Performance of Repeat Procedures

Background

Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain while simultaneously minimizing the numbers of animals needed when consistent with sound scientific practices, is imperative[1]. Furthermore, the *Guide*[2] specifies that the Institutional Animal Care and Use Committee (IACUC) should weigh study objectives against animal welfare concerns in accordance with the tenets of the Three R's. The IACUC is often confronted with a situation where procedures may have to be repeated in 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. 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 interval between the repeats as well the total number of repeats together with a statement of the anticipated long term effects of the repeated procedures on the animal clearly articulated in the protocol. 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.

The changes within this document must be handled in compliance with the VVC process described in NOT-OD-14-126 and in the IACUC reviewed and approved Protocol Review Process document.

Procedures and guidance

Oral gavage is a widely used method for safely administering known quantities substances to animals by properly trained (experienced/qualified) personnel. 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. The maximum dosing volume is 1.5 ml for a 30 gram mouse (10 ml/kg), and 16 ml for a 400 g rat (10-20 ml/kg)[3]. When performed properly, the procedure can be used to administer 50-200 µl into the stomach of mice daily for up to 20 weeks.

Fluid collection

Studies often require repeated (timed) collections of fluid e.g. blood, cerebrospinal fluid, rumenal fluid, urine, synovial fluid, intraocular fluid, semen, milk, tracheal wash, broncho-alveolar lavage, etc. 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 or even use of an assistive device e.g. electro-ejaculator or artificial vagina. Most of these procedures are relatively benign and pose little or any adverse impact when done correctly by a properly trained (qualified or experienced) individual. Animals should be monitored during and after the

[1] United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Principle III and IV.

[2] Guide for the Care and Use of Laboratory Animals (2011), National Research Council, National Academy Press, Washington, DC. P27-28.

[3] Diehl KH, Morton R, Morton D, *et al* (2001). A good practice guide to the administration of substances and removal of blood including routes and volumes. Journal of Applied Toxicology 50(5): 600-613.

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procedure, and be provided appropriate analgesia or anesthesia. A veterinarian should be notified in case of development of complications to facilitate resolution of the problem.

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, hydration, and health. Up to 10% of the total blood volume (1% of the body weight) can be safely removed in a normal healthy animal. This volume may be collected once in 3-4 weeks[4]. This period allows the animal to recover from the potential adverse effects of blood loss. Again the same 3-4 week recovery period should be followed. If you are collecting 0.75% of the body weight a recovery period of 2 weeks may be sufficient, and for 0.5% a week's recovery is okay and for 0.05% blood can be collected daily. The same total amount of blood can also be removed as multiple quantities over a 24 hour period. If necessary fluids can also be administered to support the blood volume, especially for collections at the upper end of the limit.

The table below provides examples of maximal amounts of blood that can be collected.

Species[5]	**Body weight**	**Blood volume**	**Maximum survival collection volume**
Mouse	20-63 g	1.6-3.2 ml	0.2-0.4 ml
Rat	250-520 g	20-40 ml	2.5-5.2 ml
Hamster	85-150 g	6.8-12 ml	0.85-1.5 ml
Gerbil	45-130 g	4.4-8 ml	0.45-1.3 ml
Guinea pig	700-1200 g	40-80 ml	7-12 ml

[4] Diehl KH, Hull R, Morton D, *et a*l (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology 21: 15-23.

[5] Adapted from Joslin JO (2009) Blood collection techniques in exotic small mammals. Journal of Exotic Pet Medicine 18(2): 117-139.

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The table below provides common sites for blood collection in a number of laboratory animal species.

Species	Site
Mouse	Saphenous, tail, facial and saphenous veins
Rat	Saphenous, tail and jugular veins
Hamster	Saphenous and jugular veins
Guinea pig	Saphenous, tarsal and jugular veins, vena cava
Rabbit	Ear and jugular veins
Ferret	Jugular, cephalic and saphenous veins, cranial vena cava
Cat	Jugular, cephalic and saphenous veins
Dog	Saphenous, cephalic and jugular veins
Pig	Ear, saphenous, tail, cephalic, femoral and mammary veins, vena cava
Sheep	Jugular, ear, and cephalic veins

The following table indicates the common routes for administration of substances; for Guinea Pigs, Rat recommendations can be used.[6] Suggested maximum volumes to be administered are shown in parenthesis. For most mammalian species these volumes can be safely administered: 5-20 ml/kg orally; 5 ml/kg subcutaneously; 0.05 ml/kg intramuscularly; 10 ml/kg intraperitoneally; and 5 ml/kg intravenously as a bolus or 2-4 ml/kg continuous infusion.[7] Intranasal administration for distribution into the lungs should be done on lightly anesthetized animals to prevent distress and allow for improved aspiration of fluids.[13]

	Oral (µl/g) [max dose]	**Subcutaneous (µl/g) [max dose]**	**Intraperitoneal (µl/g) [max dose]**	**Intramuscular (max dose)**	**Intravenous bolus(µl/g) [max dose]**	**Intranasal[7] (total volume)**
Frequency	Daily	No more than three times daily	Daily	No more than two sites daily	Daily	Daily[13]
Mouse Example for a 30 g mouse	10 [50] 300 µl [1.5 ml]	10 [40] 300 µl [1.2 ml]	20 [80] 600 µl [2.4 ml]	50 µl total per site	5 150 µl	35-50µl
Rat Example for a 400 g rat	10 [40] 4 ml [16 ml]	5 [10] 2 ml [4 ml]	10 [20] 4 ml [8 ml]	100 µl total per site	5 2 ml	35-50µl

[6] Adapted from Diehl KH, Hull R, Morton D, et al (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology 21: 15-23.

[7] Turner PV, Brabb T, Pekow C, Vasbinder MA (2011). Administration of substances in laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science 50 (5): 600-613.

[13]Southam DS, Dolovich M, O'Byrne PM, Inman MD. 2002. Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia. Am J Physiol Lung Cell Mol Physiol 282:L833-L839.

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	Oral (ml/kg) [max dose]	Subcutaneous (ml/kg) [max dose]	Intraperitoneal (ml/kg) [max dose]	Intramuscular (ml/kg) [max dose]	Intravenous bolus (ml/kg) [max dose]	Intranasal[7]
Frequency	Daily	No more than three times daily	Daily	No more than two sites daily	Daily	Daily[13]
Rabbit	10 [15]	1 [20]	5 [20]	0.25 [0.5]	2	200-500µl
Dog	5 [15]	1 [2]	1 [20]	0.25 [0.5]	2.5	200-500µl
Mini pig	10 [15]	1 [2]	1 [20]	0.25 [0.5]	2.5	--------

Substances to be administered should be sterile and if possible the pH should be near neutral. Changes in the experimental substance may be reviewed by VVC if the substance is in a similar class of substances to the original protocol (e.g. chemotherapeutic, antibiotic, hormone, vehicle, etc.) and there is a change in concentration (typically increased, as reduced concentrations do not require IACUC review) as long as it isn't anticipated to impact animal welfare. Other pharmaceuticals that help facilitate the research such as over-the-counter medications may be used with veterinary approval. Examples include OTC ophthalmic saline solutions, ear cleaning solutions, etc.

Cerebrospinal spinal fluid (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 magna, 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 likely 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.

Rate of CSF formation[8]

Species	Rate (µl/min)
Mouse	0.325
Rat	2.1-5.4
Guinea pig	3.5
Rabbit	10
Cat	20-22
Dog	47-66
Sheep	118
Goat	164
Calf	290

In rats, 0.1-0.15 mL can be collected in 3 minutes, every 3-7 days to avoid sample contamination with blood. Samples as large as 250 µl can be collected every 3 days for up to 3 weeks[9]. In rabbits 1.5-2 ml of fluid can be collected by direct tap of the 4th ventricle. In mice 0.025 ml of CSF can be collected. Repeated collection of 0.03-0.33 ml CSF can be performed in guinea pigs. In cats a maximum of 0.5 ml of CSF can be removed, and up to 1-2 ml in a 10 Kg dog. Frequent sampling of 0.05-0.15 ml of CSF can be performed in pigs via a catheter for several weeks.[10]

Rumen fluid is sometimes collected for research or clinical purposes. The rumen of an adult dairy cow contains 184 liters (49 gallons) of fluid. An adult sheep or goat rumen is about 3-6 gallons (11.4 – 22.8 liters). Rumen fluid can be collected either through a permanent fistula or using a stomach tube. Creating of a fistula makes it easy to collect rumen fluid and is usually the preferred method for long term repeated fluid collection. The process requires surgery to create the fistula as well as regular cleaning and maintenance of the fistula. Use of a stomach tube is

[8] Vernau W, Vernau KA, Bailey CS (2008). Cerebrospinal fluid. Chapter 26. P768-819. Clinical Biochemistry of Domestic Animals. Kaneko JS, Harvey JW, Bruss ML (eds.) 6th edition. Academic Press/Elsevier.

[9] Koch MA (2006) Experimental modeling and research methodology. Chapter 18, P 606-607. Suckow MA, Weisbroth SH, Franklin CL (eds.). 2nd edition. The Laboratory Rat. Academic Press/Elsevier, Amsterdam.

[10] Iwarsson K, Lindberg L, Waller T (194) Common non-surgical techniques and procedures. Chapter 16, P267. Svendsen P, Hau J (eds.) Handbook of Laboratory Animal Science. Volume 1. CRC Press, Inc, Boca Raton, FL.

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fairly straight forward, however, it causes a temporary discomfort to the animal, and has the potential to cause esophageal and oropharyngeal irritation or trauma, and the potential for aspiration pneumonia. Use of a stomach tube does not require surgery and a recovery period. Rumen fluid can be collected repeatedly by a trained or experienced individual provided due care is take to avoid the potential adverse impact of the procedure e.g. alteration of rumen microbiome, dehydration, digestive disturbances, trauma and/or aspiration pneumonia especially if a stomach tube is used. When 5 or more gallons of rumen fluid is removed daily, it is necessary that the fluid be replaced with water.

Aseptic technique and appropriate pain management are required for collection of synovial fluid (arthrocentesis). The amount of fluid that can be collected will be dependent on the joint cavity as well as the species. The knee joint often provides the most accessible and largest practical volume of fluid. The amount of fluid in the joint is typically very small. The human knee joint contains about 4 ml of synovial fluid. The equine stifle joint has about 20 ml of synovial fluid and the carpal joint has about 10 ml. For pharmacokinetic studies arthrocentesis can be done at 0, 6, 12, 24 and 72 hours and can be repeated with a rest of 1-2 weeks (Frisbie, 2014)[11]. Arthrocentesis can be safely performed every week for 10-20 weeks.

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 (Lappin, 2014)[12].

Swabs of mucus membranes and body surfaces or orifices can be performed non-invasively using a sterile cotton tip applicator, or similar device. They can typically be conducted with manual restraint. If the animal is uncooperative, then the procedure should be aborted. Nasal, vaginal, rectal, ocular, otic, pharyngeal, and buccal swabs can be collected up to 3 times a day. As long as there is no evidence of trauma, such as bleeding, these swabs can be collected up to 30 consecutive days. Skin surface swabs or scrapings can be taken as often as needed as long as there is no evidence of distress to the animal.

Saliva can be collected from the oral cavity. If the animals are conditioned to produce saliva using training methods as described in the protocol, saliva can be collected up to 3 times daily. If medication is needed to collect saliva, it should be given no more than once daily.

Feces can be collected up to 3 times daily by manual collection from animals larger than rabbits using a lubricated fecal loop. For larger animals a lubricated finger or hand can also be used to collect fecal samples from the rectum. Voided feces can be collected as often as desired. If needed, animals may be temporarily individually housed in appropriate caging to facilitate the collection of voided feces.

Dietary supplement changes. Experimental and nutritional components are frequently provided in the feed of animals. The composition of the experimental and nutritional components may be altered as long as the remainder of the diet maintains the proper nutritional components to meet the needs of the animals, and there is no withholding of feed.

[11] Frisbie D (2014). Personal communications.

[12] Lappin, M (2014). Personal communications.