		No	>10 mL (body weight dependent)
	Lateral saphenous vein**		No	>10 mL (body weight dependent)
	Tail vein		No	>10 mL (body weight dependent)

* A maximum of 3 procedures may be performed per eye (up to 6 collections total per animal).
**Contralateral side should be used in subsequent collections.
^ To obtain enough blood, samples from different fish may need to be pooled for analysis.

6. EXCEPTIONS

6.1. The PI may request an exception to the above standards by describing the departure in the AUP
6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

7.1. References:

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7.1.2. AAALAC International. 2016. Caveats from AAALAC's Council on Accreditation regarding the resource: 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education.

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7.1.6. Diehl, K.L., Hull, D., Phister, R., et al. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology 21: 15-23, 2001.

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7.1.8. Eshar, D and M. Weinberg. 2010. Venipuncture in Bats. Lab Animal. Volume 39, No. 6.

7.1.9. The Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Research and Teaching, 2010.

7.1.10. Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, 2004. Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research.

7.1.11. Hooper, S., Amelon, S. Handling and blood collection in the little brown bat (Myotis lucifugus). Lab Anim 43, 197–199 (2014). https://doi.org/10.1038/laban.543

7.1.12. Kelly, L.M. and L.C. Alworth. Lab Animal. Volume 42. Number 10. 2013. Techniques for Collecting Blood from the Domestic Chicken.

7.1.13. Kramer, M.H. and D.J. Harris. Journal of Exotic Pet Medicine, Vol 19, No 1 (January), 2010: pp 82-86.

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7.1.15. National Centre for the Replacement, Refinement & Reduction of Animals in Research: Blood Sampling

7.1.16. Zebrafish: CITI Working with Zebrafish (Danio rerio) in Research Settings

7.1.16.1. Web page: https://about.citiprogram.org/en/homepage/

7.1.16.2. Instructions: https://rcb.tamu.edu/animals/training

7.1.17. Sikes, R.S. and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. Journal of Mammalogy, 97(3):663–688, 2016 DOI:10.1093/jmammal/gyw078

7.1.18. Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, Maryland.

7.1.19. Wolfensohn & Lloyd, 2003, Handbook of Laboratory Animal Management and Welfare, 3rd Edition.

7.1.20. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals [published correction appears in J Pharmacol Pharmacother. 2017 Jul-Sep;8(3):153]. J Pharmacol Pharmacother. 2010;1(2):87–93. doi:10.4103/0976-500X.72350

7.1.21. Zang L, Shimada Y, Nishimura Y, Tanaka T, Nishimura N. Repeated Blood Collection for Blood Tests in Adult Zebrafish. *J Vis Exp*. 2015;(102):e53272. Published 2015 Aug 30. doi:10.3791/53272

7.2. Contact veterinary staff for hands-on training opportunities as well as animal health care. A duty supervisor and veterinarian are on call 24 hours a day.

7.2.1. CMP at (979) 845-7433

7.2.2. PAR: at (713) 677-7471

7.2.3. PRF: at (361) 221-0770

7.3. Animal Welfare Office - 979.845.1828 or animalcompliance@tamu.edu

7.3.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
7.3.2. TAMU-G-001: Guidelines on Choosing Appropriate Endpoints
7.3.3. TAMU-G-029: Guidelines on Animal Protocol Participation

7.4. Acknowledgements

7.4.1. This document was partially prepared using materials obtained from the Universities of Iowa, Tennessee Minnesota and Arizona.

8. HISTORY

Effective Date	Version #	Description
04/30/2020	000	College Station/Galveston: New Format and Updated Content; replaced unnumbered SOP ("Guidelines on Blood Collection") Reviewed and approved via email.
05/25/2020	001	Houston/Kingsville: New document. Reviewed and approved via email.
06/16/2020	002	Dallas: New Document
03/24/2022	003	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	004	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
01/01/2023	005	College Station/Dallas/Galveston/Kingsville: Renewal; updated definitions, expansion in toe clipping prohibition, addition of site preparation, addition of exceptions section; clarified units when using body weight to estimate blood volume. Reviewed and approved via email.
02/01/2023	006	Houston: Renewal; updated definitions, expansion in toe clipping prohibition, addition of site preparation, addition of exceptions section; clarified units when using body weight to estimate blood volume. Reviewed and approved via email.