University of Louisville

Institutional Animal Care and Use Committee

Meeting Minutes

Thursday, 10 December 2020, 9:00 AM

Teleconference

Members Present:
Dr. Pascale Alard*
Dr. Geoffrey Clark*
Dr. Cynthia Corbitt
Dr. Amanda LeBlanc
Dr. Ben Lovely
Dr. David Magnuson
Dr. George Pantalos
Dr. Kenneth Palmer
Dr. Karen Powell*
Dr. Mary Proctor
Dr. David Samuelson
Dr. Leslie Sherwood
Ms. Kathleen Smith
Dr. Sucheta Telang*

Members Absent:
Dr. Swati Joshi-Barve

Additional Attendees:
Ms. Stacy Cantrell
Dr. Steven Davison
Dr. Katie Emmer, *Alternate for Dr. Karen Powell*
Ms. Brigitte Foote
Dr. Torsten Hopp
Mr. Tim Mulliger
Ms. Tegan Tulloch

*Not present for the entire meeting; see notes below

I. Call to Order and Approval of the Minutes from the Previous Monthly Meeting, 19 November 2020 (Attachment 1)

Dr. Pantalos called the meeting to order at 9:05 AM with 11 voting members present.

The minutes from the previous meeting were presented for review. *A motion to accept the minutes was **approved*** (11 *"in favor," none opposed, no abstentions*).

[Drs. Powell and Telang arrived following this discussion; Dr. Emmer reverted to alternate member; voting member count = 12.]

II. Ratification of Approved *Proposals* (Attachment 2)

All Committee members had an opportunity to individually review "Proposals to Use Laboratory Animals in Research and Teaching" (*Proposals*) presented for IACUC approval. *The following proposals were ratified with all eligible votes in favor and abstentions due to a conflict of interest for the following:* 18232 (Boakye [Sherwood]), 18365 (Beverly [Sherwood]), 20739 (Powell), 20814 (Neimat [Powell]), and 20815 (LeBlanc).

New Proposals: 20815

Three Year Renewals: 19653

Modifications: 18231 18232 18265 18313 19528 19561 19664 20708 20739 20814

Annual Review: 18365

Tissue: None.

Administrative Modification: 20695 20726

III. Continuing Education, Policy Review, iRIS Improvements

A. IACUC Policy Revisions, "Rodent Breeding Colonies" (*Sherwood, Davison*) (**Attachment 3**)
Dr. Davison summarized revisions to this policy which focused on clarifying the weaning time of rodents.

[Drs. Alard, Clark and Palmer joined during this discussion; voting member count = 15.]

*A motion to approve the policy changes was **unanimously approved** (15 "in favor," none opposed, no abstentions).*

B. New IACUC Policy, "IACUC Standard Procedures for Rodents" (*Sherwood, Emmer*) (**Attachment 4**)
Dr. Emmer presented this new policy to the IACUC which outlines standard procedures for rodents in an effort to improve consistency and reduce administrative burden for researchers by incorporating these procedures into the IACUC *Proposal* form. The Committee requested a few minor changes including formatting the tables onto a single page and removing a double negative.

*A motion to approve the new policy with the minor changes was **unanimously approved** (15 "in favor," none opposed, no abstentions).*

C. New IACUC Policy, "IACUC Standard Procedures for Non-Rodents" (*Sherwood, Powell*) (**Attachment 5**)
Dr. Powell presented this new policy to the IACUC which outlines standard procedures for non-rodent mammals in an effort to improve consistency and reduce administrative burden for researchers by incorporating these procedures into the IACUC *Proposal* form. The Committee requested that Dr. Sherwood review the policy and make any additional necessary changes including removing the requirement that only CMRU staff perform certain procedures, adding removal of catheter after 3 days to ferrets, and changing the policy name to "IACUC Standard Procedures for Non-Rodent Mammals."

*A motion to approve the new policy with the additional changes was **unanimously approved** (15 "in favor," none opposed, no abstentions).*

IV. Open Discussion / Full Committee Review – *None*

V. Old Business

A. Humane Endpoints *Pseudomonas Aeruginosa* Subcommittee (*Pantalos, Palmer, Powell, Sherwood*)
The subcommittee updated the IACUC on the discussions regarding refinement of the *P. Aeruginosa* endpoints. The subcommittee met with the investigator and discussed the IACUC's expectations, as well as the investigator's contract work which has limited flexibility. The CMRU and CPM are financially supporting a pilot study to explore new humane endpoints. The subcommittee expects the pilot study to be submitted for IACUC *Proposal* review in January.

B. Laboratory and Satellite Rodent Housing Subcommittee (*Sherwood*)
The subcommittee met and had productive discussions. The current IACUC policy is under revision by the CMRU veterinarians and then it will be sent to Dr. Hopp for review. The subcommittee's goal is to have the revised policy ready for the IACUC's January meeting.

C. Surgery Training Subcommittee (*Alard, Emmer, LeBlanc, Pantalos, Sherwood*)
Dr. Sherwood summarized the subcommittee's meeting and the policy revisions and CITI courses currently under development. The subcommittee agreed that training requirements should apply to

all individuals performing surgery on rodents and non-rodent mammals. The training will consist of a custom online CITI course and hands-on surgery training or observation (for experienced individuals). Three-year refresher training will be required for both components. The subcommittee agreed that a slow roll-out, similar to the IACUC's euthanasia training requirement, is most appropriate and feasible. Initial implementation will only apply to new proposals, renewals, or personnel additions. Due to the pandemic, only the online CITI course will be required initially. Once COVID restrictions are lifted, individuals will be expected to also complete the hands-on/observation requirement. The subcommittee hopes to have the policy revisions and CITI courses ready to present to the IACUC by the January meeting.

D. Human Cell Line Testing (*Sherwood*, *Davison*)
Dr. Davison updated the IACUC on this item. A majority of the lines have been submitted and tested. There have been no positive results yet, but they are awaiting a few more results. *C. Bovis* was found in a breeding room and may have originated there, but the origin may never be identified. *C. Bovis* is very infectious and lives well in the environment. The CMRU veterinarians are meeting with the researchers next week to discuss facility decontamination and other changes aimed at protecting immunocompromised animals.

E. PETA Open Records Request (*Pantalos)*
Dr. Pantalos contacted David King of the University's legal counsel and believes he has made some headway in regard to obtaining the official response to PETA's open record request. Dr. LeBlanc recently attended the SCAW workshop and noted that the New England Anti-Vivisection Society's FOIA requests now outnumber PETA's ten to one, and the society recently hired a FOIA expert to request information from universities.

VI. New Business

A. Self-Report of an Adverse Event *Proposal* 20743 (*Pantalos, Sherwood, Powell*) (**Attach. 6**)
The Committee reviewed the self-report outlined in the letter. The IACUC noted that this was an unfortunate event that could not have been anticipated. The Committee agreed that the PI has responded appropriately and that no further action is required, but the IACUC will request that the PI update the Committee when the proper fittings and labeling have been installed and tested on the biosafety cabinet.

A motion to send a thank you letter to the PI requiring no further action other than an update when the proper fittings and labeling have been installed and tested on the biosafety cabinet was ***unanimously approved*** (*15 "in favor," none opposed, no abstentions*).

B. Community Members (*Sherwood*) (**Attachment 7**)
Dr. Sherwood informed the Committee that a new community member will be joining in January 2021, Dr. Stacy Simpson, a professional musician. Dr. Sherwood reviewed the importance of community members and the regulations that require a fully constituted IACUC in order to conduct official business. The Committee requested an information sheet with a brief description of the role and time commitment; Dr. Sherwood stated that she will put something together.

C. CMRU Update (*Sherwood*)
Dr. Sherwood provided an update on CMRU operations during the pandemic. CMRU husbandry operations had returned to the regular cage change schedule briefly, but due to staff shortages in November caused by the pandemic, husbandry returned to the pandemic plan's three-week cage change schedule with the outlined and IACUC approved exceptions. Dr. Sherwood noted that the staff is managing but is stressed with balancing homeschooling, childcare, personal stress, and their jobs; CMRU husbandry will be working throughout the holidays.

D. Recent SCAW Virtual Winter Conference (*LeBlanc*)
Dr. LeBlanc summarized the recent SCAW conference for the Committee. The conference included helpful case studies and scenarios which were analyzed and then commented on by the Director of OLAW and USDA representatives. Dr. LeBlanc suggested the IACUC review these scenarios as continuing education once they are available online.

E. End of Year Review (*Tulloch*)
Ms. Tulloch presented the 2020 End of Year review to the Committee summarizing accomplishments and other statistics.

F. Other Business
Dr. Sherwood noted that the USDA and OLAW annual reports were submitted; these will be included in the January agenda. The AAALAC 2020 form is not online yet, but the annual report will be submitted to AAALAC once the form is available.

Dr. Sherwood informed the Committee that [redacted]

Dr. LeBlanc has information regarding the COVID vaccine that she will forward to the IACUC Office for distribution to the Committee.

VII. Adjournment

The IACUC was reminded that the next meeting will occur via teleconference Thursday, 21 January 2021 at 9:00 a.m. *Meeting was adjourned at 10:55 AM.*

Investigator	Protocol	Original Approval	Submission Approval	Expiration	Species	Pain Class	Animals Approved
King, Suzanne N	IACUC 18231	2018-06-01 00:	2020-12-01 00:	2021-05-31 00:	Rat (Laboratory)	2	185
Modification					**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff	

Swallowing Disorders After Radiation Therapy to Head and Neck

Boakye, Maxwell	IACUC 18232	2018-03-12 00:	2020-11-17 00:	2021-03-11 00:	Pig (Domestic)	2	256
Modification					**Reviewers:**	LeBlanc, Amanda J - Designated/Chair Signoff	

Myelotomy with intramedullary hemorrhagic necrosis removal (MIHN) as a therapeutic strategy in a porcine model of traumatic spinal cord injury

Zhang, Huang-Ge	IACUC 18265	2018-08-20 00:	2020-12-01 00:	2021-08-19 00:	Mouse (Laboratory)	3	4230
Modification					**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff	

Fruit exosome-like particles for biomarker, biological functions and therapeutic delivery of extracellular miRNAs

Lei, Zhenmin	IACUC 18313	2018-09-10 00:	2020-11-24 00:	2021-09-09 00:	Mouse (Laboratory)	2	532
Modification					**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff	

Copy of Consequences of mouse luteinizing hormone receptor knockout

Investigator	Protocol	Original Approval	Submission Approval	Expiration	Species	Pain Class	Animals Approved
Beverly, Levi J	IACUC 18365	2019-03-21 00:	2020-12-07 00:	2022-03-20 00:	Pig (Domestic)	2	19
Annual Review					**Reviewers:**	Telang, Sucheta - Designated	
Pigs models of cancer and cancer treatments							
Li, Yan	IACUC 19528	2019-08-26 00:	2020-11-24 00:	2022-08-25 00:	Mouse (Laboratory)	2	736
Modification					**Reviewers:**	LeBlanc, Amanda J - Designated/Chair Signoff	
The mechanism of hepatocellular carcinoma (HCC) carcinogenesis and treatment							
Damodaran, Chendil	IACUC 19561	2019-09-18 00:	2020-11-24 00:	2022-09-17 00:	Mouse (Laboratory)	2	3180
Modification					**Reviewers:**	LeBlanc, Amanda J - Designated/Chair Signoff	
Chemoprevention of metastatic colon cancer							
Damodaran, Chendil	IACUC 19561	2019-09-18 00:	2020-11-24 00:	2022-09-17 00:	Mouse (Laboratory)	2	3180
Modification					**Reviewers:**	LeBlanc, Amanda J - Designated/Chair Signoff	
Chemoprevention of metastatic colon cancer							
Kakar, Sham S	IACUC 19653	2020-12-01 00:	2020-12-01 00:	2023-11-30 00:	Mouse (Laboratory)	2	680
3YR					**Reviewers:**	Magnuson, David S, Ph.D. - Designated	
Copy of Development of Therapeutics for Cancer							
Dean, Douglas C	IACUC 19664	2020-02-12 00:	2020-11-24 00:	2023-02-11 00:	Pig (Domestic)	2	1134
Modification					**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff	

Investigator	Protocol	Original Approval	Submission Approval	Expiration	Species	Pain Class	Animals Approved
Retinal Metabolism and Therapy in Swine							
-Tyagi, Suresh C	IACUC 20695	2020-03-17 00:	2020-12-01 00:	2023-03-16 00:	Mouse (Laboratory)	2	632
Admin Mod - Title Change						**Reviewers:**	Tulloch, Tegan N - Designated
Copy of Reversing Skeletal Muscle Myopathy by Hydrogen Sulfide							
Giridharan, Guruprasad A	IACUC 20708	2020-03-20 00:	2020-12-07 00:	2023-03-19 00:	Pig (Domestic)	2	8
Modification						**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff
Advanced Filtration System for Serum Potassium							
Moore, Joseph B Admin	IACUC 20726	2020-05-14 00:	2020-12-04 00:	2023-05-13 00:	Mouse (Laboratory)	2	9150
Mod - Strain Addition						**Reviewers:**	Tulloch, Tegan N - Designated
Functional studies of lncRNAs regulated in cardiovascular disease							
Powell, Karen S	IACUC 20739	2020-06-09 00:	2020-11-30 00:	2023-06-08 00:	Pig (Domestic)	2	40
Modification						**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff
Large Animal Procedures Training-Swine							
Neimat, Joseph S	IACUC 20814	2020-10-05 00:	2020-12-01 00:	2023-10-04 00:	Tissue Only	Tissue Only	
Modification						**Reviewers:**	Pantalos, George, Ph.D. - Designated/Chair Signoff

Investigator	Protocol	Original Approval	Submission Approval	Expiration	Species	Pain Class	Animals Approved
Application of a Novel Ultrasound Powered Device for Deep Brain Stimulation							
LeBlanc, Amanda J	IACUC 20815	2020-12-07 00:	2020-12-07 00:	2023-12-06 00:	Rat (Laboratory)	3	160
Initial Review					**Reviewers:**	Samuelson, David J, Ph.D. - Designated	
UroA and rat LPS-induced inflammation							
Petruska, Jeffrey C	IACUC 20821	2020-11-23 00:	2020-11-23 00:	2023-11-22 00:	Mouse (Laboratory)	2	1297
3YR					**Reviewers:**	Corbitt, Cynthia, Ph.D. - Designated	
In vitro assessment of sensory neurons from mice - RENEWAL							

IACUC Standard Procedures for Rodents

Policy: This policy describes the IACUC's approved standard procedures for rodents (mice, rats, hamsters, or guinea pigs) which investigators may easily incorporate into their IACUC *Proposals*. Principal Investigators (PI) performing procedures as described in this policy may utilize the checkboxes in the procedures section of the IACUC's *Proposal* form rather than describing the procedures in full. If the performance of the procedure will deviate from the descriptions in this policy, including administration or blood collection volumes, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. All procedures, including the IACUC's standard procedures, must be included in the IACUC *Proposal* form and approved by the IACUC prior to performance. It is the responsibility of the PI to ensure that all laboratory personnel responsible for procedures are appropriately trained and qualified. The Comparative Medicine Research Unit (CMRU) veterinary staff is available to provide training at no charge; training can be scheduled through the IACUC's website.

Rationale: This policy has been drafted to ensure consistency in the performance of common non-surgical procedures, as well as reduce the administrative burden to investigators and the IACUC in drafting and reviewing IACUC *Proposals*.

Table of Contents: Rodent Techniques [*Mice, Rats, Hamsters, Guinea Pigs*]

***NEW*:** 9 December 2020
Reviewed: 10 December 2020
Adopted: 10 December 2020

Procedures, Guidelines, and Exceptions:

I. **Agent Administration:** Common agent administration techniques for rodents are described below. Table 1 lists the recommended needle size and ***maximum*** administration volume for each route. **Note:** If administration volumes will exceed the maximum volumes stated in Table 1 below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Intraperitoneal (IP) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Restrain the animal and tilt backwards so that the head is lower than the hind end and its abdomen is exposed. Insert the needle into *the animal's* lower right quadrant of the abdomen (to avoid the cecum and urinary bladder) at about a 30-degree angle with the needle bevel up. Pull back on the plunger to ensure negative pressure and that no abdominal organs have been punctured prior to injecting. If any fluid is aspirated, the solution is contaminated and must be discarded and the procedure repeated with a new syringe and needle. If no fluid is aspirated, depress the plunger to administer the solution into the peritoneal cavity.

b. **Subcutaneous (SC) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Mice, hamsters: Insert the needle with the bevel up into the skin fold between the thumb and finger created by the restraining hand over the scapular region. Pull back the syringe plunger to aspirate the syringe. If air is aspirated, the needle has gone through the skin and out the other side and must be redirected. If negative pressure is aspirated, depress the plunger to administer the substance in a steady motion between the skin and the body wall.
Rats, guinea pigs: Restrain the animal and tent loose skin over the dorsum. Insert the needle with the bevel up into the skin tent and pull back the syringe plunger to aspirate the syringe. If negative pressure is aspirated, depress the plunger to administer the substance in a steady motion between the skin and the body wall.

c. **Intradermal (ID) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Clip a patch of hair on animal's back and clean the injection site with alcohol. Insert the needle ~1 mm into skin with the bevel up, holding the needle parallel to the skin. Do not aspirate, but administer the substance slowly. Proper injection results in a small, persistent skin welt. The substance should go between the layers of the skin and not underneath the skin.

d. **Tail Vein Intravenous (IV) Injection** [*Mice & Rats*]
Pre-warm the animal for 5-10 minutes to dilate the tail vessels using a heating device placed under the cage or a commercially available warming box, then place the animal into a restraint device. Alternatively, the tail can be submerged in warm (37-40°C) water for 30 seconds. *Animals must be constantly monitored for signs of heat distress or injury.* Clean the injection site with alcohol. Hold tail and, if desired, apply digital pressure to the vessels at the proximal tail to act as a temporary tourniquet. Insert the needle into the lateral tail vein, holding the syringe parallel to the tail. It is easiest to inject at approximately 1/3 of the tail length from the tail tip and move proximally if additional attempts are needed. If visualization is difficult, a light may be used to illuminate the vessels. *It is vital to ensure*

there are no bubbles in the syringe or injection solution. Do not aspirate the syringe, which will collapse the vessel. Administer the substance in a slow, fluid motion, releasing any digital pressure before administration. Too rapid of administration can cause vascular overload or rupture of the vein from excessive pressure. Remove the needle, gently apply pressure to the injection site with gauze, and ensure hemostasis before returning the animal to its cage.

e. **Oral Gavage (PO)** [*Mice, Rats, Hamsters, Guinea Pigs*]
Manually restrain the animal. Measure the gavage needle against animal's body to ensure proper needle length. The gavage needle should measure from the oral cavity to the xiphoid process/last rib. Mark the needle at that length and do not insert it further, which may perforate the stomach. Gavage needles can be stainless steel or plastic and straight or curved, but must have a non-traumatic tip and be appropriate size for the animal (see Table 1). Place tip of gavage needle in animal's mouth and advance it to the back of the oral cavity while extending the animal's head and neck vertically to create a straight line with the body. Pass the gavage needle slowly to the measured point, letting it fall without resistance down the esophagus. *Never force the gavage needle, which can cause traumatic injury such as esophageal perforation and require euthanasia.* If any resistance is felt, pull the needle out and place again. Once the gavage needle is properly placed, administer the substance slowly and carefully remove the needle. Return the animal to the cage and monitor for labored breathing or other signs of distress. Recheck the animal in 12-24 hours after dosing.

f. **Intramuscular (IM) Injection** [*Mice, Rats, Hamsters, Guinea Pigs*]
Restrain the animal to allow access to a hind limb. A restraint device or second technician may be needed to ensure proper placement the injection. Clean the injection site with alcohol. Palpate the quadriceps muscle and insert the needle with the bevel up into the muscle belly, directed away from the femur and sciatic nerve. Injection can be placed in either the cranial thigh musculature or caudal thigh musculature. Pull back the syringe plunger to aspirate the syringe. If no blood is aspirated, depress the plunger to slowly administer the substance. *Due to the limited muscle mass of rodents, only a very small volume can be comfortably and practically administered IM. Consequently, this technique is typically not recommended, especially not for mice or hamsters.*

Table 1: Recommended needle size and ***maximum*** administration volume for common injection techniques in rodent species. **Note:** If administration volumes will exceed the maximum volumes stated in the table below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

	IP		SC		ID		IV		PO		IM	
	Needle size	Volume (ml/kg)	Needle size	Volume (ml/kg)	Needle size	Volume (ml/ site)	Needle size	Volume (ml/kg)	Needle size, length	Volume (ml/kg)	Needle size	Volume (ml/kg /site)
Mice	25-27g	<10	25-27g	<5*	27-30g	<0.05-0.1	27-30g	<5**	18-22g 1-1.5"	<5-10	27g	<0.05
Rats	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	16-20g, 1.5-3"	<5-10	25g	<0.05

Hamsters (dwarf)	25-27g	<10	25-27g	<5	27-30g	<0.05-0.1	27-30g	<5**	18-22g 1-1.5”	<5-10	25g	<0.05
Hamsters (Syrian)	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	18-20g 1.5-2”	<5-10	25g	<0.05
Guinea Pigs	23-25g	<10	23-25g	<5	27-30g	<0.05-0.1	25-27g	<5**	14-18g, 1.5-3”	<5-10	25g	<0.05

*Up to 40 ml/kg can be administered in mice
**Up to 2-4 ml/kg/hr can be given IV for continuous rate infusions

Blood Collection: Common phlebotomy techniques for rodents are described below. Non-terminal blood collection is limited to 1% of body weight per collection or 1.5% of body weight total over a 14-day period with multiple draws. If an approved *Proposal* requires blood collection greater than this amount for scientific reasons, fluid volume replacement must be considered. It is recommended to withdraw only the minimum amount of blood required to meet experimental needs. Table 2 provides maximum collection volumes for rodent species. For estimation purposes, each drop of blood is ~50 µL; however, it is recommended to measure out the approved collection volume prior to phlebotomy if not designated on the collection tube. **Note:** If collection volumes will exceed the maximum volumes stated in Table 2 below, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

g. **Submandibular Blood Collection** [*Adult Mice*]
Restrain the animal by grasping the skin along its back and ensure the skin is taut over the mandible. The intersection of a line drawn straight down from the lateral canthus of the eye and straight back from the commissure of the lips (note: usually a hairless dot over the mandible is present here) is used as a landmark for the puncture site. Insert an 18-20 gauge needle or 5 mm lancet to the shallow depth of 1-2 mm just caudal to the dimple and then pull out to start the flow of blood from the facial vein. Collect the sample and then release manual restraint to stop the flow of blood. Ensure bleeding has stopped before returning the animal to the home cage.

h. **Tail Vein Blood Collection** [*Mice & Rats*]
Place the animal in an appropriate restraining device and clean the blood collection site with alcohol. If the lateral tail vein is difficult to visualize, the animal may need to be pre-warmed using a heating device placed under the cage, a commercially available warming box, or by submerging the tail in warm (37-40°C) water for 30 seconds. *Animals must be constantly monitored for signs of heat distress or injury.* Using a sterile needle, lancet, or scalpel blade, nick the lateral tail vein approximately 1/3 of the tail length from the tail tip. It is recommended to use a 25-gauge needle or 4 mm lancet for mice and a 22-gauge needle or 6 mm lancet for rats. Collect the approved blood volume into an appropriate container. Gently apply pressure to the injection site with gauze to facilitate hemostasis before returning the animal to its cage. A clotting agent such as styptic powder may be applied if needed. If multiple collections are needed, alternate sides of the tail and move proximal towards the base of the tail.

i. **Lateral Saphenous Vein Blood Collection** [*Mice, Rats, Hamster, Guinea Pigs*]
Restrain the animal manually or using a restraint device. Clip the hair on the lateral aspect of a hind limb. Clean collection site with alcohol. Extend the leg and apply gentle pressure at the caudal aspect of the knee joint to occlude the vessel. Prick the lateral saphenous vein with a needle, which runs dorsally and laterally over the tarsal joint. It is recommended to use a 25-27 gauge needle for mice and hamsters and a 23-25 gauge needle for larger rodents. Collect the approved blood volume into an appropriate container. Release occluding pressure and hold gentle pressure with gauze over the puncture site to stop bleeding. Ensure hemostasis before returning the animal to the home cage.

j. **Jugular Blood Collection** [*Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in dorsal recumbency, clip the hair on the ventral neck to aid in identification of landmarks, and wipe the skin with alcohol. Using the non-dominant hand, restrain the animals so the forearms are pulled back, the skin is pulled taught across the neck, and head is hyperextended upwards. Insert a 25 gauge needle with the bevel up, medial to the point of the shoulder and advance towards the jugular furrow. Once the skin has been pierced, apply negative pressure as the needle is advanced until blood is aspirated. Collect the sample and then gently apply pressure with gauze over the puncture site to facilitate hemostasis before recovering the animal and returning to the home cage.

k. **Cranial Vena Cava Blood Collection** [*Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in dorsal recumbency, clip the hair on the ventral neck to aid in identification of landmarks, and wipe the skin with alcohol. Insert a 25 gauge needle at a 30 degree angle between the manubrium and point of the shoulder, cranial to the first rib. Direct the needle towards head of the femur on the contralateral side. Once the skin has been pierced, apply negative pressure as the needle is advanced until blood is aspirated. Collect the sample and then gently apply pressure with gauze over the puncture site to facilitate hemostasis before recovering the animal and returning to the home cage.

l. **Cardiocentesis** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a deep plane of anesthesia as observed by lack of pedal reflex. Insert a 22-25 gauge needle with the bevel up through the skin and below the xiphoid process at midline at an approximately 10-30 degree angle. Direct the needle into the chest cavity and towards the heart. A 1" needle is typically sufficient for a mouse, but larger rodents may require a 1.5-2" needle to puncture the heart. Apply negative pressure by pulling back on the syringe plunger until blood is aspirated. Collect enough blood to exsanguinate the animal (4-5% of body weight) and observe cessation of heart and respiratory rate or another secondary method of euthanasia must be performed. *This technique should only be performed on a deeply anesthetized animal as a terminal collection.*
Note: Exsanguination via cardiocentesis can also be performed immediately following euthanasia (e.g., carbon dioxide asphyxiation) for both terminal blood collection and as a secondary method of euthanasia.

Table 2: Maximum blood collection volumes for common rodent species.
Note: If collection volumes will exceed the maximum volumes stated here, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used.

	Example Body Weight (BW)	1% BW per single collection	1.5% BW total over 14 days	4-5% BW terminal collection
Mice	20 g	0.2 ml	0.3 ml	0.8-1.0 ml
Rats	300 g	3.0 ml	4.5 ml	12.0-15.0 ml
Hamsters (dwarf)	30 g	0.3 ml	0.45 ml	1.2-1.5 ml
Hamsters (Syrian)	120 g	1.2 ml	1.8 ml	4.8-6.0 ml
Guinea Pigs	900 g	9.0 ml	13.5 ml	36-45 ml

II. **Animal Identification:** Common animal identification techniques for rodents are described below. More information including advantages and disadvantages of these methods is available in the IACUC's *Rodent Identification* policy. Table 3 summarizes the recommended ages for the identification methods and whether anesthesia or analgesia is recommended.

a. **Subcutaneous Transponder Placement** [*Mice, Rats, Hamsters, Guinea Pigs*]
Anesthetize the animal and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Position the animal in ventral recumbency and wet the injection site over the shoulder blades with 70% alcohol to part the fur. The microchip and implantation cannula must be appropriately sized for the species (ideally 16g or smaller for rodents), encapsulated in biocompatible material, and sterilized prior to implantation. Most microchips can be purchased sterilized and pre-loaded into disposable delivery systems. Tent the skin at the injection site and insert the implantation needle subcutaneously into the inter-scapular space. Deliver the microchip and then slowly withdraw the needle while manually pinching the skin to ensure the microchip stays under the skin. If needed, the injection site may be closed with medical grade tissue glue (e.g., Vetbond™). Recover the animal and return it to the home cage. This procedure is not recommended for neonatal mice due to the size of the implant, but is acceptable for *weanlings and adults*.

b. **Toe Tattooing** [*Mice, Rats, Hamsters, Guinea Pigs*]
Use an appropriate restraint method for the age and species. Wipe the desired paw pad with an alcohol wipe. Transfer animal tattoo paste (e.g., Ketchum Manufacturing green tattoo paste) onto a sterile surface such as gauze squares or into a sterile secondary container (e.g., Eppendorf tube) to prevent contaminating the stock tube. Dip a 27-30 gauge hypodermic needle tip into a small amount of the tattoo paste and then superficially puncture through the toe pad corresponding to the desired number. A 30 gauge needle is recommended for mice, while up to a 27 gauge needle may be used for larger rodents. A new needle should be used for each animal and replaced if it becomes dull or barbed. Return the animal to its cage and do not clean excess paste off toe pad. An example identification chart is included in Appendix I, but identification systems can vary based on needs and preferences. This procedure can be performed on all ages of rodents and is the preferred method for identifying neonatal mice.

c. **Tail Tattooing** [*Mice & Rats*]
Use an appropriate restraint method or anesthetize the animal. Wipe the tail tattoo site with an alcohol wipe. To manually apply a tattoo, use the tip of a 27-30 gauge sterile hypodermic needle to abrade the dermis of the dorsal tail in the shape of the desired identifier, careful to avoid the tail vasculature. Transfer animal tattoo paste (e.g., Ketchum Manufacturing green tattoo paste) onto a sterile surface such as gauze squares or into a sterile secondary container (e.g., Eppendorf tube) to prevent contaminating the stock tube. Dip the needle into a small amount tattoo paste and apply it to the abraded area. Gently blot the excess paste from the tail with gauze. A new needle should be used for each animal and replaced if it becomes dull or barbed. Alternatively, commercially available animal tattooing methods such as the AIMS™ or Labstamp® rodent tattoo systems may be used according to the manufacturer's specifications. A dose of analgesia such as meloxicam or buprenorphine is recommended prior to starting the procedure. This procedure can be performed on all ages of mice and rats.

d. **Ear Punching/Notching** [*Mice, Rats, Hamsters, Guinea Pigs*]
Appropriately restrain the animal so the ears are accessible and there is limited movement of the neck/head. Use an ear punch device to remove a small piece(s) of ear tissue on the pinna. The punch/notch location should correspond with a desired identification system. If bleeding occurs, apply gentle pressure to the site with gauze and ensure hemostasis before returning the animal to its cage. The ear punch device should be maintained with a sharp cutting surface to minimize tissue injury and disinfected prior to each use. Ear punches should be no larger than 2 mm in diameter. This procedure can be performed once the ears have developed and the pinna are sufficiently pronounced (*after 14 days of age*).

e. **Ear Tagging** [*Mice, Rats, Hamsters, Guinea Pigs*]
Appropriately restrain the animal so the ears are accessible and there is limited movement of the neck/head. Wipe the pinna with alcohol and then apply a unique identifier using an ear tagging device. This is traditionally a stamped nickel tag, but may include newer commercial options such as colored stainless-steel dots or plastic QR codes. The ear tag should be sterilized prior to application. Place the ear tag ~3mm inward from the margin of the pinna on the ventral half of the ear. Improper placement too central in the ear can cause excessive irritation and discomfort. Improper placement too distal on the pinna can cause the tag to become detached from the ear or torn out. This procedure can be performed once the ears have developed and the pinna are able to support the weight of the tag (*weaning age or older*).

f. **Fur Clipping** [*Mice, Rats, Hamsters, Guinea Pigs*]
A patch of fur on the back or side of the rodent may be shaved using an electric clipper. This method may be used to temporarily mark animals of all coat colors for 1-4 weeks at a time depending on the hair cycle.

g. **Temporary Marking** [*Mice, Rats, Hamsters, Guinea Pigs*]
Sharpies or other non-toxic markers may be used to temporarily mark rodents on the tail or fur. These marks usually only last 1-2 days and can be hard to see based on coat color. Non-toxic fur pigments are also available and may last up to 12 weeks (*e.g.*, Animal Markers).

III. **Genotyping:** Common rodent genotyping techniques are described below. More information about these techniques is available in the IACUC's *Tissue Harvesting for Rodent Genotyping* policy.

a. **Ear Punching/Notching** [*Mice, Rats, Hamsters, Guinea Pigs*]
Ear tissue may be collected as described in the "Animal Identification" section and used for genotyping. Any variation from this method will be described separately in the protocol.

b. **Tail Biopsy** [*Mice & Rats*]
Manually restrain or anesthetize the animal and remove up to 5mm of the distal tail tip using a sterile scalpel, razor blade, or sharp scissors. Collect the sample and gently apply pressure with gauze to the biopsy site to facilitate hemostasis before returning the animal to the home cage. A clotting agent such as styptic powder or medical grade tissue glue (e.g., Vetbond™) may be applied if needed. A new sterile blade or scissors must be used for each animal or, alternatively, the instruments can be disinfected between animals with a bead sterilizer if reused. Recommendations and requirements for anesthesia and analgesia are based on the animal's age and amount of tissue excised (see Table 3 below). Local anesthesia can be achieved via 10 second immersion of the tail in cold ethanol, application of ethyl chloride spray, or application of a lidocaine treatment. Systemic analgesia is commonly meloxicam or buprenorphine in rodents.

Table 3: Anesthesia and analgesia recommendations and requirements for tail biopsy procedures based on rodent age and amount of tissue removed.

Age, Tissue Removed	Local Anesthesia	Systemic Analgesia	General Anesthesia
≤ 12 days old, up to 5mm	Recommended		
13-21 days old, up to 5mm	Required	Recommended	
> 21 days old, up to 2mm	Required	Recommended	
> 21 days old, 2-5mm		Required (48 hours)	Required

c. **Blood** [*Mice, Rats, Hamsters, Guinea Pigs*]
Blood may be collected as described in the "Blood Collection" section and used for genotyping. Any variation from these methods will be described separately in the protocol.

d. **Saliva/Buccal Swab** [*Mice, Rats, Hamsters, Guinea Pigs*]
The animal is appropriately restrained based on age and species. A cotton swab is used to collect saliva and cheek cells from the oral cavity by rubbing the swab back and forth on the inside of the cheek. Once a sample is collected, the animal is returned to its home cage.

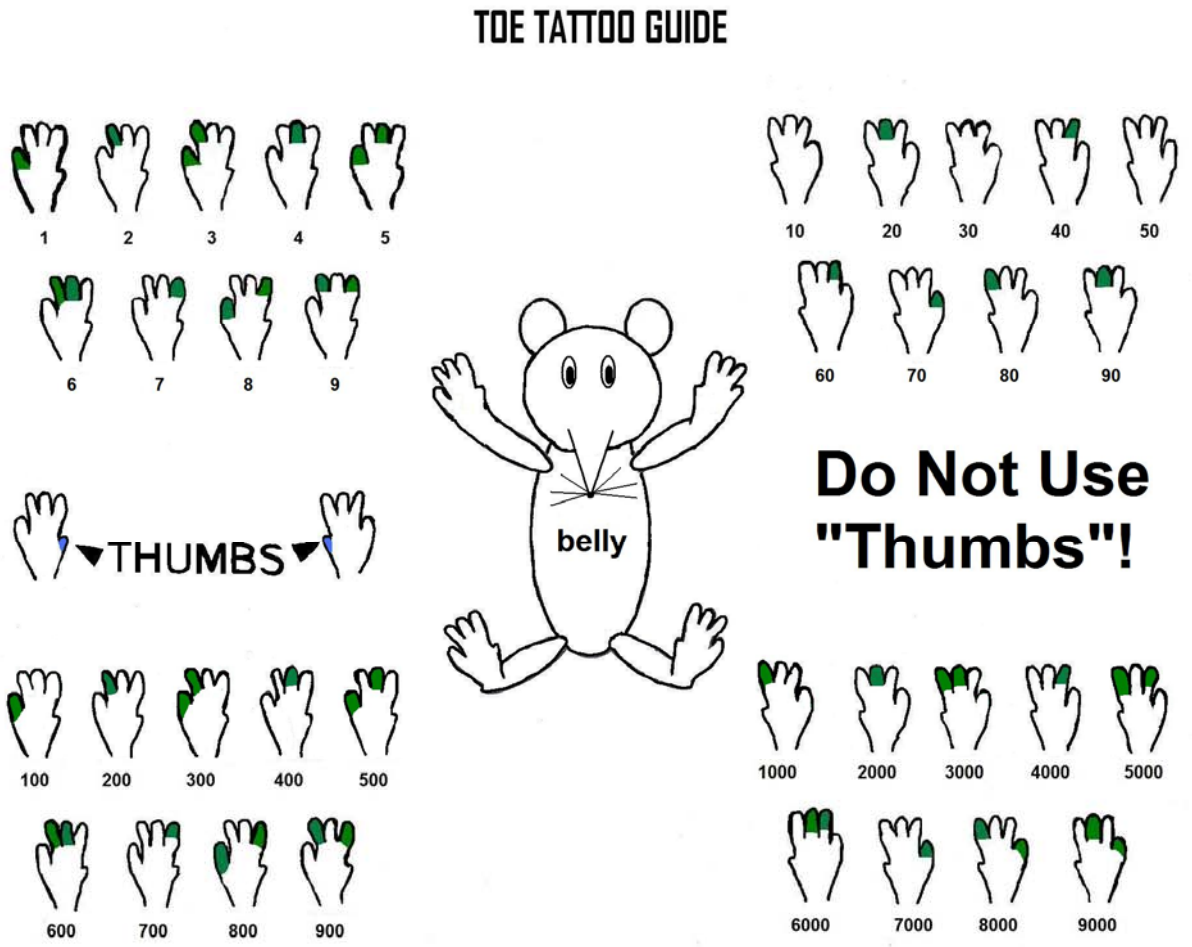
e. **Fecal Pellet** [*Mice, Rats, Hamsters, Guinea Pigs*]
Fecal pellets are either collected from the cage or collected directly from the animal. The animal may be manually restrained and handled for up to 1 minute to encourage passing and collection of a fresh stool sample. Alternatively, the animal may be placed into an empty, clean cage to encourage defecation. Once a sample is collected, the animal is returned to its home cage.

f. **Fur** [*Mice, Rats, Hamsters, Guinea Pigs*]
The animal is appropriately restrained based on age and species. Forceps are used to pluck a small amount of fur from the animal by pulling from the base of the hair shafts. Once a sample is collected, the animal is returned to its home cage.

References:

1. Diehl KH, 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.
2. Fox JG, et al. *Laboratory Animal Medicine*. San Diego (CA), Academic Press, 2015.
3. Morton DB, et al. 2001. Refining procedures for the administration of substances. *Lab Animals* 35: 1-41.
4. Turner PV, et al. 2011. Administration of substances to laboratory animals: Routes of administration and factors to consider. *JAALAS* 50(5): 600-613.
5. Turner PV, et al. 2011. Administration of substances to laboratory animals: Equipment considerations, vehicle selection, and solute preparation. *JAALAS* 50(5): 614-627

Appendix I:



***NEW*:** 9 December 2020
Reviewed: 10 December 2020
Adopted: 10 December 2020

IACUC Standard Procedures for Non-Rodent Species

Policy: This policy describes the IACUC's approved standard procedures for select non-rodent species which investigators may easily incorporate into their IACUC *Proposals*. Principal Investigators (PI) performing procedures as described in this policy may utilize the checkboxes in the procedures section of the IACUC's *Proposal* form rather than describing the procedures in full. If the performance of the procedure will deviate from the descriptions in this policy, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. All procedures, including the IACUC's standard procedures, must be included in the IACUC *Proposal* form and approved by the IACUC prior to performance. It is the responsibility of the PI to ensure that all laboratory personnel responsible for procedures are appropriately trained and qualified. The Comparative Medicine Research Unit (CMRU) veterinary staff is available to provide training at no charge; training can be scheduled through the IACUC's website.

Rationale: This policy has been drafted to ensure consistency in the performance of common non-surgical procedures, as well as reduce the administrative burden to investigators and the IACUC in drafting and reviewing IACUC *Proposals*.

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Reviewed: 10 December 2020
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Procedures, Guidelines, and Exceptions:

Cat Techniques

I. **Agent Administration:** Common agent administration techniques for cats are described below. **Note:** If administration volumes will exceed the maximum volumes stated in the procedures below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Oral Administration of Liquids** [*Cats*]
When possible, flavored liquids especially formulated for cats should be used for a better experience. The animal will be gently restrained manually or using a commercial cat bag if needed. Liquid medication will be slowly administered orally by letting the cat readily ingest or by gently inserting a syringe into the corner of the mouth. The liquid is slowly dispensed as the animal is observed to comfortably swallow it. The animal is observed to ensure medication was ingested. Maximum volume 2 ml/kg.

b. **Oral Administration of Pills and Capsules** [*Cats*]
When possible, flavored tablets that animals will readily eat should be used for a better experience. Pills may also be crushed up or capsules opened and mixed into a small amount (1

to 2 teaspoons) of wet food. The animal must be seen ingesting all of the medication/food to consider the treatment complete. If not available, the animal will be gently restrained manually or using a commercial cat bag if needed and the medication will be administered by placing the pill/capsule in the back of the throat or by using a commercial pilling device. Administer at least 3-6 ml of tap water after oral capsules or tablets are given to ensure the animal swallows, and to aid in transit of medication to the stomach.

c. **Subcutaneous Injection** [*Cats*]

Standard injections are given in the loose skin of the dorsal cervical region, dorsal interscapular area, or dorsal thoracic area. Restrain the animal in the most appropriate position to ensure the animal and the handler's comfort and safety as well as the best access to the chosen injection site. In some instances, a commercially available cat bag may be needed. A 22-25 gauge 1/2 to 1-inch needle is used. The smallest size and length needle possible should be used for animal comfort and is dependent upon the animal size and viscosity of the solution. Skin is tented and the needle is placed at a 45-degree angle. The plunger is pulled back to ensure the needle has not inadvertently entered a blood vessel. If no blood is seen the substance can be slowly injected. The maximum volume is 5 ml/kg of a substance or 25 ml/kg of isotonic fluid. Multiple sites may need to be used if necessary to deliver the required volume. No more than 20 ml should be injected per site. Fluids should be warmed.

d. **Intramuscular Injection** [*Cats*]

Standard intramuscular (IM) injections are given in the quadriceps, hamstrings, or dorsal lumbar muscle groups. The animal will be gently restrained manually or by using a commercial cat bag if needed. A 22-25 gauge 1/2 to 1-inch needle is used but may only need to be inserted part of the length. For all locations, pull back on the syringe to ensure that the needle has not entered a blood vessel, and slowly inject the solution. Maximum volume is 1 ml for animals 2 kg or less and 2 ml for an animal greater than 2 kg.

Due to the potential for muscle damage, multiple injections should be avoided.

- The ***quadriceps muscle group*** can be located by palpating along the cranial femur. It lies cranial to the greater trochanter and proximal to the patellar ligament. The needle is inserted perpendicular into the proximal 1/2 to 1/3 of the muscle mass.
- The ***hamstrings or caudal thigh muscles*** are located on the caudal femur in a triangular area demarcated by the greater trochanter, ischial tuberosity, and stifle joint. The needle should be directed perpendicular to the muscle mass in the proximal 1/2 to 1/3 of the muscle group. Care should be taken to avoid the sciatic nerve.
- The ***dorsal lumbar muscle group*** can be palpated along the spine between the last rib and the wing of the ileum. The correct injection site is at the level of the 3rd to 5th lumbar vertebrae. Insert the needle parallel to the spine and at a 45-degree angle to the animal's back.

e. **Intravenous Injections** [*Cats*]

The animal will be gently restrained manually or using a commercial cat bag if needed. Common venipuncture sites in the cat include the external jugular vein, cephalic vein, lateral saphenous vein, and the medial saphenous vein. Fur may be clipped to aid in the visualization of the peripheral vein. The site is cleaned with 70% alcohol. The vein is occluded with a tourniquet as applicable or digital pressure. A 22-25 gauge 1/2 to 1-inch needle is inserted into the vessel at a 25-degree angle. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once

correct needle placement is confirmed, release occlusive pressure on the vein, and slowly inject the agent monitoring for signs of perivascular infiltration. The fluid should flow easily with no resistance. If resistance is encountered, stop and reposition the needle. Once the injection is complete, remove the needle and place gently pressure on the site to promote hemostasis. A light bandage may be used for cephalic and saphenous puncture sites. Maximum volume is 2-5 ml per animal slow IV bolus or 10 ml/kg/hour for continuous rate infusions.

- The ***jugular vein*** is located between the angle of the mandible and the thoracic inlet. Manual restraint for jugular blood collection requires some skill and involves positioning the cat at the edge of a surgery or prep table, holding the front legs over the edge with one hand, and tipping the neck back and nose up with your other hand. The front legs and neck up to the point of the mandible should be in a vertical plane. The venipuncture should be performed in the middle 1/3 of the jugular vein.
- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal can be restrained in dorsal recumbency or a sitting/standing position. The vessel is occluded and rolled laterally at the antecubital area.
- The ***lateral saphenous vein*** is located on the craniomedial side of the back leg between the ankle (talus) and the knee. The animal should be placed in lateral recumbency with the intended site on the upper leg. The vessel is occluded and rolled from caudal to lateral to isolate it on the upper-facing aspect of the leg.
- The ***medial saphenous vein*** is located on the medial aspect of the hind limb between the knee and the inguinal area. The animal should be restrained in lateral recumbency with the intended venipuncture site on the down leg. The vessel is occluded at the inguinal area.

II. **Blood Collection via Venipuncture** [*Cats*]

Note: If collection volumes will exceed the maximum volumes stated here, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

The animal will be gently restrained manually or using a commercial cat bag if needed. Common venipuncture sites in the cat include the external jugular vein, cephalic vein, lateral saphenous vein, and the medial saphenous vein. Fur may be clipped to aid in the visualization of the peripheral vein. The site is cleaned with 70% alcohol. The vein is occluded with a tourniquet as applicable or digital pressure. A 22-25 gauge 1/2 to 1-inch needle is inserted at a 25-degree angle. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once correct needle placement is confirmed, continue to gently pull back in the syringe plunger to collect blood. Once blood withdrawal is complete, remove the needle and place gentle pressure on the site to promote hemostasis. A light bandage may be used for cephalic and saphenous puncture sites. Animals are monitored until hemostasis is achieved. Felines have an average blood volume of 55 ml/kg. Maximum blood volumes to be collected for survival procedures should not exceed 1% complete blood volume per 24 hours, 7.5% complete blood volume weekly, or 10% complete blood volume every 2 to 4 weeks.

- The ***jugular vein*** is located between the angle of the mandible and the thoracic inlet. Manual restraint for jugular blood collection requires some skill and involves positioning

the cat at the edge of a surgery or prep table, holding the front legs over the edge with one hand, and tipping the neck back and nose up with your other hand. The front legs and neck up to the point of the mandible should be in a vertical plane. The venipuncture should be performed in the middle 1/3 of the jugular vein.

- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal can be restrained in dorsal recumbency or a sitting/standing position. The vessel is occluded and rolled laterally at the antecubital area.
- The ***lateral saphenous vein*** is located on the craniomedial side of the back leg between the ankle (talus) and the knee. The animal should be placed in lateral recumbency with the intended site on the upper leg. The vessel is occluded and rolled from caudal to lateral to isolate it on the upper-facing aspect of the leg.
- The ***medial saphenous vein*** is located on the medial aspect of the hind limb between the knee and the inguinal area. The animal should be restrained in lateral recumbency with the intended venipuncture site on the down leg. The vessel is occluded at the inguinal area.

III. **Peripheral IV Catheter Placement** [*Cats*]

The animal will be gently restrained manually or using a commercial cat bag if needed. The most common peripheral catheter placement site is the cephalic vein with the medial saphenous being a lesser option due to undesirable positioning in awake animals and the difficulty of securing the catheter in place. The hair is clipped from the site and the area is surgically prepped. Insert a 22-24 gauge 1-inch catheter into the vein. Once blood is observed in the hub of the needle, hold the needle in place while advancing the catheter off the needle and into the vein. The catheter should glide easily with no resistance. If resistance is met, stop and reposition the catheter. Release occlusive pressure on the vein once the catheter is in place. Remove the needle from the catheter and flush with heparinized saline to verify position. Monitor site for signs of perivascular infiltration. Connect fluids to the catheter or place an injection port or cap on the end of the catheter. Securely tape the catheter in place. If the catheter will not be used immediately, lock it with heparin to avoid clotting. *Peripheral catheters should not be maintained more than 3 days.*

- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal is placed in dorsal recumbency and the vessel is identified. The vessel is occluded and rolled laterally at the antecubital area.
- The ***medial saphenous vein*** is located on the medial aspect of the hind limb between the knee and the inguinal area. The animal should be restrained in lateral recumbency with the intended venipuncture site on the down leg. The vessel is occluded at the inguinal area.

IV. **Intubation** [*Cats*]

The animal must be anesthetized before intubation. The appropriately sized cuffed endotracheal tube is chosen for the size of the animal and the cuff is checked on the tube before placement in the animal (see Table 1 below). The end of the tube is lubricated with sterile lubricant or oral lidocaine gel. The animal is placed in sternal or lateral recumbence and the mouth is gently opened and the tongue is retracted for visualization of the epiglottis. The epiglottis is depressed with the tip of an appropriately sized laryngoscope blade. The endotracheal tube is passed through the glottis and into the trachea until the tip of the tube is midway between the larynx and thoracic inlet. The tube placement is checked by auscultating both sides of the patient's chest for breathing sounds. Also, the neck can be palpated to ensure the presence of the tube, and the tube is inspected for condensation. Once confirmed in the trachea, the tube is secured with a tie around the endotracheal tube behind the

adaptor and then tied around the animal's head behind ears to the upper jaw or the lower jaw, depending on the surgical procedure. The endotracheal tube is connected to the inhalation anesthesia machine, respirator, or Ambu bag. The cuff is inflated with sufficient air to prevent leakage.

Table 1: Guidelines for endotracheal tube size in cats

1 KG	3.0 mm
2 KG	3.5 mm
3.5 KG	4.0 mm
4+KG	4.5 mm

V. **Microchip Implantation for Identification and/or Temperature Monitoring** [*Cats*]
Microchip implantation may be performed in an awake animal, but it is best practice to perform implantation in conjunction with planned anesthesia, if possible, due to the size of the trocar used for insertion. The animal will be restrained manually or using a commercial cat bag if needed (if not under anesthesia). The skin is tented or pulled taught between shoulder blades or behind the ear. The microchip applicator is inserted subcutaneously for the full length of the applicator needle. This helps to ensure that the transponder will not back out of the track. The plunger of the microchip applicator is depressed. The skin is pinched as the applicator is removed. The animal is scanned to ensure proper placement and function of the microchip.

VI. **Prolonged Physical Restraint for IACUC Standard Procedures** [*Cats*]
Prolonged restraint, defined by the IACUC as over 15 minutes for rodents and over 30 minutes for non-rodent mammals, must be described in the IACUC *Proposal* and reviewed and approved by the IACUC prior to performance. Prolonged restraint should not be used for convenience. The below description detailing necessary restraint for the performance of the IACUC's approved standard procedures to prevent injury to animals, as well as personnel, has been included for investigator convenience. Investigator's may copy and paste the below information into the prolonged restraint subsection (NonStandard Housing Section) of the IACUC *Proposal* form. See the IACUC's *Prolonged Restraint* policy.

Device description	Commercially available cat bag made of a soft nylon material with Velcro closures. The animal is completely enclosed except for the head and neck.
Justification for necessity and duration of restraint	Used to restrain cats for minor procedures such as injections and venipuncture. Restraint will be for the shortest duration possible to safely perform the procedure.
Duration of confinement	Anticipated less than 30 minutes but may run slightly longer if unforeseen difficulties occur. No animal will remain in the restraint device more than 1 hour per time.
Acclimation procedures	Not applicable. Cat bags are only used when necessary and then for the shortest duration of time.
Monitoring procedures	Animals will be monitored by lab staff the entire time they are in the bag.
Provision for veterinary care if there are adverse clinical events	A vet will be called to examine the animal immediately if there is an adverse event.

Criteria for removing animals that do not adapt to the restraint device	If an animal does not settle down and continuous to struggles against device after 15 minutes of being placed in the bag it will be removed.
# of attempts before animals are permanently removed from restraint due to failure to acclimate	Two.

VII. **Other Information: Fasting Prior to Planned Anesthesia Event** [*Cats*]
The below information is intended to serve as guidance to investigators in the planning of procedures and drafting of *Proposals*.

- Less than 8 weeks of age or less than 2 kg **-** Do not withhold water. Withhold food for 1 to 2 hours.
- Greater than 2 kg healthy adult - Do not withhold water. Withhold food for 4 to 6 hours.

Swine Techniques

I. **Agent Administration:** Common agent administration techniques for swine are described below. **Note:** If administration volumes will exceed the maximum volumes stated in the procedures below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Oral Administration of Liquids** [*Swine*]
The animal will be gently manually restrained if needed. Liquid medication will be slowly administered orally by gently inserting a syringe into the corner or front of the mouth. The liquid is slowly dispensed as the animal is observed to ingest it. Pills will be placed inside food treats for administration. The animal is observed to ensure medication was ingested. Volume limit 1 ml/kg.

b. **Subcutaneous Injection** [*Swine*]
Subcutaneous administration is not easily executed in swine because the skin tightly adheres to the underlying tissue over most of the body surface. Injections are often inadvertently administered into subcutaneous fat which may alter the drug's release/absorption. Standard injections are given in the loose skin of the lateral cervical area directly caudal to the ear. They may also be given in the loose skin of the axillary or inguinal area in small animals or in larger animals already under anesthesia for other procedures. The needle should be inserted at a 45-degree angle to the skin. Aspiration is performed to verify that needle is not inadvertently in a blood vessel prior to slowly administering the agent. Injections can be given in awake animals. Awake animals should be injected in familiar surroundings and offered a small amount of food or treat if possible to distract them and avoid stress. Younger animals may be gently held for injections. For animals up to 25 kgs, a 19-23 gauge 1/2 to 1-inch needle is used. For animals over 25 kgs, an 18-20 gauge 1/2 to the 1-inch needle is used. The smallest size and length needle possible are used for animal comfort and is dependent upon the animal size and viscosity of the solution. If the animal is awake and not restrained, it is recommended that a butterfly catheter or an extension set be connected to the needle to allow the pig to move freely while the drug is

being administered. Maximum volume is 1-2 ml/kg. Multiple sites may need to be used if necessary to deliver the required volume.

c. **Intranasal Administration** [*Swine*]
A mucosal atomization device or 20-24 gauge plastic catheter sheath can be used on the end of a syringe to deliver medications intranasal in animals less than 25 kg. Alternatively, if the animal is very calm and the head can be tilted upward, medication can be slowly dripped into the nostrils directly from a syringe. For animals greater than 25 kg, substances can be administered directly into the nostrils by using a syringe only. The maximum dose is 1 ml/10kg. Total volume should be divided between both nostrils.

d. **Intramuscular Injection** [*Swine*]
Standard intramuscular (IM) injections are given in the cervical muscle group which is located in the triangular area behind the ear and before the angle of the shoulder. Due to the thick subcutaneous layer of fat, true IM injections must be administered very deeply using long needles inserted at a 90-degree angle to the musculature. Aspiration is performed to verify that the needle is not inadvertently in a blood vessel before slowly administering the agent. Injections can be given in awake animals but proper restraint is required. Animals should be injected in familiar surroundings and offered a small amount of food or treat if possible to distract them and avoid stress. Younger animals may be gently held for injections and animals up to 40 kg can be placed in a sling-type restraint device. For animals up to 25 kgs. An 18-22 gauge 1/2 to 3/4-inch needle is used. For animals 25-70 kgs, a 16-19 gauge 3/4 to 1-inch needle is used. For animals greater than 70 kg, a 14-18 gauge 1 to 2-inch needle is used. The smallest size and length needle possible is used for animal comfort and is dependent upon the animal size and viscosity of the solution. For animals greater than 70 kg, due to the size and strength of the animal and the size of the needle required, prior sedation is recommended for safe handling. If the animal is awake it is recommended that a butterfly catheter or an extension set be connected to the needle to allow the pig to move freely while the drug is being administered. Maximum volume is 0.25 ml/kg. Multiple sites can be used if necessary to deliver the required volume.

e. **Intravenous Injection** [*Swine*]
Animals must have an intravenous (IV) catheter in place to do IV injections in awake animals. IV injections are usually given in the ear veins or through central lines placed in the jugular vein. They can also be administered through catheters placed in the cephalic or saphenous vein. The catheter is flushed to ensure patency and correct position. When verified, the substance is administered slowly. If the animal is awake and not restrained, it is recommended that an extension set be connected to the needle to allow the pig to move freely while the drug is administered. The maximum volume for IV bolus delivery is 2.5 ml/kg. The maximum volume for slow IV injection is 5 ml/kg.

II. **Blood Collection via Venipuncture** [*Swine*]
Note: If collection volumes will exceed the maximum volumes stated here, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

Blood collection in swine requires that the animal be sling trained (less than 40 kg limit), have a central catheter in place, or the animal is anesthetized. Venous blood can be collected from the cranial vena cava, ear vein, lateral or medial saphenous vein, or cephalic veins. The site is cleaned and an appropriately sized needle is inserted into the vein according to animal and vessel size. The plunger of the syringe is gently and steadily retracted to collect the sample. Once the sample is collected, the needle is removed from the vein and skin, and gentle but firm pressure is applied to the site to ensure hemostasis. If applicable, the site may be wrapped with a temporary bandage to aid in hemostasis. Animals are monitored until hemostasis is achieved. Swine have an average blood volume of 56 ml/kg. Maximum blood volumes to be collected for survival procedures should not exceed 1% complete blood volume per 24 hours, 7.5% complete blood volume weekly, or 10% complete blood volume every 2 to 4 weeks.

III. **Catheter Placement** [*Swine*]

a. **Peripheral Intravenous Catheter Placement** [*Swine*]

Catheter placement in swine requires that the animal be anesthetized before placement except in very small piglets that can be manually restrained or in animals that are sling trained (less than 40 kg limit). Catheters can be placed in cephalic, saphenous, or ear veins. Hair will be clipped and the site will be surgically prepped. Occlusive pressure will be applied or a constricting band will be placed proximal to the infusion site. An 18-24g IV catheter of appropriate length will be used, depending upon animal and vessel size, to directly pierce the skin and vein. Once blood is observed in the hub of the needle, hold the needle in place while advancing the catheter centrally off the needle and into the vein. The catheter should glide easily with no resistance. If resistance is met, stop and reposition the catheter. Release occlusive pressure on the vein once the catheter is in place. Remove the needle from the catheter and flush with heparinized saline to verify position. Monitor site for signs of perivascular infiltration. Connect fluids to the catheter or place an injection port or cap on the end of the catheter. Securely tape the catheter in place. If the catheter will not be used immediately, lock it with heparin to avoid clotting. When not in use, the catheter should be flushed and relocked for maintenance at least daily. *Peripheral catheters should not be maintained for more than 3 days.*

b. **Urinary Catheter Placement** [*Female Swine Only*]

Urinary catheter placement in swine requires that the animal be anesthetized before placement except in animals that are sling trained (less than 40 kg limit). The perineal area is aseptically prepped and sterile gloves are donned. A sterile appropriately sized (8-14 French) Foley urinary catheter will be lubricated and placed within the urethra and fed into the urinary bladder until urine is liberated. The retention bulb will be inflated with normal saline to prevent premature removal. The urinary catheter is connected to a sterile urine collection bag. If to be maintained long term, the urinary catheter will be secured with a commercial patch adhered to the animal's hip. If only used short term, the bulb will be deflated and the catheter will be removed before the animal waking or being removed from the sling. It is not possible to pass urinary catheters in male swine due to the anatomy of the penile urethra.

IV. **Intubation** [*Swine*]

Intubation of swine requires advanced veterinary technical skills due to the shape and size of the head and mouth. They are also very prone to laryngospasms, thus requiring deep anesthesia and careful monitoring. <u>All swine intubation should be performed by CMRU veterinary staff</u>. The appropriately sized cuffed endotracheal tube is chosen for the size of the animal and the cuff is checked on the tube prior to placement in the animal. The end of the tube is lubricated with sterile

lubricant or oral lidocaine gel. The animal is placed in sternal or lateral recumbence and the mouth is gently opened and the tongue is retracted for visualization of the epiglottis. The epiglottis is depressed with the tip of the laryngoscope blade. The endotracheal tube is passed through the glottis and into the trachea until the tip of the tube is midway between the larynx and thoracic inlet. The tube placement is checked by auscultating both sides of the patient's chest for breathing sounds. Also, the neck can be palpated to ensure the presence of the tube, and the tube is inspected for condensation. Once confirmed in the trachea, the tube is secured with a tie around the endotracheal tube behind the adaptor and then tied around the animal's head behind ears, to the upper jaw or the lower jaw depending on the surgical procedure. The endotracheal tube is connected to the inhalation anesthesia machine, respirator, or Ambu bag. The cuff is inflated with sufficient air to prevent leakage.

V. **Microchip Implantation for Identification and/or Temperature Monitoring** [*Swine*]

The animal will be restrained appropriately (if not under anesthesia). The skin is tented or pulled taught between shoulder blades or behind the ear. The microchip applicator is inserted subcutaneously if only used for identification. The microchip applicator is inserted intramuscularly if also used for temperature. The microchip applicator is inserted for the full length of the applicator needle. This helps to ensure that the transponder will not back out of the track. The plunger of the microchip applicator is depressed. The skin is pinched as the applicator is removed. The animal is scanned to ensure proper placement and function of the microchip.

VI. **Prolonged Physical Restraint for IACUC Standard Procedures** [*Swine*]

Prolonged restraint, defined by the IACUC as over 15 minutes for rodents and over 30 minutes for non-rodent mammals, must be described in the IACUC *Proposal* and reviewed and approved by the IACUC prior to performance. Prolonged restraint should not be used for convenience. The below description detailing necessary restraint for the performance of the IACUC's approved standard procedures to prevent injury to animals, as well as personnel, has been included for investigator convenience. Investigator's may copy and paste the below information into the prolonged restraint subsection (NonStandard Housing Section) of the IACUC *Proposal* form. See the IACUC's *Prolonged Restraint* policy.

Device description	Commercially available swine sling made of a soft hammock with holes for the limbs to go through attached to a frame. The animal can be trained to walk into the hammock when it is lowered or, if small, it can be gently placed in the hammock. The hammock can be raised to lift the animal off the ground while maintaining a normal postural position. Weight limit 40 kg.
Justification for necessity and duration of restraint	Used to restrain pigs for minor procedures such as urine collection, injections, and venipuncture. Restraint will be for the shortest duration possible to safely perform the procedure.
Duration of confinement	Anticipated less than 30 minutes but may run slightly longer if unforeseen difficulties occur. No animal will remain in the sling more than 1 hour per time.
Acclimation procedures	Pigs will be acclimated to the sling by allowing them to investigate the sling for 1-2 days. The pig will then be placed in the sling for 5 minutes daily, increasing by 5 minutes/day until a maximum restraint time of 30 minutes is achieved when necessary.
Monitoring procedures	Animals will be monitored by lab staff the entire time they are in the sling.

Provision for veterinary care if there are adverse clinical events	A vet will be called to examine the animal immediately if there is an adverse event.
Criteria for removing animals that do not adapt to the restraint device	If pigs do not tolerate (evidenced by restlessness and vocalization that does not cease within a couple of minutes) the restraint they will be removed from the sling and the next training session time will be reduced. Restraint time will not be increased until the animal tolerates the previous training session. Food rewards will be given to the animals during acclimation and training such as marshmallows, cereal, fruit, or flavored dog treats.
# of attempts before animals are permanently removed from restraint due to failure to acclimate	Five.

VII. **Other Information: Fasting Prior to Planned Anesthesia Event** [*Swine*]
The below information is intended to serve as guidance to investigators in the planning of procedures and drafting of *Proposals*.

- Babies on milk – no fasting
- Weaning to 3 months of age – at least 3 hours
- Greater than 3 months of age with procedures not involving abdominal organs – 6 to 12 hours
- Greater than 3 months of age with procedures involving abdominal organs – at least 12 hours

Rabbit Techniques

I. **Agent Administration:** Common agent administration techniques for rabbits are described below. **Note:** If administration volumes will exceed the maximum volumes stated in the procedures below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Oral Administration of Liquids** [*Rabbits*]
When possible, flavored liquids especially formulated for rabbits should be used for a better experience. The animal will be gently restrained manually or using a commercial rabbit snuggle if needed. Liquid medication will be slowly administered orally by letting the rabbit readily ingest or by gently inserting a syringe into the corner of the mouth. The liquid is slowly dispensed as the animal is observed to comfortably swallow it. The animal is observed to ensure medication was ingested. Volume limit 2 ml/kg.

b. **Oral Administration of Pills and Capsules** [*Rabbits*]
When possible, flavored tablets that animals will readily eat should be used for a better experience. Pills may also be crushed up or capsules opened and mixed into a small amount (1 to 2 teaspoons) of yogurt, honey, or other treats. The animal must be seen ingesting all of the medication/food to consider the treatment complete. If not available, the animal will be gently restrained manually or using a commercial snuggle if needed and the medication will be administered by placing the pill/capsule in the back of the throat or by using a commercial pilling device. Administer at least 3-6 ml of tap water after oral capsules or tablets are given to ensure the animal swallows, and to aid in transit of medication to the stomach.

c. **Subcutaneous Injection** [*Rabbits*]
The animal may be gently restrained manually or using a commercial rabbit snuggle if needed. Standard injections are given in the loose skin of the dorsal neck and shoulder area. Injections can be given in awake animals. A 22-25 gauge 1/2-1 inch needle is used. The smallest size and length needle possible is used for animal comfort and is dependent upon the animal size and viscosity of the solution. Skin is tented and the needle is placed at a 45-degree angle. The plunger is pulled back to ensure the needle has not inadvertently entered a blood vessel. If no blood is seen the substance can be slowly injected. If the animal is awake and not restrained, it is recommended that a butterfly catheter or an extension set be connected to the needle to allow the animal to move freely while the drug is being administered. The maximum volume is 5 ml/kg of a substance or 25 ml/kg of isotonic fluid. Multiple sites may need to be used if necessary to deliver the required volume. No more than 20 ml should be injected per site. Fluids should be warmed.

d. **Intramuscular Injection** [*Rabbits*]
The animal may be gently restrained manually or using a commercial rabbit snuggle if needed. Standard IM injections are given in the quadriceps or dorsal lumbar muscle groups. Injections can be given in awake animals but secure restraint is required to prevent the animal from injuring itself. A short 1/2 to 5/8-inch 25 gauge needle is used. Pull back on the syringe to ensure that the needle has not entered a blood vessel, and slowly inject the solution. Maximum volume is 1 ml for an adult animal or 0.05 ml/kg. Due to the potential for muscle damage, multiple injections should be avoided.

- If using the **quadriceps muscle group**, the muscle is palpated along the cranial femur and lies cranial to the greater trochanter and proximal to the patellar ligament. The needle is inserted perpendicular into the proximal 1/2 to 1/3 of the muscle mass.
- If using the **dorsal lumbar muscle group**, palpate the muscle group along the spine between the last rib and the wing of the ileum. The correct injection site is at the level of the 3rd to 5th lumbar vertebrae. Insert the needle parallel to the spine and at a 45-degree angle to the animal's back.

e. **Intravenous Injections** [*Rabbits*]
Animals must have an intravenous (IV) catheter in place to do IV injections in awake animals. IV injections are usually administered in the ear veins or through central lines placed in the jugular vein. They can also be administered through catheters placed in the cephalic or saphenous vein. The catheter is flushed to ensure patency and the substance is administered slowly. Maximum volumes to be injected is dependent upon the animal size and whether a peripheral or central catheter is used. Maximum volume for IV bolus delivery is 2 ml/kg true bolus or 5 ml/kg as a slow bolus over several minutes. The maximum volume for slow IV injection is 20 ml/kg slowly infused over 10 minutes or longer.

II. **Blood Collection via Venipuncture** [*Rabbits*]
Rabbits have a 44 ml/kg blood volume. Maximum blood volumes to be collected for survival procedures should not exceed 1% complete blood volume per 24 hours, 7.5% complete blood volume weekly, or 10% complete blood volume every 2 to 4 weeks. **Note:** If collection volumes will exceed the maximum volumes stated here, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are

strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

- **3 ml or less per sample:** The most common site to collect small volumes of blood is the ***aural (ear) vessels (artery and vein)***. Twenty minutes before attempting to take blood, while the animal is still in its home cage, a local anesthetizing cream will be applied to the back of both ears. The animal will be manually restrained or placed in a snuggle if needed immediately before taking the blood sample to minimize the time of restraint. The back of the ear will be cleaned with 70% alcohol. Shaving is not required. The vessel is manually occluded by applying gentle pressure at the base of the ear. A 22-25 gauge ½ to ¾ inch needle is inserted at a 25-degree angle. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once correct needle placement is confirmed, continue to gently pull back in the syringe plunger to collect blood. Once blood withdrawal is complete, remove the needle and place gentle pressure on the site to promote hemostasis. A light bandage may be used. Animals are monitored until hemostasis is ensured before placing the animal back in the home cage.

- **Greater than 3 ml per sample:** If larger blood samples are needed the animal should be anesthetized and blood is withdrawn from the jugular vein. The cervical area overlying the jugular vein is shaved and aseptically prepped. The rabbit is placed in dorsal recumbency and the neck is extended. Gently pressure is applied at the base of the neck to occlude the vessel for visualization. The ***jugular vein*** is located between the angle of the mandible and the thoracic inlet. A 22-25 gauge 1/2 to 1-inch needle is inserted at a 25-degree angle in a caudal direction. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once correct needle placement is confirmed, continue to gently pull back in the syringe plunger to collect blood. Once blood withdrawal is complete, remove the needle and place gentle pressure on the site to promote hemostasis. A light bandage may be used. Animals are monitored until hemostasis is ensured before placing the animal back in the home cage.

III. **Peripheral IV Catheter Placement** [*Rabbits*]

The animal may be gently restrained manually or using a commercial rabbit snuggle if needed. Should any animal not tolerate the snuggle, they will be briefly sedated with isoflurane for catheter placement. Catheters are most often placed in the aural (ear) vessels (artery and vein) but can be placed in the cephalic or saphenous veins. The hair is clipped from the site if applicable and the area is surgically prepped. Insert a 22-24 gauge 1-inch catheter into the vein. Once blood is observed in the hub of the needle, hold the needle in place while advancing the catheter off the needle and into the vein. The catheter should glide easily with no resistance. If resistance is met, stop and reposition the catheter. Release occlusive pressure on the vein once the catheter is in place. Remove the needle from the catheter and flush with heparinized saline to verify position. Monitor site for signs of perivascular infiltration. Connect fluids to the catheter or place an injection port or cap on the end of the catheter. Securely tape the catheter in place. If the catheter will not be used immediately, lock it with heparin to avoid clotting. *Catheters should not be maintained for more than 3 days.*

- The ***aural vessels*** are located on the dorsal pinna. The veins are small and run marginally along the pinna. The artery is larger and more centrally located. The vessels are occluded by applying pressure at the base of the ear.

- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal is placed in dorsal recumbency and the vessel is identified. The vessel is occluded and rolled laterally at the antecubital area.
- The ***lateral saphenous vein*** is located on the craniomedial side of the back leg between the ankle (talus) and the knee. The animal should be placed in lateral recumbency with the intended site on the upper leg. The vessel is occluded and rolled from caudal to lateral to isolate it on the upper-facing aspect of the leg.

IV. **Intubation** [*Rabbits*]

Intubation of rabbits requires advanced clinical skills due to the shape and size of the head and mouth. They are also very prone to laryngospasms thus requiring deep anesthesia and careful monitoring. All rabbit intubation should be performed by CMRU veterinary staff or laboratory personnel with advanced clinical skills as outlined in the IACUC Participant Training Log. The appropriately sized cuffed endotracheal tube is chosen for the size of the animal and the cuff is checked on the tube before placement in the animal. The end of the tube is lubricated with sterile lubricant or oral lidocaine gel. The animal is placed in sternal or lateral recumbence and the mouth is gently opened and the tongue is retracted for visualization of the epiglottis. The epiglottis is depressed with the tip of the laryngoscope blade. The endotracheal tube is passed through the glottis and into the trachea until the tip of the tube is midway between the larynx and thoracic inlet. The tube placement is checked by auscultating both sides of the patient's chest for breathing sounds. Also, the neck can be palpated to ensure the presence of the tube, and the tube is inspected for condensation. Once confirmed in the trachea, the tube is secured with a tie around the endotracheal tube behind the adaptor and then tied around the animal's head behind the ears, to the upper jaw or the lower jaw depending on the surgical procedure. The endotracheal tube is connected to the inhalation anesthesia machine, respirator, or Ambu bag. The cuff is inflated with sufficient air to prevent leakage.

V. **Microchip Implantation for Identification and/or Temperature Monitoring** [*Rabbits*]

Microchip implantation can be performed in an awake animal but it is best practice to perform it in conjunction with planned anesthesia, if possible, due to the size of the trocar used for insertion. The animal will be restrained manually or using commercial snuggle if needed (if not under anesthesia). The skin is tented or pulled taught between shoulder blades or behind the ear. The microchip applicator is inserted subcutaneously for the full length of the applicator needle. This helps to ensure that the transponder will not back out of the track. The plunger of the microchip applicator is depressed. The skin is pinched as the applicator is removed. The animal is scanned to ensure proper placement and function of the microchip.

VI. **Prolonged Physical Restraint for IACUC Standard Procedures** [*Rabbits*]

Prolonged restraint, defined by the IACUC as over 15 minutes for rodents and over 30 minutes for non-rodent mammals, must be described in the IACUC *Proposal* and reviewed and approved by the IACUC prior to performance. Prolonged restraint should not be used for convenience. The below description detailing necessary restraint for the performance of the IACUC's approved standard procedures to prevent injury to animals, as well as personnel, has been included for investigator convenience. Investigator's may copy and paste the below information into the prolonged restraint subsection (NonStandard Housing Section) of the IACUC *Proposal* form. See the IACUC's *Prolonged Restraint* policy.

Device description	Commercially available rabbit snuggle.
Justification for necessity and duration of restraint	Restraint is necessary to prevent animal injury during the procedure and the snuggle comfortably immobilizes the animal while also reducing handling stress. Restraint will be for the shortest duration possible to safely perform the procedure.
Duration of confinement	Anticipated less than 30 minutes but may run slightly longer if unforeseen difficulties occur. No animal will remain in the snuggle for more than 1 hour per time.
Acclimation procedures	None is required. These soft snuggles are designed to calm rabbits and allow non-invasive procedures such as blood collection to be done without the use of anesthesia.
Monitoring procedures	Animals will be monitored by lab staff the entire time they are in the snuggle.
Provision for veterinary care if there are adverse clinical events	A vet will be called to examine the animal immediately if there is an adverse event.
Criteria for removing animals that do not adapt to the restraint device	If the animal stresses or is distressed in the snuggle it will be removed immediately.
# of attempts before animals are permanently removed from restraint due to failure to acclimate	Three.

VII. **Other Information: Fasting Prior to Planned Anesthesia Event** [*Rabbits*]
Fasting is not recommended for rabbits.

Ferret Techniques

VIII. **Agent Administration:** Common agent administration techniques for ferrets are described below. **Note:** If administration volumes will exceed the maximum volumes stated in the procedures below, the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Administration volumes in excess of the maximum volumes listed here must be included in the procedural description and scientifically justified in the IACUC *Proposal*.

a. **Oral Administration of Liquids** [*Ferrets*]
When possible, flavored liquids that are palatable to the ferret should be used for a better experience. The animal will be gently restrained manually or wrapped in a towel. Liquid medication will be slowly administered orally by letting the ferret readily ingest or by gently inserting a syringe into the corner of the mouth. The liquid is slowly dispensed as the animal is observed to comfortably swallow it. The animal is observed to ensure medication was ingested. Volume limit 5 ml/kg.

b. **Oral Administration of Pills and Capsules** [*Ferrets*]
The pills should be crushed up or capsules opened and mixed into a small amount (½ to 1 teaspoon) of wet food, nutrical, or laxatone/ferretone. The animal must be seen ingesting all of the medication/food to consider the treatment complete.

c. **Subcutaneous Injection** [*Ferrets*]
Standard injections are given in the loose skin of the dorsal cervical region. Gently manually restrain, wrap in a towel, or place the animals in a deep container for unrestrained administration. A 22-25 gauge 1/2 to 1-inch needle is used. The smallest size and length needle possible is used for animal comfort and is dependent upon the animal size and viscosity of the solution. It is recommended that a butterfly or extension set should be used to accommodate animal movement if unrestrained. Skin is tented and the needle is placed at a 45-degree angle. The plunger is pulled back to ensure the needle has not inadvertently entered a blood vessel. If no blood is seen the substance can be slowly injected. The maximum volume is 5 ml/kg of a substance or 25 ml/kg of isotonic fluid. Multiple sites may need to be used if necessary to deliver the required volume. No more than 20 ml should be injected per site. Fluids should be warmed.

d. **Intramuscular Injection** [*Ferrets*]
Standard IM injections are given in the quadriceps. The animal will be gently restrained manually, wrapped in a towel, or scruffed if needed. A 22-25 gauge 1/2 to 5/8-inch needle is used but may only need to be inserted part of the length. Pull back on the syringe to ensure that the needle has not entered a blood vessel, and slowly inject the solution. The maximum volume is 0.5 ml/kg or a total volume of 1 ml per animal. Due to the potential for muscle damage, multiple injections should be avoided.

- The ***quadriceps muscle group*** can be located by palpating along the cranial femur. It lies cranial to the greater trochanter and proximal to the patellar ligament. The needle is inserted perpendicular into the proximal ½ to 1/3 of the muscle mass.

e. **Intravenous Injections** [*Ferrets*]
The animal will be gently restrained manually, wrapped in a towel, or briefly sedated. Common venipuncture sites in the ferret include the external jugular vein, cephalic vein, and lateral saphenous vein. Fur may be clipped to aid in the visualization of the vein. The site is cleaned with 70% alcohol. The vein is occluded with a tourniquet as applicable or digital pressure. A 22-25 gauge 1/2 to 1-inch needle is inserted into the vessel at a 25-degree angle. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once correct needle placement is confirmed, release occlusive pressure on the vein, and slowly inject the agent monitoring for signs of perivascular infiltration. The fluid should flow easily with no resistance. If resistance is encountered, stop and reposition the needle. Once the injection is complete, remove the needle and place gently pressure on the site to promote hemostasis. A light bandage may be used for cephalic and saphenous puncture sites. Maximum volume is 2-5 ml per animal slow IV bolus or 10 ml/kg/hour for continuous rate infusions.

- The ***jugular vein*** is located between the angle of the mandible and the thoracic inlet. Manual restraint for jugular blood collection requires some skill and involves positioning the ferret at the edge of a surgery or prep table, holding the front legs over the edge with one hand, and tipping the neck back and nose up with your other hand. The front legs and neck up to the point of the mandible should be in a vertical plane. The venipuncture should be performed in the middle 1/3 of the jugular vein.
- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal can be restrained in dorsal recumbency or a sitting/standing position. The vessel is occluded and rolled laterally at the antecubital area.

- The ***lateral saphenous vein*** is located on the craniomedial side of the back leg between the ankle (talus) and the knee. The animal should be placed in lateral recumbency with the intended site on the upper leg. The vessel is occluded and rolled from caudal to lateral to isolate it on the upper-facing aspect of the leg.

IX. **Blood Collection via Venipuncture** [*Ferrets*]

Note: If collection volumes will exceed the maximum volumes stated below, then the procedure ***must*** be described in full and the standard procedure checkboxes within the *Proposal* form cannot be used. Collection volumes in excess of the maximums outlined in this policy may require fluid replacement; investigators are strongly encouraged to consult with a CMRU veterinarian in regard to fluid replacement volumes and types prior to *Proposal* submission.

The animal will be gently restrained manually, wrapped in a towel, or briefly anesthetized with isoflurane if needed. Common venipuncture sites in the ferret include the external jugular vein, cephalic vein, lateral saphenous vein, and the brachiocephalic trunk/vena cava. Fur may be clipped to aid in the visualization of the peripheral vein. The site is cleaned with 70% alcohol unless using the vena cava which must be aseptically prepped since the needle enters the thoracic cavity. The vein is occluded with a tourniquet or digital pressure as applicable. A 22-25 gauge 1/2 to 1-inch needle is inserted at a 25-degree angle. To ensure access within the vessel, draw back on the syringe plunger to create negative pressure, and watch for a flash of blood in the needle hub or within the syringe. Once correct needle placement is confirmed, continue to gently pull back in the syringe plunger to collect blood. Once blood withdrawal is complete, remove the needle and place gentle pressure on the site to promote hemostasis. A light bandage may be used for cephalic and saphenous puncture sites. Animals are monitored until hemostasis is achieved. Ferrets have an average blood volume of 70 ml/kg. Maximum blood volumes to be collected for survival procedures should not exceed 1% complete blood volume per 24 hours, 7.5% complete blood volume weekly, or 10% complete blood volume every 2 to 4 weeks.

- The ***jugular vein*** is located between the angle of the mandible and the thoracic inlet. Manual restraint for jugular blood collection requires some skill and involves positioning the cat at the edge of a surgery or prep table, holding the front legs over the edge with one hand, and tipping the neck back and nose up with your other hand. The front legs and neck up to the point of the mandible should be in a vertical plane. The venipuncture should be performed in the middle 1/3 of the jugular vein.
- The ***brachiocephalic trunk/cranial vena cava*** is located within the thoracic cavity. The animal should be in dorsal recumbency with the arms held down near the chest. The needle is inserted between the manubrium and the first rib on the right side at a 30-45 degree angle and directed towards the left hip. Once the needle pierces the skin, pull back on the plunger to create a slight vacuum. Insert the needle to the hub (only use a ½ inch needle for this technique) and slowly start retracting while maintaining a vacuum on the syringe until blood appears. Once blood appears, stop retracting and collect the sample. Actual vessel penetration is dependent upon the insertion point.
- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal can be restrained in dorsal recumbency or a sitting/standing position. The vessel is occluded and rolled laterally at the antecubital area.
- The ***lateral saphenous vein*** is located on the craniomedial side of the back leg between the ankle (talus) and the knee. The animal should be placed in lateral recumbency with the intended site on

the upper leg. The vessel is occluded and rolled from caudal to lateral to isolate it on the upper-facing aspect of the leg.

X. **Peripheral Intravenous Catheter Placement** [*Ferrets*]

The animal will be gently restrained manually or by wrapping it in a towel. The most common peripheral catheter placement site is the cephalic vein. The hair is clipped from the site and the area is surgically prepped. Insert a 22-24 gauge 1-inch catheter into the vein. Once blood is observed in the hub of the needle, hold the needle in place while advancing the catheter off the needle and into the vein. The catheter should glide easily with no resistance. If resistance is met, stop and reposition the catheter. Release occlusive pressure on the vein once the catheter is in place. Remove the needle from the catheter and flush with heparinized saline to verify position. Monitor site for signs of perivascular infiltration. Connect fluids to the catheter or place an injection port or cap on the end of the catheter. Securely tape the catheter in place. If the catheter will not be used immediately, lock it with heparin to avoid clotting.

- The ***cephalic vein*** is located along the dorsal aspect of the front limb between the elbow and the carpus in a slightly medial to lateral orientation. The animal is placed in dorsal recumbency and the vessel is identified. The vessel is occluded and rolled laterally at the antecubital area.

XI. **Intubation** [*Ferrets*]

The animal must be anesthetized before intubation. The appropriately sized endotracheal tube (see Table 1 below) is chosen for the size of the animal and the cuff is checked (if applicable) on the tube before placement in the animal. The end of the tube is lubricated with sterile lubricant or oral lidocaine gel. The animal is placed in sternal or lateral recumbence and the mouth is gently opened and the tongue is retracted for visualization of the epiglottis. The epiglottis is depressed with the tip of an appropriately sized laryngoscope blade. The endotracheal tube is passed through the glottis and into the trachea until the tip of the tube is midway between the larynx and thoracic inlet. The tube placement is checked by auscultating both sides of the patient's chest for breathing sounds. Also, the neck can be palpated to ensure the presence of the tube, and the tube is inspected for condensation. Once confirmed in the trachea, the tube is secured with a tie around the endotracheal tube behind the adaptor and then tied around the animal's head behind ears to the upper jaw or the lower jaw, depending on the surgical procedure. The endotracheal tube is connected to the inhalation anesthesia machine, respirator, or Ambu bag. The cuff is inflated with sufficient air to prevent leakage.

Table 1: Guidelines for endotracheal tube size in ferrets

1 KG	2.0 mm
2 KG	3.0 mm

XII. **Microchip Implantation for Identification and/or Temperature Monitoring** [*Ferrets*]

Microchip implantation can be performed in an awake animal but best to do it in conjunction with planned anesthesia, if possible, due to the size of the trocar used for insertion. The animal will be restrained manually, scruffed, or wrapped in a towel if needed (if not under anesthesia). The skin is tented or pulled taught between shoulder blades or behind the ear. The microchip applicator is inserted subcutaneously for the full length of the applicator needle. This helps to ensure that the transponder will not back out of the track. The plunger of the microchip applicator is depressed. The skin is pinched as the applicator is removed. The animal is scanned to ensure proper placement and function of the microchip.

XIII. **Other Information: Fasting Prior to Planned Anesthesia Event** [*Ferrets*]
The below information is intended to serve as guidance to investigators in the planning of procedures and drafting of *Proposals*.

- Less than 8 weeks of age or less than 2 kg **-** Do not withhold water. Withhold food for 1 to 2 hours.
- Greater than 1 kg healthy adult - Do not withhold water. Withhold food for 3 to 4 hours.

References:

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4. Fox JG, et al. *Biology and diseases of the ferret.* Ames (IA), John Wiley & Sons, Inc. 2014.
5. National Centre for the Replacement & Reduction of Animals in Research (NC3Rs) Blood sample volumes. https://www.nc3rs.org.uk/blood-sample-volumes.
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7. Silvestein DC. et al. *Small animal critical care medicine*. St. Louis (MO), Elsevier Saunders. 2015.
8. Swindle, MM et al. *Swine in the laboratory.* Boca Roton (FL), CRC Press, 2015.
9. Suckow MA, et al. *The laboratory rabbit, guinea pig, hamster, and other rodents*. San Diego (CA), Academic Press, 2012.
10. Turner PV, et al. 2011. Administration of substances to laboratory animals: Routes of administration and factors to consider. *JAALAS* 50(5): 600-613.

Center for Predictive Medicine
for Biodefense and Emerging Infectious Diseases
UNIVERSITY OF LOUISVILLE

November 24, 2020

Institutional Animal Care and Use Committee
University of Louisville

Dear IACUC,

This letter is being submitted to report an adverse event that occurred to five animals associated with procedures approved in Protocol 20743. On October 27, 2020 (Day 0 of the study), a research staff member anesthetized and infected five animals with SARS-CoV-2 that had been treated with test article approximately 2 hours prior. Unfortunately, these five animals did not recover from anesthesia and expired. This adverse event was reported on the evening of October 27th in an email to me, the research staff involved with the study, CMRU staff and the Facilities Operations Manager (FOM).

Upon investigation, the staff researcher stated they used a large anesthesia chamber that was connected to a dedicated oxygen gas line valve on the right side of a 6-foot biosafety cabinet (BSC) located in an ABSL3 procedure room. The staff member anesthetized the five animals and when in a stable plane of anesthesia, challenged with virus by intranasal administration. This person noted the animals were slightly gasping at the time of infection. The animals were returned to their cage after infection, where they did not recover from anesthesia. The research staff member documented this adverse event on the individual USDA records for each animal affected. Speculating that the oxygen and CO_2 gas lines may be reversed on the right side of the BSC they then connected the anesthesia chamber to the oxygen gas line valve on the opposite (left) side of the BSC. All remaining animals in the study who were anesthetized and infected with virus or vehicle, recovered from anesthesia. On October 28th, CMRU staff stated they used the same BSC approximately 3 weeks prior to anesthetize animals without incident, however, the anesthesia chamber was connected to the left side oxygen gas line valve of the cabinet. Therefore, the vivarium manager utilized a handheld Thermo Scientific IR-CO_2 gas tester and determined that, in fact, the oxygen and CO_2 lines leading into the right side of the BSC were reversed. They reported their findings to me, the UofL veterinarian and the FOM. The FOM noted that at the time of facility commissioning by a third-party contractor there was a checklist which included testing of all lines leading from the gas manifold to individual biosafety cabinets, but documentation was not found by searching the two thousand plus page final draft. In the ten years of operation this particular gas line had never been utilized.

Corrective Actions:

1. The oxygen and CO_2 gas lines to the BSC where the adverse event occurred have signage posted DO NOT USE and it is valved out.
2. Annual shutdown for the facility will commence on December 7th at which time the proper fittings and labeling will be installed and tested.
3. All other lines in BSCs have been tested to ensure proper labeling of oxygen and CO_2 ports to BSCs and equipment.

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Therefore, we believe the incorrect labeling of the oxygen and CO_2 gas lines leading to the right side of the BSC that were connected to the anesthesia chamber resulted in CO_2 asphyxiation and death of the five animals. We feel we have taken appropriate actions to prevent any additional future incidents, but welcome additional suggestions/requirements by the committee.

Sincerely,

William E. Severson

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How does OLAW define nonscientific IACUC member?

The PHS Policy IV.A.3.b.(3) describes the nonscientific member as one member whose primary concerns are in a nonscientific area (for example, ethicist, lawyer, member of the clergy). The intent of the Policy is to have a diversity of perspectives in the membership of the committee and have an individual with a naïve attitude with regard to science and scientific activities. A person without scientific training meets the intent of the Policy, such as an ethicist, lawyer, or member of the clergy, as the Policy gives as examples. Some other examples include librarians, those working in business or finance, or instructors in English, history, or other liberal arts disciplines. When the rationale for categorizing an individual as a nonscientist is not apparent based on their occupation or training, the institution should maintain written documentation of the reason for the categorization. (See NOT-OD-15-109)

How does OLAW define nonaffiliated IACUC member?

The PHS Policy IV.A.3.b.(4) describes the nonaffiliated member as "one individual who is not affiliated with the institution in any way other than as a member of the IACUC, and is not a member of the immediate family of a person who is affiliated with the institution." The nonaffiliated member represents the general community interests in the proper care and use of animals. The nonaffiliated member is (1) not a laboratory animal user or former user, (2) not affiliated with the institution, or (3) not an immediate family member of an individual affiliated with the institution. Immediate family includes parent, spouse, child, and sibling.

In evaluating the qualifications of an individual to serve as a nonaffiliated member, the Chief Executive Officer should confirm the appointee has no discernible ties or ongoing affiliation with the institution. Regarding service of former employees or students as nonaffiliated members, the appointing official must be assured that the person is not in any way obligated to the institution. Real or perceived conflicts of interest must be avoided to ensure the IACUC's and the institution's integrity. Appointment of an individual who is unambiguously unaffiliated is the most effective way to fulfill the intent of the Policy. Public member is another term for nonaffiliated member. (See NOT-OD-15-109)

May one individual fulfill the requirement for both a nonaffiliated and a nonscientific member?

Yes, as long as that individual meets the requirements for each position. The PHS Policy states, "An individual who meets the requirements of more than one of the categories detailed in IV.A.3.b.(1)-(4) of this Policy may fulfill more than one requirement. However, no committee may consist of fewer than five members." (See NOT-OD-15-109)