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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY
Performance of Repeat Procedures
Approval Date: February 1, 2021

BACKGROUND
Research protocols are routinely amended as research evolves. To facilitate research progression and efficient amendment processing, the IACUC has developed specific criteria based upon NIH's NOT-OD-14-126 that are detailed in the Review and Approval of IACUC Submissions policy. In support of the use of performance standards and professional judgment and to reduce regulatory burden, the information below provides additional guidance when determining if the requested change meets the criteria for administrative review with verified veterinary consultation (VVC) and to the veterinarian, who is serving as the subject matter expert, when verifying compliance with the policy is appropriate for the animals in the requested circumstance.

The IACUC is often confronted with a situation where procedures may have to be repeated for an individual animal in order to achieve study objectives. This document discusses examples of common scenarios that can be used as a roadmap to IACUC decision making and provides the required guidance for changes in the duration, frequency, type (e.g., blood collection site or volumes, route of administration, volumes, and dosages), or number of approved procedures. Changes that result in increased pain or distress to the animal, impact approved endpoint criteria, or impact approved personnel safety considerations cannot be processed by VVC. Also, VVC cannot be used to add new procedures, but only to make specific modifications to approved procedures.

Regardless of the procedure to be performed there should be:
- appropriate justification as to why a procedure needs to be repeated
- an indication of the approximate interval range between the repeats
- the approximate number of repeats
- a statement of the anticipated long term effects of the repeated procedures on the animal

The total numbers of procedures that can be performed is dependent on the skills of the individual performing it, the nature of the procedure, other procedures previously performed on the animal, future procedures that may need to be performed, the temperament of the animal, and health as well as physiological status of the animal.

This policy offers direction on the following topics:
- Sample Collection: Blood; Cerebral Spinal Fluid; Urine; Nasal, Buccal, Vaginal, Rectal Swabs; Tracheal Wash & Broncho-Alveolar Lavage; Saliva; Feces
- Substance Administration
- Behavioral Tasks

REGULATORY GUIDANCE
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- Animal Welfare Act (AWA) and Regulations (AWR)

- PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy)
- NOT-OD-14-126: Guidance on Significant Changes to Animal Activities
- Guide for Care and Use of Laboratory Animals (*Guide*)

SAMPLE COLLECTION

Studies often require repeated (timed) collections of fluids (e.g., blood, cerebrospinal fluid, urine, nasal/buccal/vaginal/rectal swabs, tracheal wash, broncho-alveolar lavage, saliva, feces). In some rare instances this can be achieved by "free catch" such as for urine or collecting feces after defecation, however, for the most part the process requires penetration of a cavity typically with a needle, cannula, catheter or trocar with or without anesthesia and/or analgesia. Most of these procedures are relatively benign and pose little or no adverse impact when done correctly by a properly trained (qualified or experienced) individual. Animals must be monitored during and after the procedure, and be provided appropriate analgesia or anesthesia as detailed in the protocol, and a veterinarian notified in case of development of complications to facilitate resolution of the problem. This guidance is based upon the normal hydration status of the animal, as collection of fluids such as blood or cerebral spinal fluid in a dehydrated animal can adversely affect the animal's health and well-being.

- Blood: The circulating blood volume for most common laboratory species is 55-70 ml/Kg (5-7% of the body weight). The value is influenced by the status of the animal including age, body condition and health. Up to 10-15% of the total blood volume (approximately 1% of the body weight) can be safely removed in a normal healthy animal, with an approximate recovery period of two to four weeks. This volume (either as a single sample or combination of repeated samples) may be collected every 3 weeks. This period allows the animal to recover from the potential adverse effects of blood loss. The table below and *Appendix A: Common Sites for Blood Collection* provide species specific guidance regarding the maximum amounts of blood and specific anatomic locations that can be used for collection. Maximum volume limits do not apply to blood collected under anesthesia for a terminal procedure.

Species	Example Weight	Maximum blood volume over a 3 week period (1% body weight)
Mouse	25 g	0.250 mL
Rat	250 g	2.5 mL
Rabbit	4 kg	40 mL
Macaque	8 kg	80 mL
Sheep	40 kg	400 mL
Pig	30 kg	300 mL

- Cerebrospinal spinal fluid (CSF): CSF can be collected by a direct tap (use smallest gauge needle possible) into one of the ventricles, or cerebromedullary cistern most often through the foramen magnum, or by direct cannulation of a ventricle; and/or lumbar puncture or cannulation in the larger species. Repeated collection is enhanced by using a catheter. Implantation of a catheter involves a surgical procedure under general anesthesia, however, subsequent collections will not require anesthesia. Direct taps are simpler, however, they will require anesthesia each time it is performed. Regardless of the approach used, collections must be done aseptically. The total

volume that can be collected at any one time represents a balance between the extraction (collection) rate and the production rate. For repeated collections, each collection should represent a much smaller volume than from a single collection. Collection of fluid should take into account the risk of brain or spinal cord injury (trauma), brain herniation, cerebrospinal fluid leakage, bleeding into the cranial space, and possibility of infection. Only the smallest amount of fluid required should be collected, and no more frequently than every 3-7 days for up to 3 weeks.
    - In rats (rate of CSF formation: 2.1-5.4 µl/min), 0.1-0.15 mL can be collected.
    - In mice (rate of CSF formation: 0.325 µl/min), 0.025 mL of CSF can be collected.

- Urine: Collection of voided samples can occur as frequently as needed for study. Cystocentesis should be performed after cleaning the puncture site with antiseptic solution. If urine is not obtained at the first attempt, change the needle before making the second attempt. The procedure should be aborted after the third unsuccessful attempt. The needle should not be redirected during the procedure. Cystocentesis may induce mild transient hematuria, bruising and urine leakage. Healthy bladders heal relatively quickly. It is possible to collect urine daily by cystocentesis especially if the procedure is ultrasound guided, which minimizes the possibility of trauma.
- Nasal, Buccal, Vaginal & Rectal Swabs: Nasal, buccal, vaginal and rectal swabs can be performed non-invasively using a sterile cotton tip applicator, or similar device. They can typically be conducted with manual restraint, or with approved chemical restraint based upon the species and temperament of the individual animal. If the animal is uncooperative, then the procedure should be aborted or performed with chemical restraint as described in the protocol.
- Tracheal Wash & Broncho-Alveolar Lavage (BAL): Tracheal wash and BAL procedures require administration of agents to prevent pain and distress associated with the procedures for sample collection. These procedures can be performed as described within the protocol up to once weekly.
- Saliva: Saliva can be collected from the oral cavity. Animals may be conditioned to produce saliva using training methods as described in the protocol or medication may be needed to collect saliva.
- Feces: Feces can be collected by manual collection from animals larger than rabbits using a lubricated fecal loop, or may be collected when voided for all species.

SUBSTANCE ADMINISTRATION

Injections and oral routes of administration are used to deliver experimental and pharmaceutical agents to patients. Understanding the different techniques with regards to rate of delivery as well as volume limits is important when choosing the appropriate injection method. Properties of the article being injected must also be considered to ensure it does not cause irritation if given via the selected technique, increase pain or distress, impact the approved endpoint criteria, or impact the approved personnel safety considerations. Substances to be administered should be pharmaceutical grade, unless justified and approved by the IACUC, sterile, and pH near neutral if possible. Certain injection sites may require physical or chemical restraint of the animal. Please consult with the DCM or OAW veterinarians for more information and recommendations. The needle gauge required is species and route specific and guidance is provided in the tables below. A ≥ 25 G needle represents any needle that is 25G or of smaller diameter (e.g., 27G). Route specific guidance is included:

- Oral:
    - *Drinking H_2O, feed, pill/capsule consumed*: Oral administration by these routes allows for non-invasive continuous or frequent dosing of agents. Supplements to the water or feed need to be communicated and coordinated with DCM staff.

- *Oral Gavage*: Oral gavage is part of accepted routine toxicological testing accepted by agencies like Food and Drug Administration and Organization for Economic Cooperation and Development's 28 or 90 day oral toxicity, where substances are administered daily during that time period.

- Subcutaneous (SC): Larger volumes need to be divided between locations. The number of sites is based upon the species and volume to be administered. No more than three injection sites should be used per day; and administrations can occur as frequently as twice daily for chronic administration.
- Intraperitoneal (IP): Daily IP injections can be administered for up to 30 days without adverse effects.
- Intramuscular (IM): The smallest volume possible should be administered. No more than two IM injection sites should be used per day and sites should be rotated, taking into account inflammation and sequelae associated with the substance administered. Anesthesia and analgesia administration may be needed based upon the species and anticipated sequelae.
- Intravenous (IV): IV administration may be a single bolus (larger dose administered over a short period of time) or slower administration. Single boluses may be administered with a needle and syringe or through a secured IV catheter depending on the species and substance being administered. Slower administration, including continuous rate infusions (CRIs), usually require placement of a secured IV catheter. Catheters need to be placed and maintained using aseptic technique.
- Intradermal (ID): Intradermal injections are typically associated with assessment of inflammatory or sensitization responses. Depending on the skin thickness at the site of injection, a volume of 0.05-0.1 mL can be administered.
- Transdermal: The site for transdermal administration frequently requires preparation of the site (e.g., clipping or fur, depilatory, alcohol swab to remove oils) or immersion (aquatics). Depending on the location of administration, the animal or a cage mate may ingest the agent. Consideration of any adverse impact to the animal and measures that may be necessary to prevent or minimize ingestion should be considered.
- Inhalational & Intranasal: Inhalational and intranasal administration may require manual or chemical restraint, as described in the protocol.

Determining Injection Volumes

To determine calculated injection volume (typically measured in "mL"): $\frac{\text{animals' weight (kg)} \times \text{dose (mg/kg)}}{\text{concentration (mg/mL)}}$

Route of Administration	**Sites for administration**
IV – Intravenous	*mouse/rat*: tail vein, saphenous vein, retro-orbital sinus *macaque*: cephalic vein, saphenous vein jugular or femoral vein via existing indwelling catheters
IP – Intraperitoneal	lower left or right quadrant
IM – Intramuscular	*mouse/rat*: quadriceps, hamstring; *macaque/sheep/pig*: quadriceps, hamstring, gluteal, epaxial
SC – Subcutaneous	back (scruff), lateral abdomen
ID – Intradermal	lateral abdomen, dorsum
PO – by mouth	oral gavage, drinking H_2O, feed, pill/capsule

Mouse Injection Limits in mL/ kg

Route	*PO*	*SQ*	*IP*	*IM**	*IV(bolus**)*	*IV (slow)*
Dose: mL/ kg (maximum dose)	**10 (50)**	**10 (40)**	**20 (80)**	**0.05 mL/site (0.1 mL/site)**	**5**	**25**
Needle Size	**Species-specific gavage needle**	**≥ 25 G**	**≥ 25 G**	**≥ 26 G**	**≥ 25 G**	**≥ 25 G**
Weight (in kg)						
0.020	0.20 mL	0.20 mL	0.40 mL	0.05 mL	0.10 mL	0.50 mL
0.025	0.25 mL	0.25 mL	0.50 mL	0.05 mL	0.13 mL	0.63 mL
0.030	0.30 mL	0.30 mL	0.60 mL	0.05 mL	0.15 mL	0.75 mL
0.035	0.35 mL	0.35 mL	0.70 mL	0.05 mL	0.18 mL	0.88 mL
0.040	0.40 mL	0.40 mL	0.80 mL	0.05 mL	0.20 mL	1.00 mL
0.045	0.45 mL	0.45 mL	0.90 mL	0.05 mL	0.23 mL	1.13 mL
0.050	0.50 mL	0.50 mL	1.00 mL	0.05 mL	0.25 mL	1.25 mL

*Technique is discouraged in mice due to small muscle mass
**A bolus is a larger dose given over a shorter period of time

Rat Injection Limits in mL/ kg

	PO	SQ	IP	IM*	IV(bolus**)	IV (slow)
Dose: mL/ kg (maximum dose)	**10** (40)	**5** (10)	**10** (20)	**0.1 mL/site** (0.2 mL/site)	**5**	**20**
Needle Size	**Species-specific gavage needle**	**≥ 25 G**	**≥ 25 G**	**≥ 25 G**	**≥ 24 G**	**≥ 24 G**
Weight (in kg)						
0.200	2.0 mL	1.0 mL	2.0 mL	0.1 mL	1.0 mL	4.0 mL
0.250	2.5 mL	1.2 mL	2.5 mL	0.1 mL	1.25 mL	5.0 mL
0.300	3.0 mL	1.5 mL	3.0 mL	0.1 mL	1.5 mL	6.0 mL
0.350	3.5 mL	1.7 mL	3.5 mL	0.1 mL	1.75 mL	7.0 mL
0.400	4.0 mL	2.0 mL	4.0 mL	0.1 mL	2.0 mL	8.0 mL
0.450	4.5 mL	2.2 mL	4.5 mL	0.1 mL	2.25 mL	9.0 mL
0.500	5.0 mL	2.5 mL	5.0 mL	0.1 mL	2.5 mL	10.0 mL

*Technique is discouraged in rats due to small muscle mass
**A bolus is a larger dose given over a shorter period of time

Macaque Injection Limits in mL/ kg

	PO	SQ	IP	IM	IV(bolus**)	IV (slow)
Dose: mL/ kg (maximum dose)	**5** (15)	**2** (5)	**10** (20)	**0.25** (0.5)	**2.5**	**10**
Needle Size		**≥ 22 G**	**≥ 22 G**	**≥ 22 G**	**≥ 22 G**	**≥ 22 G**
Weight (in kg)						
3	15 mL	6 mL	30 mL	0.75 mL	7.5 mL	30 mL
5	25 mL	10 mL	50 mL	1.25 mL	12.5 mL	50 mL

7	35 mL	14 mL	70 mL	1.75 mL	17.5 mL	70 mL
10	50 mL	20 mL	100 mL	2.50 mL	25.0 mL	100 mL
13	65 mL	26 mL	130 mL	3.25 mL	32.5 mL	130 mL
16	80 mL	32 mL	160 mL	4.00 mL	40.0 mL	160 mL

**A bolus is a larger dose given over a shorter period of time

Rabbit Injection Limits in mL/ kg

	PO	SQ	IP*	IM	IV(bolus**)	IV (slow)
Dose: mL/ kg (maximum dose)	**10** (15)	**1** (2)	**5** (20)	**0.25** (0.5)	**2**	**10**
Needle Size		**≥ 20 G**	**≥ 20 G**	**≥ 20 G**	**≥ 21 G**	**≥ 21 G**
Weight (in kg)						
3	30 mL	3 mL	15 mL	0.75 mL	6 mL	30 mL
5	50 mL	5 mL	25 mL	1.25 mL	10 mL	50 mL
7	70 mL	7 mL	35 mL	1.75 mL	14 mL	70 mL

*Technique is discouraged in rabbits due to large cecum
**A bolus is a larger dose given over a shorter period of time

BEHAVIORAL TASKS

Category C pain and/or distress behavioral assessments (e.g., radial arm, elevated plus, and elevated T mazes; Active Place Avoidance; Passive Avoidance; tail flick; flinch jump; incline plane) that are detailed in the protocol can be performed multiple times as needed for study, as long as the duration (animals may not be outside of the animal facility for more than 12 hours) and frequency do not result in increased pain or distress to the animal or impact approved endpoint criteria.

ADDITIONAL REFERENCES

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