



## Title: Counting and Reporting Animal Use Numbers

### I. Purpose

The purpose of this policy is to provide guidance to Principal Investigators (PIs) on the determination of animal number needs in protocols and the Ross University School of Veterinary Medicine (RUSVM) required reporting of animal use numbers. The Public Health Service (PHS) and the Animal Welfare Act Regulations (AWAR) require that IACUC protocols specify, and include a rationale for, the number of animals to be used. In addition, AAALAC International expects institutions to provide numbers for all vertebrate species used in the annual report. The guidance contained herein will enable the accurate tracking of animals which is crucial for ensuring that animal usage does not exceed animal numbers in the approved IACUC protocol, which could result in a deviation and/or the suspension of animal use privileges.

### II. Scope and Responsibilities

This policy applies to all PIs using animals in research or teaching at RUSVM, regardless of physical location or species of the animals. It is the PI's responsibility to keep track of the number of animals used on the protocol, to not exceed the total number or reuses requested and report the animal numbers to the IACUC each semester. The PI can receive assistance from technicians and Animal Resources (AR) to track animal use. AR is responsible for managing rest periods across protocols based on use and reuse.

### III. Abbreviations

AR: Animal Resources

PI: Principal Investigator

### IV. Definitions

- **Animal Numbers:** An exact count of animals (the exception is fish where an approximation is acceptable) that should be reported semesterly to the IACUC.
- **Animal Use:** animals who undergo any type of activity for research or teaching.
- **Re-use:** The sequential use of the same animal(s) within a single IACUC protocol or on more than 1 protocol. If a palpation lab is done 6 times in a semester and the same cow is in all 6 labs, that cow was re-used 6 times.

- **Continued or repeated use:** There are circumstances where the first experimental procedure (first 'use') is essential for the second (i.e. making a transgenic, surgical preparation in one protocol which is required for the second or sequential PK studies with unrelated compounds). These circumstances should be written into the protocol and approved.
- **Other repeated uses:** Repeated measurements or cross-over studies are a part of the protocol and are not considered reuse. Cross-over study example: the animal receives each treatment in a random order and each animal serves as its own control.

#### V. General Guidelines and Requirements

- When determining animal numbers the PI should refer to the **Policy Guidelines for Categories of Animal Use and Rest Periods** for RUSVM's Teaching Animals.
- Given the number of animals used on multiple protocols, consult with Animal Resources regarding animal availability prior to submitting a protocol to IACUC.
- Within their protocol, PIs must request animal numbers calculated within the protocol period (3 years). Within the protocol, re-use can be presented on a semesterly basis.
- Using fewer animals and having fewer reuses than requested within a protocol period (3 years) is better than deviating from an approved protocol.
- Reuse is one means of decreasing animal numbers. However, too much reuse can result in welfare concerns. Reuse and total numbers must be balanced.
- **It is the PI's responsibility to report animal numbers every semester to IACUC.**
- If a PI is replaced, it is important for the new PI to evaluate and understand the number of animals used to date.
- It is the PI's responsibility to assess changes in animal number needs and reuse when changes to labs are made, and to apply for protocol amendments as needed.
- Exceeding animal numbers or reuses must be reported in a deviation.

#### VI. Justification of Animal Numbers

In protocol applications, a clear description of how the number of animals and reuse were determined is required. Below are common types of animal use protocols and means of justifying animal numbers. See also Appendix II and III.

- **Teaching** (e.g. laboratories): Animal numbers must take into account the minimum needed without impacting hands-on-teaching experience, the student-to-animal ratio, animal rest periods and reuse welfare.
- **Exploratory Study Requiring No Statistical Analysis** (e.g. marine production colony): Animal sample sizes are justified based on the probability of success of the experimental procedure.
- **Epidemiological Studies** (intervention and observational): Animal sample size calculators can be used to estimate a population, test a hypothesis (intervention and observational), or obtain results that support the detection of an event.
- **Behavioral Studies**: Animal sample size calculations depend on the type of animal behavioral study employed (e.g., field work vs. human-animal bond)
- **Pilot Studies**: Animal numbers are determined by researchers' experience and peer reviewed publications. Animal numbers are generally small. Data obtained can be used to determine statistically relevant sample size calculations for future experiments.

## VII. Semesterly Reporting

- At the end of each semester, PIs must report the following information for each protocol: Number of total animals used, their name/ID, date used and reuses per animal within the protocol.
- PIs may utilize tools such as ProVet or a form (see example in Appendix).

## VIII. Reuse of Animals Guidelines

- An animal that has already been used in one or more procedures can only be reused under the following conditions, even if another animal that has not undergone any procedures is available:
  - the actual severity of the previous procedures was 'mild' to 'moderate' (i.e. Category I to III of the IACUC Guidelines for Categories of Animal Use);
  - it is demonstrated that the animal's general state of health and well-being has been fully restored;
  - the further procedure is classified as 'mild' to 'moderate' (i.e. Category I to IV of the IACUC Guidelines for Categories of Animal Use) or 'non-recovery'; and
  - it is in accordance with veterinary advice and final disposition guidelines within the

species-specific acquisition/processing SOP's.

- In special cases, with permission from the Attending Veterinarian, the IACUC may allow an animal to be reused again in a category IV or V procedure, as outlined in the IACUC Guidelines for Categories of Animal Use. This is allowed only if the animal has been used for no more than once for a Category V procedure. Specific conditions must be met (laid out below) for the continued use of animals in these cases.
- Continued use of an animal is allowable when:
  - the experimental design/ teaching objective requires it, and it is justified in the protocol;
  - approved by the IACUC; and
  - determination of suitability for further use through following questions:
    - is the animal healthy?
    - have there been any adverse events?
    - is there any obvious reason why the animal should not be used?
    - has the animal been exposed to procedures outlined in paragraph b above?
  - for category IV or V studies limits on the number of uses should be defined in the protocol
  - scientific questions are appropriately addressed (i.e. has there been a sufficient washout period, will compound given interfere with later studies).

#### IX. Review

This document is subject to biannual review.

#### X. Appendices

Appendix I: Example Semesterly Reporting Form procedures in a SOP

Appendix II: Additional guidelines in justifying animal numbers

Appendix III: Hypothetical examples for animal number calculations for teaching protocols

Appendix IV: References

#### **Policy/SOP Approval:**



**Signed**

**Dr. Jennifer Ketzis, IACUC Chair**

**Date 7 July 2024**

**Appendix I: Example Semesterly Reporting Form**

PIs may use this form or another template that they prefer. The key items to be included are the protocol number, animal used (name or number), and date used.

IACUC protocol number:

Semester:

Animals used (name or number):	How many times was the animal used?	Date or time frame of animal usage:

\*If necessary add rows and pages

Below are examples of the completed form for different types of protocols

IACUC protocol number: 21.12.40 - EVS-7 ITC Equine Rotation Semester: Summer 23

Animals used (name or number):	How many times was the animal used?	Date or time frame of animal usage:
Buddy	15	5/8/2023-8/19/2023

IACUC protocol number: 20.10.26 - VMS 5796 Surgery Laboratory II Sheep Castration  
Semester: Summer 23

Animals used (name or number):	How many times was the animal used?	Date or time frame of animal usage:
O23	1	6/14/2023

## Appendix II: Additional Guidelines in Justifying Animal Numbers

Animal number justification begins with a clearly stated, hypothesis-driven outline of the experimental design and/or learning objectives. **The PI should consider the following:**

1. Experiment's purpose / Learning objective(s)
2. Number of experimental groups/subgroups by species/strain per group/subgroup / number of student groups/number of students per animal
3. Total number of control and experimental animals from the experimental design (Consider including a grid or flowchart)
4. If tissue harvest is required, the relationship between the amount of tissue needed to the number of animals required to produce that tissue
5. Anticipated animal losses or removal due to morbidity, mortality or other expected difficulties with the experimental procedures.

### Techniques for sample size calculation

1. Power analysis: This is the most popular technique, for this researcher should have information and knowledge on the effect size, standard deviation, type 1 error, power, direction of effect, the appropriate statistical tests, and attrition or death of animal.
2. Resource equation method: This technique can be used if the PI is unable to determine statistical justification such as effect size or standard deviation and if there are multiple factors involved.
3. Guidelines: Guidelines for some study types specify animal numbers.

### Studies Requiring inferential statistical analysis

1. Protocol justification statement must include the values of alpha, beta, sigma, and effect size used in the power analysis to determine sample size or the appropriate literature and guideline references.
2. Websites helpful in determining appropriate sample size:
  - <https://stats.oarc.ucla.edu/other/mult-pkg/seminars/intro-power/>
  - <https://epitools.ausvet.com.au>
  - <https://epitools.fp7-risksur.eu/tools/index?toolId=46>
  - <https://epidemiology.sruc.ac.uk/shiny/apps/samplesize/>
  - <http://statpages.org>
  - <https://arxiv.org/abs/1707.00222>

## **Appendix III: Hypothetical examples for animal number calculations for teaching protocols**

### **Example 1. Kennel dogs in a physical examination**

#### Assumptions about the lab & students

- There are up to 200 students per semester.
- Each semester, the canine PE lab is delivered in 12 sessions.
- Students are divided into lab groups of 3-4 students per animal.
- 4-6 dogs are used in each lab.

#### Assumptions about dogs

- There are 30 dogs in the kennel
- No dog stays on campus for more than 6 semesters (2 years)
- 6 new dogs arrive on campus each semester and 6 leave the program

#### A dog “use”

- 1 dog in a single lab session for 1 hour = 1 use.
- 1 “use” results in a 24 h rest period

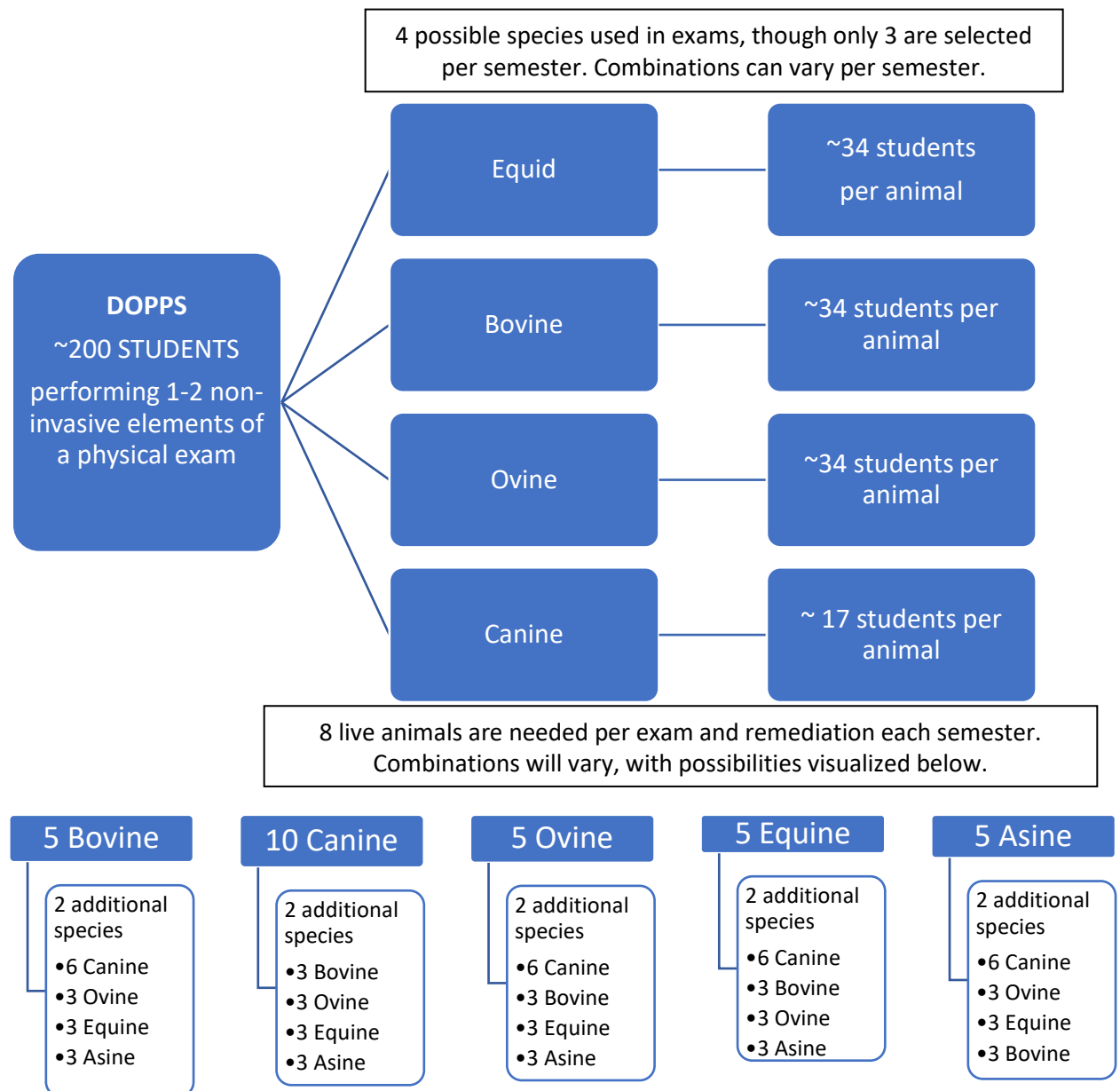
#### Number of dogs needed and number of uses

The RUSVM canine teaching colony consists of 30 dogs with an average of 6 dogs leaving/entering each semester. This means over a 3-yr period there is a maximum of 78 dogs in the colony that can be used on this protocol.

To fulfill the needs of the classes, dogs will need to be reused each semester. Depending on scheduling, rest periods and availability, the same dog could potentially be re-used in up to 12 labs per semester.

**Example 2. Multi-species calculation for DOPPS**

Each semester a cohort of students is expected to take the DOPPS. Historically, around half need to remediate and so a second exam is held. For academic integrity, the two exam days must be identical even if there is a smaller cohort on the second day.



Per semester, the exams use 8 canines, 4 bovine, 4 ovine, 4 equine, and/or 4 asine. However, one animal is needed in reserve (2 for canine) to replace an animal if it becomes stressed; therefore, the maximum count is 10 canine, 5 bovine, 5 ovine, 5 equine, and/or 5 asine.



### **Animal Number Request**

- 1.** The RUSVM canine teaching colony consists of 30 dogs with an average of 6 dogs leaving/entering each semester. This means over a 3-yr period there is a maximum of 78 dogs in the colony that can be used on this protocol.
- 2.** The RUSVM bovine EVS teaching herd consists of 8 bovines that are available for EVS labs. Over the course of 3-yrs, additional bovines may be added to the EVS herd dependent on colony management, therefore this protocol requests a total of 11 bovines.
- 3.** The RUSVM horse teaching herd consists of 7 horses that are available to be used on this protocol. RUSVM is trying to grow the colony, so this protocol is requesting a total of 11 horses over a 3-yr period.
- 4.** If a different donkey is used each semester for both exam days, the maximum number is 90 (5 animals per one exam, two exams a semester = 10 animals x 3 semesters/yr x 3 yrs =90).
- 5.** If a different sheep is used each semester for both exam days, the maximum number is 90 (5 animals per one exam, two exams a semester = 10 animals x 3 semesters/yr x 3 yrs =90).

### **Animal Number Reuse -*Calculated per individual animal***

In a semester, an individual animal can be reused up to 2 times.

## Appendix IV: References

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16. Stevenson, M.A. (2021). Sample Size Estimation in Veterinary Epidemiologic Research. *Frontiers in Veterinary Science* 7. doi: 10.3389/fvets.2020.539573.