ROSS UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

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Title: Counting and Reporting Animal Use Numbers	
Issued Date: Nov 2020	
Revised Date: July 2023	
Authority: IACUC	

I.   Purpose

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 accurate tracking of animals 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 Principal Investigators (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 to track animal use. Animal Resources is responsible for managing rest periods across protocols based on use and reuse.

III.   Definitions

a. **Animal Numbers** – An exact count of animals (the exception is fish where an approximation is acceptable) that should be reported semesterly to the IACUC.

b. **Animal Use** – animals who undergo any type of activity for research or teaching.

c. **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.

d. **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.

e. **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.

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IV. General Guidelines

a. 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.](#)

b. Given the number of animals used on multiple protocols, consult with Animal Resources regarding animal availability prior to submitting a protocol to IACUC.

c. Using fewer animals and having fewer reuses than requested within a protocol period (3 years) is better than deviating from an approved protocol.

d. Reuse is one means of decreasing animal numbers. However, too much reuse can result in welfare concerns. Reuse and total numbers must be balanced.

e. **It is the PI's responsibility to report animal numbers every semester to IACUC.**

f. If a PI is replaced, it is important for the new PI to evaluate and understand the number of animals used to date.

g. It is the PI's responsibility to assess changes in animal number needs and reuse when changes to labs are made and apply for a protocol amendment as needed.

h. Exceeding animal numbers or reuses must be reported in a deviation.

V. 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.

a. **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.

b. **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.

c. **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.

d. **Behavioral Studies:** Animal sample size calculations depend on the type of animal behavioral study employed (e.g., field work vs. human-animal bond)

e. **Pilot Studies**: Animal numbers are determined by researchers' experience and peer reviewed publications. Animal numbers are generally small. Data obtained can be used statistically relevant sample size calculations for future experiments.

VI. Semesterly Reporting

a. At the end of each semester, PIs must report for each protocol the following information: Number of total animals used, their name/ID, date used and reuses per animal within the protocol.

b. PIs may utilize tools such as ProVet or a form (see example in Appendix).

VII. Re-use of Animals Guidelines

a. An animal already used in one or more procedures, when a different animal on which no procedure has previously been carried out could also be used, may only be reused provided that the following conditions are met:

   o 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);

   o it is demonstrated that the animal's general state of health and well-being has been fully restored;

   o 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

   o it is in accordance with veterinary advice and final disposition guidelines within the species-specific acquisition/processing SOP's.

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b. In exceptional circumstances, by way of derogation from the previous paragraph, and after consultation with the Attending Veterinarian, the IACUC may allow reuse of an animal previously used in category IV or V procedure of the IACUC Guidelines for Categories of Animal Use, provided the animal has not been used more than once for a Category V procedure and under the conditions laid out down below for continued use of animals.

c. Continued use of an animal is allowable when:

- the experimental design/ teaching objective requires it and it is justified in the protocol; and

- 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 3.2?

- for category IV or V studies limits on the number of uses should be defined in the protocol

- scientific questions (i.e. has there been a sufficient washout period, will compound given interfere with later studies) are appropriately addressed

VIII.   Review

This SOP is subject to annual review.

SOP Approval:

Jennifer K Ketzis

7 July 23

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I.   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

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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: Most popular and 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: if statistical justification such as effect size, standard deviation is unable to be determined or 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

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

Example 1. Kennel dogs in a physical examination

Assumptions about the lab & students

- Each student will conduct a physical examination
- Students are divided into groups of 20
- There are 2 groups of students per day
    - Week 1: 2 groups Monday, 2 on Tuesday (80 students)
    - Week 2: 2 groups Monday, 2 on Tuesday (80 students)
    - Week 3: 1 group Monday (20 students)
- There are 180 students per semester

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 and examined by 4 students = 1 use.
- 1 "use" results in a 24 h rest period

Number of dogs needed and number of uses

- Calculating per group: 20 students, 4 per dog = 5 dogs
- Per day/week: since dogs can only be used once in a day and then must have a 24+ h rest, different dogs will be used on Monday and Tuesday. Hence 5 different dogs for each group = 5 dogs x 4 groups = 20 dogs each used once.
- If the same dogs are used all 3 weeks, total is 20 dogs with a maximum of 3 uses
- *However*, some dogs might not work well, so best to add 2 dogs in case 2 have to be removed: 22 dogs with a maximum of 3 uses: this is semester 1 of the 9 semester protocol.
- Total dogs semesters 2-9: add 6 dogs per semester: 6*8 = 48
- **Total protocol dogs: 22+ 48 = 70**
- Total uses: if one dog's stay on campus (6 semesters) overlaps with the protocol, that dog could be used 3 times per semester = 3 uses per semester * 6 semesters = 18 uses.
- **Maximum reuses: 18**

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Example 2. Kennel dog in optho exam

Assumptions about the lab & students
- Each student will conduct an optho exam
- Students are divided into groups of 30 with one group per day
    - Week 1: 1 group Monday, 1 on Tuesday (60 students)
    - Week 2: 1 group Monday, 1 on Tuesday (60 students)
    - Week 3: 1 group Monday, 1 on Tuesday (60 students)
- There are 180 students per semester

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 and examined by 2 students = 1 use.
- 1 "use" results in a 24 h rest period

Number of dogs needed and number of uses
- Calculating per group: 30 students, 2 per dog = 15 dogs
- Per week: since dogs cannot be used 2 days in a row, different dogs will be used on Monday and Tuesday. Hence 30 dogs will be used each week, once.
- Since the kennel only has 30 dogs, every dog will have to be used every week: 3 uses per dog
- Semester 1 of the protocol is 30 dogs; 3 uses per dog
- Total dogs semesters 2-9: add 6 dogs per semester: 6 dogs*8 semesters = 48
- **Total protocol dogs: 30+ 48 = 78**
- Total uses: if one dog's stay on campus (6 semesters) overlaps with the protocol, that dog could be used 3 times per semester = 3 uses per semester * 6 semesters = 18 uses.
- **Maximum reuses: 18**

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Appendix IV: References

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