

Policy 355

Blood Collection

Responsible Official:	Research Administration
Administering Division/Department:	Research Compliance and Regulatory Affairs / IACUC
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355.1 Preface:

Blood collection (venipuncture, phlebotomy) is a common and important specimen collection procedure in the conduct of research. In many protocols, multiple blood draws are an important part of collecting and analyzing data. The Emory University Institutional Animal Care and Use Committee (IACUC) developed a policy to best enable blood collection while minimizing the potential for pain, unnecessary stress, distress, or untoward effect in research animals. These are articulated by way of this general overview supplemented by companion documents appropriate to certain species.

The general standards are followed by species-specific sections. These sections are more precise, offering specific alternatives and guidance regarding:

- the frequency and total number of blood collection events;
- maximum collectable volumes allowed based upon specific physiology;
- detailing allowable routes particular to each species;
- differentiating between terminal and survival circumstances;
- disclosing requirements for anesthesia or restraint;
- scientific qualifiers and addressing conditionally permissible methods or
- settings germane to a species.

This list is not exhaustive and persons requiring information regarding the following items are encouraged to contact the Training Coordinators for their specific site.

- the supplies and equipment needed,
- specifics of restraint or anesthesia,
- requirements for ancillary care,
- habituation requirements,
- application to study in the field, etc.



o **DAR Training Request:**

- SOM DAR Training: dartrn@emory.edu
- Emory Primate Center (EPC) DAR Training: epc-dartrn@emory.edu

355.2 General Requirements

355.2.1 Blood collection procedures must be appropriately documented in pertinent sections of the IACUC application and specifically approved by the IACUC.

355.2.2 The protocol must include a description of:

- all potential collection sites/methods,
- any surgical preparation (if applicable),
- the amount(s) to be withdrawn,
- the frequency of blood collection and/or interval between blood collection events,
- the total number of blood collection events planned per subject,
- the maximum number of needle sticks attempts per collection site to not exceed three,
- and the type of restraint needed (including animal training techniques).

It is important to take into account the total blood volume yielded from the chosen blood collection technique when addressing the combination of frequency and volume of blood collection.

355.2.3 If the protocol involves more than one species, blood collection procedures must be addressed for each species.

355.2.4 The IACUC may require competency demonstration and/or training in the blood collection techniques necessary for the conduct of the work, as a condition of protocol approval.

355.2.5 The standards and species-specific guidelines detailed in this document, apply to adult subjects in apparent good health, normal body condition (i.e., within normal body weight for sex and age), and adequate plane of nutrition, with an uneventful experimental history and under non-intensive blood collection schemes. In the case of subjects expected to not meet these criteria, the protocol must include a program of supportive care and monitoring that takes into consideration the subject's status and experimental needs.

355.2.6 To prevent complications associated with multiple blood collections such as anemia and the adverse effect it can have on experimental outcomes, any deviations to the policy will require IACUC review and approval based upon acceptable scientific justification.

355.3 General Standards

355.3.1 For optimal health and to prevent hypovolemic shock, physiological stress and death, blood draws should be limited to the lower end of the volume allowances provided. Maximum blood volumes should be taken only from healthy animals or as terminal procedures.

355.3.2 No animal may be left unattended until hemostasis (i.e., bleeding has stopped) is achieved and, if applicable, it has recovered sufficiently from anesthesia.

355.3.3 To prevent infection or other complications at blood collection sites, clip away fur/hair (if applicable) and disinfect blood collection area. No needle reuse between animals.



355.3.4 Although there may be species-specific differences, as a general rule the approximate blood volume of an animal is in the range of 6-8% of body weight (i.e., 60-80 ml/kg). In the case of species regulated under the federal Animal Welfare Act Regulations, the maximum blood volume recognized by USDA for each species in its "Animal Welfare Inspection Guide" are applied as follows:

- Cat: 66 ml/kg
- Dog: 86 ml/kg (USDA, 2013)
- Gerbil: 67 ml/kg (USDA, 2013)
- Goat: 70 ml/kg (USDA, 2013)
- Guinea pig: 75 ml/kg (USDA, 2013)
- Hamster: 78 ml/kg (USDA, 2013)
- Sheep: 66 ml/kg (USDA, 2013)
- Swine: 65 ml/kg
- Rabbit: 62 ml/kg (USDA, 2013)
- Nonhuman primates: refer to specific guidelines

In the case of species not regulated under the federal Animal Welfare Act Regulations, like mice and rats, the maximum blood volume recommended by USDA for each species in its "Animal Welfare Inspection Guide" are as follows:

- Mouse: 80 ml/kg (USDA, 2013)
- Rat: 64 ml/kg (USDA, 2013)

355.3.5 For purposes of these guidelines, grams in body weight are equivalent to milliliters of blood. Each drop of blood is the equivalent to 0.05 milliliters (i.e., 50 microliters).

355.3.6 With the exception of nonhuman primates, which are addressed within the species-specific section, the maximum blood volume that can be safely removed from all other species for a one-time sample **without** fluid replacement is 10% of the total circulating blood volume (CBV), in the range of 6-8 ml/kg, and as stipulated in 355.3.4 above.

- With fluid replacement, up to 15% of the CBV or approximately 12 ml/kg can be removed.
- In the case of fluid replacement therapy, for every 1 ml of blood collected, 3 ml of crystalloid fluids (e.g., lactated Ringer's solution, 0.9% saline) should be administered SC or slowly IV immediately thereafter. IV administration may be necessary under certain circumstances and requires appropriate skills and/or training.
- Fluid replacement therapy does not allow for blood collection more frequently than the established guidelines.

355.3.7 Exsanguination may only be used as a terminal procedure under adequate anesthesia. It is possible for exsanguination to yield approximately half of the total circulating blood volume. This is equivalent to about 35 ml/kg but varies depending upon species. All non-survival procedures must be followed by a primary method of euthanasia.

355.3.8 Multiple Sample Collection

355.3.8.1 General: If the recommended maximum blood draw is performed, there are several published suggestions as well as regulatory guidance from USDA, where applicable, on how much time should elapse between blood draws. The Emory IACUC requires that where the maximum amount of blood is drawn on one occasion, a recovery period of at least 2 weeks between blood draws is necessary. For experiments that do not



require the suggested maximum blood draw, blood can safely be drawn more frequently as detailed in the table below:

Circulating Blood Volume	Body Weight Equivalent	Volume per Unit Body Weight	Recovery Time	Replacement Fluids
15%	1.2%	12 ml/kg	4 weeks	Required
10%	0.8%	8 ml/kg	2 weeks	Recommended
7.5%	0.6%	6 ml/kg	1 week	Optional

Calculations:

Mean species blood volume x body weight* x volume per unit body weight = Maximum volume for a single sampling

*Body weight should be measured precisely except for mice and gerbils where the approximate representative body weight considering the age, gender, and species may be used.

355.3.8.2 Depending upon the combination of frequency and volume of collection, the IACUC may require monitoring for anemia and other health indicators or specific treatments.

355.3.8.3 The use of vascular access ports are recommended when serial samples are required over a period of days or weeks. Animal training to facilitate blood collection may reduce stress for certain species during the procedure.

355.3.8.4 Serial micro-sampling in rodents, such as blood glucose monitoring for example, can be accomplished by using a combination collection technique consisting of a single, initial tail vein nick (50uL blood drop) with subsequent scab removal (10uL blood/drop). When using this approach, replacement fluids may not be necessary provided appropriate post-sampling hemostasis, gentle scab removal, and weight-specific calculations defining the maximum number of scab removal blood collections are appropriately addressed. It should be noted that chronic, continuous micro-sampling studies may still be required by IACUC to monitor or treat animals as described in 355.3.8.2. Serial sampling in rodents that require the removal of larger volumes, such as pharmacokinetics studies, will be reviewed by IACUC on a case-by-case basis.

It is recommended that serial sample collections sites be alternated in any species, if multiple needle sticks are required for blood collection.

355.3.9 Sources

- Animal Welfare Inspection Guide, United States Department of Agriculture. September 2021 [https://www.aphis.usda.gov/animal_welfare/downloads/Animal-Care-Inspection-Guide.pdf]
- Diehl K-H, et al. 2001. A good practice guide to the administration of substances and removal of blood including routes and volumes. J Appl Toxicol 21: 15-23.
- Wolfensohn, S., & Lloyd, M. (2008). Handbook of laboratory animal management and welfare. John Wiley & Sons



355.4 Species-specific Guidance

(Note: Navigation around this large pdf document is best accomplished using the bookmarks function.)

355.4.1 Birds

355.4.1.1 The most frequently sampled sites for small birds are the brachial and right-sided jugular veins. Toenail clipping can be residually painful, often results in skewed cell distributions, limits the amount of blood that can be collected and is not an approved method.

355.4.1.2 As with other species, the avian blood volume is approximately 6-11% of body weight

(Campbell, 1994; Dein, 1986; Samour, 2006) and, also as with many other species, most healthy Passeriformes and Psittaciformes can lose 10% of the blood volume, or the equivalent of 1% of the body weight, without ill effects

355.4.1.3 As birds may be delicate and can be highly stressed during restraint, careful handling by skilled personnel is a requisite.

355.4.1.4 Hematomas occur more frequently in birds than in mammals during venipuncture due to the relatively loose connective tissue surrounding veins and high venous pressure (Clark, et al., 2009). Hematoma formation may interfere with subsequent attempts at venipuncture.

355.4.1.5 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.1.6 Table 1: Survival Blood Collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures

Table with 3 columns: Collection Site, Indications, and Additional Considerations. It details two methods: Brachial (alar, cutaneous ulnar, basilic) vein and Right side Jugular vein.



Femoral vein (punctured at knee level)	Anesthesia not necessary	Requires two persons Yields small volumes
Medial metatarsal vein	Hematoma formation is rare Can be done with one person	General or local anesthesia may be necessary Yields only small volumes Not useful in small birds

355.4.1.7 Table 2: Non-survival Blood Collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Advantages	Disadvantages
Cardiac Puncture/cardiocentesis	Allows for maximum blood volume collection	Non-survival procedure only Requires anesthesia
Decapitation	Allows for large volumes of mixed blood to be collected.	Sample may be contaminated Aesthetically displeasing Special equipment necessary Potentially hazardous for operator
Occipital sinus	May yield moderate samples in the hands of a skilled operator	Training required Requires anesthesia
Exsanguination from surgically accessed internal vessels	Allows for maximum blood volume collection Sterile samples possible	Non-survival procedure only Requires surgical approach Requires anesthesia

355.4.1.8 Sources

- Campbell TW. 1994. Hematology. In Ritchie BW, Harrison GJ, Harrison LR (Eds.): Avian Medicine: Principles and Application. Wingers Publishing Inc. , Lake Worth, FL, pp176-198
- Harrison, G. J., & Harrison, L. R. (1986). Clinical avian medicine and surgery: including aviculture (No. V605 HARc). pp. 174-191.
- Djojogugito, A. M., Folkow, B., & Kovách, A. G. (1968). The mechanisms behind the rapid blood volume restoration after hemorrhage in birds. Acta Physiologica Scandinavica, 74(1-2), 114-122. pp.114-22.
- Sheldon SD, et. al. 2008. Effects of blood collection on wild birds: an update. J Avian Biol 39(4): 369-78.
- Zimmermann, N. G., & Dhillon, A. S. (1985). Blood sampling from the venous occipital sinus of birds. Poultry Science, 64(10), 1859-1862.
- Samour, J. (2006). Diagnostic value of hematology. Clinical avian medicine, 2, 587-610. pp587- 628
- Krautwald-Junghanns, M. 2007. Aids to diagnosis. Essentials of Avian Medicine and Surgery. 3rd ed. Oxford, UK: Wiley-Blackwell, 57-102.



- Harcourt-Brown, N. H. (2000). Psittacine birds. Avian Medicine. Edinburgh: Butterworth-Heimann, 1, pp 122.
- Dorrestein, G. M. (2000). Passerines and exotic softbills. Avian medicine, pp 155-156.
- Clark, P, Boardman, W, Raidal, S. 2009. Chapter 1: Collection and handling of blood samples. In Clark, P, Boardman, W, Raidal, S. Atlas of Clinical Avian Hematology. Wiley-Blackwell. Ames, IA, pp 1-15

355.4.2 Dogs

355.4.2 .1 Dogs are very smart and sociable, and it is usually possible to collect blood while the animal is awake, especially if positive reinforcement training is used. In general, two people are required to collect blood- one to restrain the dog and one to perform venipuncture.

355.4.2.2 The cephalic and jugular veins are the most common vessels used. Lateral saphenous and femoral vessels may also be used depending upon the skill of the operators, willingness of the subject, and degree of restraint required.

355.4.2.3 Where multiple samples are taken, the use of the limbs should be alternated. A temporary cannula can be used for multiple samples taken over a short period of time (more than four samples from one vein in a 24-hour period). No more than eight blood samples (four from either limb or from either jugular) should be taken in any 24-hour period. The number of attempts to obtain blood should be minimized, with no more than 3 needle sticks for each sample site.

355.4.2.4 Cardiac puncture is only allowed under anesthesia as a terminal procedure.

355.4.2.5 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.2.6 Table 1: Survival Blood Collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures

Collection Site	Indications	Additional Considerations
Cephalic Vein	Anesthesia not necessary Easily accessed Repeated collection possible Can be used for small to moderate volume collection	Requires 2 people
Lateral Saphenous Vein	Anesthesia not required Repeated sampling possible	Yields smaller volume Vessel is mobile and easily collapses. Hematoma risk May require 2 people
Jugular Vein	Moderate to large volumes can be collected Anesthesia not required	Sedation may be necessary for irritable animals Requires 2 people and some specialized training



Femoral vein	Moderate to large volumes can be collected	Anesthesia may be required Hematoma is a risk. Internal vessel laceration is a risk.
Carpal Pad / Ear Pinna	Possible refinement for frequent, repeated sampling (such as for daily blood glucose measurements) Anesthesia not required Can be done by 1 person	Will only yield a few drops

355.4.2.7 Table 2: Non-survival Blood Collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac puncture/cardiocentesis	Allows for maximum blood volume collection	Non-survival procedure only Requires anesthesia
Aorta, caudal vena cava or other internal vessel by surgical approach	Allows for maximum blood collection Sterile sample possible	Non-survival procedure only Requires anesthesia Requires surgical approach

355.4.2.8 Sources

- Borin-Crivellenti S, LZ Crivellenti, and M Tinucci-Costa. 2012. The carpal pad as an alternative sampling site for blood glucose testing in dogs. J Small Animal Pract 53: 684-6.
- NC3Rs microsite: <https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-dog>. Last updated Aug 20 2021. [accessed on April 11, 2022]
- Fox, JG, et. al. 2015. Laboratory Animal Medicine, 3rd edition. Academic Press: San Diego, CA. Chapter 25–Techniques of Experimentation, pg. 1209.

355.4.3 Frogs

355.4.3.1 Quantity of blood collection recommended: 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's recommended that collection be limited to 60-80ml/kg body weight.

355.4.3.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.3.3 To protect the integrity of the mucus layer, gloves should be powder-free, non-talc, latex or nitrile and pre-moistened with tank water.



355.4.3.4 Site of Collection

For survival blood collections, the following guidelines refer to the sites and procedures most frequently used: a) ventral abdominal vein; b) femoral vein; c) lingual vein. A list of the issues that should guide the choice of blood collection route(s) is provided below in Table 1 and Table 2. Digit amputation (of a hind toe) has been used as a means of collecting blood samples from *Xenopus* in the field; however, this method is painful and puts the frog at risk for infection. Therefore, this site is not approved for blood collection.

355.4.3.5 *Xenopus* spp., unlike most other amphibians, lack a tongue. This and other anatomical limitations make it challenging to use antemortem blood collection routes other than survival cardiac puncture. For all other species, the heart is only approved as a non-survival method and requires anesthesia for blood collection.

355.4.3.6 Table 1: Survival Blood Collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures.

Collection Site	Indications	Additional Considerations
Ventral Abdominal Vein	Anesthesia not required Repeated collection possible when experienced	Not prominent in <i>Xenopus</i> Needle should be <27G Limited to two attempts at any one time point to minimize risk of internal damage
Femoral Vein	Anesthesia not required Repeated collection possible when experienced	Special preparation of skin required Needle should be <27G
Lingual Vein	Anesthesia not required Repeated collection possible when experienced	Not possible in <i>Xenopus</i> Needle should be <27G Limited to one attempt per side of tongue at any one time point Contamination likely
Heart/Cardiac Puncture Requires specific and plausible scientific justification and case-by-case IACUC approval as well as competency assessment/training.	Allowed only in <i>Xenopus</i> as a survival procedure Sterile sample possible	Anesthesia required Special preparation of the skin is required High risk of post-collection phlebotomy complications or mortality

355.4.3.7 Table 2: Non-survival Blood Collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.



Collection Site	Indications	Additional Considerations
Heart/Cardiac Puncture	Allows for maximum blood volume collection Sterile sample possible	Anesthesia required Special preparation of skin required Needle should be <23G unless the animal is >100g weight

355.4.3.8 Sources

- SL Green. 2010. The Laboratory Xenopus sp. MA Suckow Ed. CRC Press, pp. 128-130.
- EJ Gentz. 2007. Medicine and surgery of amphibians. ILAR J. 48(3):255-9.

355.4.4 Gerbils

355.4.4.1 F For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.4.2 The lateral saphenous and jugular veins are the most used. The lateral tail vein and retro-orbital plexus are less commonly used. Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in terminal blood collection procedures.

355.4.4.3 Table 1: Survival blood collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures.

Collection Site	Indications	Additional Considerations
Lateral Saphenous Vein	Anesthesia not required Repeated blood collection possible by removal of the scab or blood can be collected into syringe	Yields only small quantities Requires restraint
Lateral Tail Vein	Anesthesia not required but may aid collection Repeated blood collection possible by removing the scab	Yields small volumes Restraint required Proper handling required to reduce the risk of tail injury (e.g., degloving)
Retro-orbital Sinus Requires scientific justification, case-by-case IACUC approval, and competency assessment/training	Yields moderate to large volumes Rapid Sterile sample possible	Anesthesia required It is discouraged due to high risk of injury to the eye and surrounding structures 2 blood collections per eye maximum, using an alternate eye per collection 2 weeks minimum between successive bleeds on any one eye.



Jugular Vein	Yields large volumes	Anesthesia required Should not be used for frequent small sampling or serial sampling due to the potential for large volumes
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355.4.4.4 Table 2: Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac puncture/cardiocentesis	Yields maximum blood volume	Non survival procedure only Anesthesia required
Aorta or Caudal Vena Cava	Yields maximum blood volume Sterile sample possible	Non survival procedure only Anesthesia required Surgical approach (laparotomy) required

355.4.4.5 Sources

- Suckow, MA, Stevens, KA, and Wilson, RP. 2012. The laboratory rabbit, guinea pig, hamster, and other rodents. Amsterdam: Elsevier Academic Press.
- Joslin JO. 2009. Blood collection techniques in exotic small mammals. *Journal of Exotic Pet Medicine*. 18(2): 117-139.
- Parasuraman, S, Raveendran, R, Kesavan, R. 2010. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother*. 1(2): 87–93.
- Donovan, J and Brown, P. 2006. Blood collection. *Care and Handling of Laboratory Animals in Current Protocols in Immunology*. John Wiley & Sons, Inc, pp. 1.7.1-1.7.9.
- Alworth, L., et al. Unassisted blood collection from unanesthetized rats and gerbils. *Lab Anim* 41, 155–156 (2012). <https://doi.org/10.1038/labon0612-155>
- Harkness, J, et. al. 2010. Clinical Procedures. In: *Biology and Medicine of Rabbits and Rodents*. Wiley-Blackwell, pp107-115
- Fried JH, et. al. Type, duration, and incidence of pathologic findings after retroorbital bleeding of mice by experienced and novice personnel. *J Am Assoc Lab Anim Sci*. 2015;54(3):317-327.

355.4.5 Guinea Pigs

355.4.5.1 Blood sampling in guinea pigs can be difficult. Guinea pigs prefer to be help upright and can get distressed if placed on their sides or back. The site chosen for venipuncture depends on the size of animal, expertise of handler and phlebotomist, and volume of blood required.

355.4.5.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.5.3 Table 1 Survival blood collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures.



Collection Site	Indications	Additional Considerations
Marginal Ear Vein	Easy to perform Anesthesia not required	Yields small volume
Lateral Saphenous or Tarsal Vein	Anesthesia not required Repeated blood collection possible by removing the scab or blood clot	Yields small volume Requires proper restraint; may require 2 people
Cephalic and Tarsal Veins	Anesthesia not required Repeated blood collection possible by removal of the scab	Yields small volume Requires proper restraint; may require 2 people Bruising and hemorrhage can occur
Jugular Vein	Yields large volume	Anesthesia required Requires specialized training Proper restraint is essential; 2 people required Should not be used for frequent or serial sampling due to the potential for large volumes
Cranial Vena Cava	Yields large volume	Anesthesia required Risk of cardiac puncture Requires specialized training and proper positioning
Femoral Vein	Allows Yields larger volume collection	Requires anesthesia Requires some specialized training

355.4.5.4 Table 2: Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac puncture/cardiocentesis	Yields maximum blood volume	Non survival procedure only Anesthesia is required



Aorta or Caudal Vena Cava	Yields maximum blood volume Sterile sample possible	Non survival procedure only Anesthesia is required Surgical approach (laparotomy) required
Decapitation	Yields large volume of mixed blood	Sample may be contaminated Aesthetically displeasing Special equipment and training required to reduce potential hazards for the operator

355.4.5.5 Sources

- Huneke RB. 2012. Guinea Pigs: Basic experimental methods. In: The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. (Suckow MA, Stevens KA, and Wilson RP Eds). Elsevier, pp. 621-37. 2012.
- Joslin JO. Blood collection techniques in exotic small mammals. J Exotic Pet Med April 2009 18(2): 117-139.
- NC3Rs microsite: <https://nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-guinea-pig>
- Parasuraman S, R Raveendran, and R Kesavan. 2010. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 1(2): 87–93.
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- Birck, M. M. et al. Non-Terminal Blood Sampling Techniques in Guinea Pigs. J. Vis. Exp (92), e51982, doi:10.3791/51982 (2014).

355.4.6 Hamsters

355.4.6.1 Hamsters tend to be more aggressive than mice. Manual restraint or a restraint device can be successfully used for blood collection.

355.4.6.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.5.6.3 Table 1 Survival blood collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures.

Collection Site	Indications	Additional Considerations
Lateral Saphenous or Tarsal Vein	Anesthesia not required Repeated blood collection possible by removing the scab	Yields small quantities Requires proper restraint
Cephalic Vein	Anesthesia not required Repeated blood collection possible by removing the scab	Yields small quantities Requires proper restraint; may require 2 people



Sublingual Vein	Yields small to moderate volumes	Anesthesia required Requires 2 people for proper restraint
Retro-orbital Sinus Requires scientific justification, case-by-case IACUC approval, and competency assessment/training.	Yields moderate to large volume Rapid Sterile sample possible	Anesthesia required Is discouraged due to high risk of injury to the eye and surrounding structures 2 blood collections per eye maximum using an alternate eye per collection) 2 weeks minimum between successive bleeds on any one eye.
Jugular Vein	Yields large volume	Anesthesia required Requires specialized training Should not be used for frequent or serial sampling due to the potential for large volumes
Cranial Vena Cava	Yields large volume	Anesthesia required Risk for cardiac puncture Specialized training and proper restraint required

355.4.6.4 Table 2: Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac puncture/cardiocentesis	Yields maximum blood volume	Non survival procedure only Anesthesia required
Aorta or Caudal Vena Cava	Yields maximum blood volume collection Sterile sample possible	Non survival procedure only Anesthesia required Surgical approach (laparotomy) required



355.4.6.5 Sources

- Donovan J and P Brown. 2006. Blood collection. Care and Handling of Laboratory Animals in Current Protocols in Immunology. John Wiley & Sons, Inc, pp. 1.7.1-1.7.9.
- Joslin JO. 2009. Blood collection techniques in exotic small mammals. Journal of Exotic Pet Medicine. April 2009 18(2): 117-139.
- NC3Rs microsite: <https://nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-hamster>
- Parasuraman S, R Raveendran, and R Kesavan. 2010. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 1(2): 87–93.
- Silverman J. 2012. Hamster: Biomedical Research Techniques. In: The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. (Suckow MA, Stevens KA, and Wilson RP Eds). Elsevier, pp. 779-97.
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355.4.7 Mice

355.4.7.1 The following guidelines refer to the most frequently used survival sampling sites for mice: a) submandibular vein, b) tail vein/artery, c) saphenous vein, and d) jugular vein. Less common survival blood collection sites requiring specific scientific justification and case-by-case IACUC approval include retro-orbital plexus, sublingual vein, penile vein, femoral artery, tail tip removal, and dorsal pedal vein.

355.4.7.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.7.3 Table 1: Survival Blood Collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures.

Collection Site	Indications	Additional Considerations
Submandibular Vein	Preferred blood collection method Maximum allowable sample volume with minimal trauma Sterile sample possible Anesthesia not required	Must be securely restrained Requires some specialized training Should not be used for frequent small sampling or repeated serial sampling
Medial or Lateral Saphenous Vein	Anesthesia not required Excellent technique for serial blood sampling Small to moderate volume of blood can be collected	Requires some specialized training Requires specialized equipment



Jugular Vein	Maximum volume of blood can be collected Result in high quality sample Sterile sample possible Moderate to large volume of blood can be collected	Anesthesia recommended Requires some specialized training Requires specialized equipment Should not be used for frequent sampling or repeated serial sampling
Lateral Tail Vein or Ventral/Dorsal Artery	Anesthesia not required Repeated blood collection possible by removal of the scab Vein is easily accessed Sterile sample possible	Must be securely restrained Yields only small quantities Some specialized equipment needed
Tail Tip Amputation	Serial blood sampling is possible via scab removal	Local or general anesthesia is required Only 1-3 mm length single amputation allowed Yields only a few drops of blood.
Retro-orbital Sinus Requires specific and plausible reasonable scientific justification and case-by-case IACUC approval as well as competency assessment/training.	Yields moderate to large volume of blood Rapid Sterile sample collection possible	Anesthesia is required Is a discouraged technique due to high risk of injury to the eye and surrounding structures 2 blood collections per eye maximum (eyes must be alternated between collections) 10 days minimum between successive bleeds on an eye

355.4.7.4 The following guidelines refer to the most frequently used non-survival sampling sites for mice: a) Retro-orbital plexus, b) heart, c) axillary plexus, d) aorta or vena cava, and e) decapitation.

355.4.7.5 A list of the issues that should guide the choice of non-survival blood collection routes is provided in Table 2.

355.4.7.6 Table 2: Non-survival Blood Collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Decapitation	Allows for large volumes of mixed blood to be collected	Sample may be contaminated Aesthetically displeasing



		Requires special equipment Potentially hazardous for operator
Heart/Cardiac Puncture	Allows for maximum blood volume collection	Anesthesia is required
Retro-orbital Sinus	Yields moderate to large volume of blood collected	Anesthesia is required
Axillary Plexus	Yields moderate to large volume of blood collected	Requires cut down Anesthesia is required
Aorta or Vena cava	Allows for maximum blood volume collection Sterile sample possible	Anesthesia is required Requires surgical approach (laparotomy)

355.4.7.7 Sources

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355.4.8 Nonhuman Primates

355.4.8.1 Nonhuman Primates (other than Mangabey)

355.4.8.1.1 This Policy provides guidelines for safe blood withdrawal from non-human primates (NHP) and takes into account the blood volume to body weight ratio measured in milliliters of blood to kilogram of body weight. These guidelines are for normal healthy animals. Animals who are aged, stressed, have spontaneous or experimental disease, or females that are menstruating may not tolerate blood collections at maximum volumes. For specific information on techniques, recommended supplies and equipment, and pre- and post- procedural care considerations, as well as to arrange for training, please contact the EPC DAR training coordinator at epc-dartrn@emory.edu

For specific information on animal training techniques, please contact: Behavioral Management Department at the Emory National Primate Research Center:

Mollie Bloomsmith: mabloom@emory.edu
Phone: 404-727-8809.

355.4.8.1.2 Restraint during blood collection

Instructions for blood collection and restraint procedures can be obtained from the EPC veterinary department. Instruction on applying animal training techniques for blood collection and restraint can be obtained from the behavioral management department.

Blood collection from NHP is most often performed while animals are restrained with chemical anesthesia. If smaller volumes are needed, NHP may be trained to cooperate by presenting a limb or other site for conscious sampling using techniques such as acclimation, desensitization, and positive reinforcement training.

A list of the considerations that should guide the choice of survival blood collection route(s) is provided in Table 1.

355.4.8.1.3 Table 1 Survival blood collection

Table 1 lists considerations guiding the choice of survival blood collection route(s).

Collection Site	Indications	Additional Considerations
Femoral	Preferred blood collection method Maximum allowable sample volume with minimal trauma Sterile sample collection is possible	Requires "blind stick" based on anatomical landmarks Requires anesthesia Requires specialized training
Saphenous	Site can be used for conscious blood collection Sterile sample collection possible Vein is easily visible	Yields small quantities (<5ml) Must be securely restrained (conscious collection) Animal requires specialized training (conscious collection) Personnel requires specialized training (conscious collection)



Cephalic	Sterile sample collection is possible	Yields small quantities (<5ml) Requires anesthesia
Ear/Digit	Site can be used for conscious blood collection Minimal trauma	Yields small quantities (few drops) Animal requires specialized training (conscious collection) Personnel requires specialized training (conscious collection)

355.4.8.1.4 The typical sites for blood collection include the femoral, saphenous, and cephalic veins. The saphenous vein is the site used for conscious sampling of small amounts (<5ml) of blood. If larger volumes are needed, the flow rates in the cephalic or saphenous veins are usually inadequate to prevent the vein from collapsing. Bleeding from the femoral vein requires a “blind stick” based on anatomical landmarks in that the femoral vein is not normally visible subcutaneously. All blood collection techniques require training and competence but conscious collections also require extensive training of the animal as well. Conscious sampling is currently only recommended for macaques and New World Primates.

355.4.8.1.5 General Considerations

Calculation of a safe, maximum blood draw that has been used successfully in non-human primates is the 10%-10% rule, which states that the maximal blood sample size is 10% of a blood volume estimated to be 10% of the animal’s body weight. The maximum blood collection (see Table 2) volume allowed is 10ml/kg for adult macaques and 6ml/kg for macaques less than 4 months old and adult new world monkeys based on the most recent lean body weight. The number of attempts to obtain blood should be minimized to a maximum of three needle sticks for each site/vessel before seeking assistance from a senior individual. In general a 20-22g 1in to 1.5in needle can be used for adults and 25g 5/8in needle can be used on infant or new world monkeys.

355.4.8.1.6 When all blood components are not needed, blood samples may exceed the calculated limits if the plasma or red cells are returned to the animal after separation, along with appropriate fluid replacement. This must be determined in consultation with the veterinary department.

355.4.8.1.7 Table 2 Maximum blood volume allowed

Species	Max Blood volume	Time range *	Special Considerations@
Macaques >4 months of age	10ml/kg	Total amount collected over 28 days	Max amount may be reduced to 6-8ml/kg
Macaques <4 months of age and adult New world monkeys	6ml/kg	Total amount collected over 28 days	Max amount may be reduced per veterinary direction

* Blood can be collected over a 28-day rolling cycle that will start at a predetermined start date period that should be documented and accessible to the PI, research resources, and the veterinary department. The 28-day period is not restricted to a calendar month so that in the case of maximum blood collection, the amount of blood collected on day 29 must be less than or equal to the amount collected on day 1.

@ The maximum blood volume for collection is contingent on Hct or/and Hbg, health status, age, and time of female cycle. Animals who are aged, stressed, have spontaneous or experimental disease, or females that are menstruating may not tolerate blood collections at maximum volumes.



355.4.8.1.8 Monitoring of animals

355.4.8.1.8.1 The blood collection site should be closely monitored for bleeding to stop at the end of the collection during the recovery period, as well as after the recovery period.

The blood collection site should be checked frequently during recovery and especially when the animal is returned to the cage. The animal should be checked again the following morning and closely monitored for any signs of abnormality at that time.

355.4.8.1.8.2 By monitoring the hematocrit (Hct) and/or hemoglobin (Hgb), it is possible to evaluate whether the animal has sufficiently recovered from a single or sequential blood draws.

An acute blood loss can cause hypovolemic shock, physiological stress, or even death. Sequential blood sampling that occurs too frequently may result in anemia. The amount of blood collected is also contingent upon the animal's health status determined by the veterinarian. The volume may be decreased to 6-8ml/kg based on spontaneous or experimental disease or in menstruating females.

355.4.8.1.8.3 Anemic animals will be treated based on the anemic status and according to veterinary recommendation. A veterinarian may reduce the allowable blood collection volumes based on the level of anemia (e.g. Mild anemia may be restricted to 8ml/kg, moderate anemia may be restricted to 6ml/kg and severe anemia may require collections to be stopped until hemoglobin and hematocrit increase).

Hematocrit level and/or	Hemoglobin level	Anemic Status
25-30%	8.5-10 g/dl	Mild
20-25%	8.0-8.5 g/dl	Moderate
<20%	<8.0 g/dl	Severe

355.4.8.1.8.4 If anemia develops the investigator should review the blood collection volumes with a veterinarian prior to the next blood collection.

355.4.8.1.8.5 Animals with concurrent illness may not fall within described classifications and will be evaluated by a veterinarian for treatment. Veterinary assessment may warrant decreased blood collection volumes until the anemia improves or resolves.

355.4.8.2 Blood Collection Guidelines (Mangabey)

355.4.8.2.1 Restraint during blood collection

All personnel must be approved by a veterinarian before collection of blood samples from mangabeys can be taken. Additional authorization is required prior to blood collection that uses direct puncture of the vein with a vacutainer needle and sleeve (holder) system.

355.4.8.2.2 Approved methods and sites for blood collection

The typical sites for collection include the femoral, saphenous, and cephalic veins. The saphenous and cephalic veins are the sites used for sampling of small amounts (3-5) of blood. If larger volumes are needed, the femoral vein should be used. Bleeding from the femoral vein requires a "blind stick" in that the femoral vein is not normally visible. The following guidelines should be followed when collecting blood samples from sooty mangabeys:

- The standard needle size should be 22G. If a larger needle is absolutely essential it should not exceed 20G.
- The needle for the vacutainer should be no longer than 1" in length.
- Whenever possible the saphenous vein should be used for collections. (i.e. for small volumes)



- Whenever feasible, a butterfly needle should be used to collect multiple tubes from an individual via the saphenous or femoral vein.
- The phlebotomist should alternate between the left and right side when collecting from the femoral vein. Documentation of blood collection should be clearly recorded and maintained on a central animal records system.
- Individuals should only attempt two needle sticks total with minimal “fishing for the vein” on an animal before seeking assistance from a senior individual.
- Upon completion of routine blood collection or attempted sticks with some bleeding the site should be “held off” with manual pressure for a minimum of 5 minutes that is counted with a timer.
- If the following conditions occur during blood collections then the site should be held off with manual pressure for a minimum of 10 minutes that is counted with a timer:
 - If a larger gauge needle is used for collections
 - If a large volume of blood is collected
 - If an arterial stick is suspected.
 - If the collection had any complications

355.4.8.2.3 Table 3 Maximum blood volume allowed

Species	Max Blood Volume	Time Range	Special Considerations @
Mangabey	10ml/kg	Total amount collected over 28 days	Max amount may be reduced per veterinary direction

@ The maximum blood volume for collection is contingent on Hct or/and Hbg, health status, age, and time of female cycle. Animals who are aged, stressed, have spontaneous or experimental disease, or females that are menstruating may not tolerate blood collections at maximum volumes.

355.4.8.2.4 Monitoring of Animals

See previous section 355.4.8.1.8 Monitoring of animals

355.4.8.2.5 The leg should be checked again the following morning and the animal closely monitored for any signs of lameness at that time. Mangabeys over 16 years of age are considered at higher risk for complications and will have additional collection restraints

Mangabeys over 16 years of age should only have large research blood collections once every 2 months

Mangabeys over 16 years of age will be limited to 30mls of blood collection at a single time point, regardless of body weight

355.4.8.2.6 All non-survival procedures must be followed by a primary method of euthanasia.

355.4.8.2.7 Sources

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355.4.9 Rabbits

355.4.9.1 The most common method of survival blood collection is to access the central artery of the ear. The most common terminal technique is exsanguination via central artery of the ear, heart, or internal vessel by surgical access.

355.4.9.2 The guidelines in Table 1 refer to the most frequently used survival collection sites for rabbits:

For universal guidance, please refer to the introduction of this policy.

The use of topical compounds or techniques that may enhance the volume of blood collected from the ear requires approval from IACUC.

Rabbits are highly prone to back injuries. For safe handling of the animal, training of the operators and conditioning of non-sedated rabbit is required for restraint and sample collection.

355.4.9.3 Circulating blood volume

Some evidence suggests the safe maximum circulating blood volume is 56 ml/kg (Harkness and Wagner, 1989; McLaughlin and Fish, 1994; Suckow, et al, 2002) although USDA recognizes 62 ml/kg.

355.4.9.4 Blood collection limits for rabbits are 7 ml/kg every 3 weeks (USDA, 2013).

355.4.9.5 Where the IACUC approves >7 ml/kg/month cumulative, non-terminal blood collection, the rabbit should be monitored regularly for distress by checking, at minimum, the hematocrit prior to blood collection to ensure that it is in the normal 30-50% range (USDA, 2013).

355.4.9.6 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.9.7 Table 1: Survival Blood Collection

Table 1 lists considerations guiding the choice of survival blood collection route(s). These methods may also be used in non-survival blood collection procedures

Collection Site	Advantages	Disadvantages
Ear Arteries and Veins	Preferred blood collection method Easily accessible from the dorsal ear Sterile sample possible Blood-gas analysis and direct blood pressure measurement possible Anesthesia not required Catheterization possible	Requires some specialized training Can obtain small volumes (0.1-5.0 ml)



Indwelling Catheters	Anesthesia not required for sample collection after catheter implantation Repeated collections possible Port is easily accessed Sterile sample possible Excellent technique for serial blood sampling Yields potentially large quantities	Requires specialized training Surgery is required for deep implantation of catheter Specialized equipment needed
Jugular Vein	Can collect large volume Results in high quality sample Sterile sample possible	Anesthesia highly recommended Requires some specialized training Requires two people Yields a large sample so should not be used for frequent small sampling or repeated serial sampling
Saphenous Vein	Especially useful for small subjects where the vein is larger than the aural vessels. Can be done without anesthesia. not required Reasonable blood volumes can be obtained (< 3 ml) Sterile sample possible Catheterization possible	Lateral recumbency for vein access requires specialized training Anesthesia/sedation are advised unless the rabbit is conditioned to handling and/or the procedure Vein is fragile; risk of hematoma risk

355.4.9.8 Table 2: Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Carotid Artery	Large volume of blood can be collected Sterile sample possible	Non-survival procedure only Anesthesia required Requires surgical approach
Heart/Cardiac Puncture	Yields maximum volume of blood Rapid Sterile sample possible	Non-survival procedure only Anesthesia required



355.4.9.9 Sources

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355.4.10 Rats

355.4.10.1 The most frequently used survival sampling sites for rats not requiring surgical intervention are lateral tail vein, lateral saphenous vein, jugular vein, ventral tail artery, and tail tip snip. The use of the retro-orbital plexus requires reasonable scientific justification, demonstration of competency, and case-by-case IACUC approval.

355.4.10.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.10.3 A list of the issues that should guide the choice of blood collection route(s) is provided in the two tables below with Table 1 for survival collections and Table 2 for terminal collections. Note that any survival method listed may be used as a terminal method as well. All terminal methods of blood collection must be followed by a primary method of euthanasia.

355.4.10.4 Table 1: Survival Blood Collection

Collection Site	Indications	Additional Considerations
Lateral tail vein nick	Anesthesia not necessary Easily accessed Repeated collection possible Needle or lancet used	Requires restraint device Yields only small quantities Tail warming may be needed Special equipment may be needed



Lateral saphenous vein	Anesthesia not required Repeated sampling possible Small to moderate volumes can be obtained	Variable sample quality/quantity Special equipment needed
Ventral tail artery puncture	Yields moderate to large volumes	General anesthesia may be necessary Special equipment may be needed
Jugular vein	Moderate to large volumes can be collected. Results in high quality sample Sterile samples possible	Not useful for repeated purposes General anesthesia required
Sublingual vein	Allows for repeated sampling, even same day. Yields moderate volumes	Requires anesthesia Training necessary May risk sublingual hematoma
Retro-orbital Sinus Requires reasonable scientific justification and case- by-case IACUC approval as well as competency assessment/training.	Yields moderate to large volume of blood Rapid Sterile sample collection possible Use of lateral or medial canthus is possible.	Anesthesia is required Is discouraged due to high risk of injury to the eye and surrounding structures 2 blood collections per eye maximum (eyes must be alternates between collections) 10 days minimum between successive bleeds on an eye.
Tail tip amputation (Tail snipping) Segmental tail amputation is only allowed under very special circumstances when scientifically justified. Contact the Training Coordinator for SOM DAR: dartrn@emory.edu	Anesthesia not necessary Allows for sequential sampling by removal of the scab at the tail tip.	Requires restraint No more than 5mm of tail can be collected over the life of the animal Yields only a few drops of blood.
Submandibular Vein Sampling	Provides good quality sample Minimal distress and tissue trauma to the animal Easy to learn	Requires training Anesthesia is required Manual pressure necessary to stop bleeding Yields a large sample so should not be used for frequent small sampling/repeated serial sampling



355.4.10.5 The following guidelines refer to the most frequently used non survival sampling sites for rats: a) cardiac puncture; b) abdominal surgical approach to the caudal vena cava; c) axillary plexus cut down, and d) decapitation. A list of the issues that should guide the choice of non-survival blood collection route(s) is provided in Table 2.

355.4.10.6 Table 2: Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac Puncture/cardiocentesis	Allows for maximum blood volume collection	Non-survival procedure only Requires anesthesia
Caudal vena cava	Allows for maximum blood volume collection Sterile samples possible	Non-survival procedure only Requires surgical approach Requires anesthesia
Axillary plexus cut down	Yields relative large volumes	Non-survival procedure only Sample may be contaminated
Decapitation	Allows for large volumes of mixed blood to be collected.	Sample may be contaminated Aesthetically displeasing Special equipment necessary Potentially hazardous for operator

355.4.10.7 Sources

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355.4.11 Swine

355.4.11.1 Restraint

Physical restraint can be stressful to swine that are unconditioned to human handling. Additionally, it may be physically challenging and dangerous to manually restrain large swine. Consequently, short-term chemical restraint is typically preferred for blood collection in order to reduce stress on both the animal and personnel, facilitate smooth collection of the sample, and reduce the risk of vascular trauma. An exception to this rule of thumb is that small to medium sized swine can be physically restrained in a sling apparatus. When using a sling, swine must first be acclimated to the device, otherwise animals may struggle and quickly become stressed. Very small swine may also be manually restrained in the handler's arms.

355.4.11.2 Refinements: The use of vascular access ports are recommended when serial samples are required over a period of days or weeks.

355.4.11.3 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.11.4 Table 1: Site of Collection

Collection Site	Indications	Additional Considerations
Cranial vena cava	>20ml volume can be collected from this site	Must be taken from the right side to avoid injury to the vagus nerve and thoracic duct. This is a blind technique requiring additional training. One week recommended between collections due to the propensity for hematomas at this site.
Jugular vein	Up to 20ml can be collected from this site	This is a blind technique requiring additional training. Right side is preferred to avoid injury to the vagus nerve and thoracic duct.



Lateral saphenous/cephalic vein	Useful on smaller pigs where the vessel is more easily visible	<5ml volumes can be collected from this site Requires sedation
Marginal ear vein	May be used for serial collections over a short period by alternating ears and moving the puncture site successively towards the ear base	<2ml volumes can be collected from this site Requires sedation/anesthesia

355.4.11.5 Table 2: Non-Survival Blood Collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac Puncture/cardiocentesis	Allows for maximum blood volume collection	Non-survival procedure only Requires anesthesia
Aorta, caudal vena cava or other internal vessel by surgical approach	Allows for maximum blood collection	Non-survival procedure only Requires anesthesia Requires surgical approach

355.4.11.6 Sources

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355.4.12 Voles

355.4.12.1 Voles tend to be more aggressive than mice, but can be successfully restrained manually by properly trained, experienced personnel or by using a restraint tube for blood collection.



355.4.12.2 For specific information on collection volumes and universal guidance, refer to the introduction of this policy. Contact the Training Coordinator (SOM DAR: dartrn@emory.edu, or EPC DAR: epc-dartrn@emory.edu) for additional information on techniques, recommended supplies and equipment, pre- and post-procedural care considerations, and to arrange training.

355.4.12.3 A list of the considerations that should guide the choice of survival blood collection route(s) is provided in Table 1. Note that any survival method may be used as a terminal method as well.

The use of the retro-orbital plexus requires reasonable scientific justification, demonstration of competency, and case-by-case IACUC approval.

355.4.12.4 Table 1 Survival blood collection

Collection Site	Indications	Additional Considerations
Lateral Saphenous Vein	Anesthesia not required Repeated bleeding possible by removal of the scab Small to moderate volume of blood can be collected	Requires some specialized training Requires proper restraint and restrainer
Submandibular Vein	In theory, offers the potential for large volumes and sterile samples.	Not yet demonstrated to be a useful technique in voles. Will require secure restraint and specialized training Volumes yielded maybe in excess of need.
Sublingual Vein	Good for small volumes	Anesthesia required Requires 2 people for proper collection Excessive bleeding is common; proper blood stopping must be ensured
Retro-orbital Sinus Requires specific and plausible scientific justification and case- by- case IACUC approval as well as competency assessment/training.	Yields moderate to large volume of blood Rapid Sterile sample collection possible	Anesthesia is required Is discouraged due to high risk of injury to the eye and surrounding structures 2 blood collections per eye maximum (eyes must be alternates between collections) 10 days minimum between successive bleeds on an eye.
Jugular Vein	Allows larger volume collection Result in high quality sample Sterile sample possible	Requires anesthesia Requires specialized training Not suitable for frequent small samplings or serial sampling due to potential for vascular damage



355.4.12.5 Table 2 Non-Survival blood collection

Table 2 lists considerations guiding the choice of blood collection methods that are only acceptable for non-survival blood collection. All non-survival procedures must be followed by a primary method of euthanasia.

Collection Site	Indications	Additional Considerations
Cardiac puncture/cardiocentesis	Allows for maximum blood volume collection Sterile sample is possible	Anesthesia is required
Aorta or Caudal Vena Cava	Allows for maximum blood volume collection Sterile sample possible	Anesthesia is required Requires surgical approach (laparotomy)
Decapitation	Allows for large volumes of blood to be collected	Deep level of anesthesia is required or can be done immediately after euthanasia Sample may be contaminated and mixed. Aesthetically displeasing Special equipment and training necessary Potentially hazardous for operator Low throughput technique
Retro-orbital Sinus	Allows for moderate to large volume of blood to be collected	Anesthesia is required
Axillary plexus cut down	Allows for moderate to large volume of blood to be collected	Non survival procedure only Anesthesia is required

355.4.12.6 Sources

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Appendix 1 NIH Guidelines for Blood Collection in Mice and Rats



Contact Information

Subject	Contact	Phone	Email
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Revision History

- Revision: 09/20/2017
- Revision: 03/16/2022

Appendix 1 NIH Guidelines for Blood Collection in Mice and Rats

Table 1: Calculated Blood Sample Volumes for Species and Range of Body Weights					
Species	Body weight (g)	*CBV(ml)	~1% CBV every 24 hrs†	~7.5% CBV every 7 days†	~10% CBV every 2 - 4wks†
Mouse	20	1.10 - 1.40	11 - 14 µl	90 - 105 µl	110 - 140 µl
	25	1.37 - 1.75	14 - 18 µl	102 - 131 µl	140 - 180 µl
	30	1.65 - 2.10	17 - 21 µl	124 - 158 µl	170 - 210 µl
	35	1.93 - 2.45	19 - 25 µl	145 - 184 µl	190 - 250 µl
	40	2.20 - 2.80	22 - 28 µl	165 - 210 µl	220 - 280 µl
Rat	125	6.88 - 8.75	69 - 88 µl	516 - 656µl	690 - 880 µl
	150	8.25 - 10.50	82 - 105 µl	619 - 788 µl	820 - 1000 µl
	200	11.00 - 14.00	110 - 140 µl	825 - 1050 µl	1.1 - 1.4 ml
	250	13.75 - 17.50	138 - 175 µl	1.0 - 1.3 ml	1.4 - 1.8 ml
	300	16.50 - 21.00	165 - 210 µl	1.2 - 1.6 ml	1.7 - 2.1 ml
	350	19.25 - 24.50	193 - 245 µl	1.4 - 1.8 ml	1.9 - 2.5 ml
		*Circulating blood volume (1ml = 1000µl)	†Maximum sample volume for that sampling frequency		

https://oacu.oir.nih.gov/system/files/media/file/2021-02/b2_blood_collection_in_mice_and_rats.pdf