SINGLE ANIMAL PROTOCOL TEMPLATE FOR DUKE & VA

After several months of cooperative efforts, the animal care and use programs of Duke and the VAMC have approved the use of a single protocol template for studies where Duke is the primary grant recipient and the animal procedures are performed at the VA. Direct VA funding continues to require the use of the VA Protocol Template (as per VA Handbook 1200). The new single protocol procedures will decrease the submission workload on researchers (only one protocol form to complete rather than two), improve consistency between the Duke and VA documents (decreasing the risk of non-compliant activity of tracking two different protocols), and improve communication between all parties (a single set of documents to review and a single set of data to share).

Researchers who are funded through Duke but performing work at the VAMC will complete the Duke protocol template and include Section V (VAMC Supplement to the Duke Protocol Template). The protocol template should be submitted to the Duke Office of Animal Welfare Assurance. The Duke IACUC will perform the first approval, and upon granting approval by the Duke IACUC, the Duke OAWA will forward the protocol to the VA Office of Research Support for review by the VA IACUC.

At present this consolidated process applies only to the protocol, but the institutions continue to work toward consolidation of the Annual Progress Report.

INSTRUCTIONS FOR COMPLETION & PEER REVIEW OF THE VETERBRATE ANIMAL SECTION (VAS) IN THE NIH GRANT APPLICATION

The NIH has issued a clarification notice (March 17, 2010) intended to standardize the minimum information required on the NIH’s Vertebrate Animal Section (VAS) of grant applications and cooperative agreements. For a full text review of this notice, visit url: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-027.html

The Five Points: The NIH notice references the instructions for completing the VAS in the PHS398 and SF424, by noting that if live vertebrate animals are to be used, federal policy requires that the following five points be addressed in all applications:

- Detailed description of the proposed use of the animals, including species, strains, ages, sex and number to be used;
- Justification for the use of animals, choice of species, and numbers to be used;
- Information on the veterinary care of the animals;
- Description of procedures for minimizing discomfort, distress, pain, and injury; and
- Method of euthanasia and the reasons for its selection

A concise (e.g., 1-2 pages), complete description of the animals and proposed procedures must also be provided within the VAS of the Research Plan. While additional details may be included in the Research Strategy, the responses to the five required points within the VAS must be cohesive and must include sufficient detail to allow evaluation.

When preparing animal use protocols for Duke IACUC approval which may be associated with federally funded projects, researchers must assure that ALL work (all 5 years) which will be described in the VAS is included in the animal protocol.

Upcoming Dates & Deadlines

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
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<tr>
<td>April 15</td>
<td>BB Seminar: Harlan Support for Mouse Users</td>
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<td>April 20</td>
<td>BB Seminar: FBI Agent Discussing Laboratory and Personal Security</td>
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<td>April 26</td>
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<td>May 3</td>
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<td>May 4</td>
<td>BB Seminar: Cancer Models in Mice</td>
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<td>May 10</td>
<td>Amendment Deadline</td>
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<tr>
<td>May 24</td>
<td>Amendment Deadline</td>
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*Deadlines are 5 PM on the date listed!*
IMPORTANT POINTS ON EFFECTIVE ANESTHESIA: MONITORING DEPTH OF ANESTHESIA

According to federal regulations, all anesthetized animals must be monitored continually to assess adequate level of anesthesia. Monitoring must begin with the first injection (or inhalation) of anesthesia and continue until the animal is fully recovered (or euthanized). There are as many acceptable methods to monitor anesthesia as there are species of animals. Certain methods, commonly used in most species, include:

• Toe pinch: A gentle pinch, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal requires more anesthesia. Any observed movement (e.g. withdrawing the paw) indicates that the animal is not sufficiently anesthetized to do surgery, or any painful procedure.

• Skin pinch: More sensitive areas of skin work best. A gentle pinch of a small fold of skin, which does not break the skin or cause any deep tissue damage, is sufficient to show if the animal requires more anesthesia. Any observed movement (e.g. twitching of the skin) indicates that the animal is not sufficiently anesthetized to do procedures.

• Jaw "tone": Generally a good indicator of muscle relaxation. The lower jaw is gently opened. Resistance by the animal (e.g. closing of the mouth) is an indicator that the animal is too light to do surgery.

• Respiratory rate: Good indicator of depth of anesthesia. Rapid, shallow respirations usually indicate the animal is too "light". Normal respiration rate varies among species, consult DLAR veterinarians if necessary for determination of normal rates.

• Heart rate/Blood pressure: An increase in heart rate and/or blood pressure usually indicates a need for supplemental anesthesia. Normal rates/pressures vary greatly among species, consult DLAR veterinarians if necessary for determination of normal rates/pressures.

• Palpebral: While useful in large species, the blink reflex is quite variable and difficult to assess in small animals (i.e. mice and rats). DO NOT USE THE PALPEBRAL REFLEX FOR MONITORING RODENT ANESTHESIA!

• Corneal: The cornea can be damaged, if not protected, but when used carefully, it is a good reflex. Touch the edge of the cornea with a gauge sponge or cotton q-tip. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient to do surgery.

• Body Temperature: Most anesthetic agents depress body temperature to a significant degree. Therefore, it is important that anesthetized animals be maintained on some type of material which shields them from contact with cold surfaces and reduces heat loss. The use of a supplemental heat source is a good idea, but must be used with caution, since burns can occur from electric blankets or water bottles that are too hot.

IACUC REVIEW OF ANIMAL USE APPLICATIONS

From the Institutional Animal Care and Use Committee Guidebook**

In order to approve a protocol that involves the use of animals, the IACUC must review the proposed care and use of animals and determine that federal criteria have been met. The Public Health Service (PHS) requires that the project be conducted in accordance with the PHS Policy, the AWA, the Guide, the institution’s Assurance, and all other applicable federal statutes and regulations related to animals. The project should also comply with all institutional policies.

As stipulated in the NIH guidance, the Duke IACUC requires use of a standardized protocol application form.

The NIH also requires institution have a mechanism to verify that consistency. The Duke IACUC performs a side-by-side comparison of the information contained in the IACUC protocol review form and the information submitted to the NIH. It is imperative that the protocol which is IACUC approved is consistent with the information submitted to the NIH!

If the protocol and grant are not consistent, then before the IACUC can issue a concordance memo to the NIH, either the protocol or the grant must be modified. If the IACUC requires changes to the protocol which are not reflected in the grant application, then the PHS funding component must be notified. Duke is required to provide PHS with the date of IACUC approval.

In most cases, the PHS allows a 60-day grace period following the receipt deadline date during which the investigator may secure IACUC approval; otherwise, the application cannot be peer reviewed. If the IACUC review occurs subsequent to the grant submission, then a letter verifying IACUC approval, and stating any modifications required by the IACUC, must be submitted to the funding agency. This grace period is nonexistent for some non-federally funded projects and investigators are required to submit evidence of IACUC approval coincident with the grant or contract submission.

The Duke OAWA & IACUC will process all requests for protocol or concordant reviews as quickly as allowed by regulation. It is critical for researchers that notices and requirements be advertised to OAWA as quickly as you learn of them. The minimum period required for a protocol review/approval is 3 weeks. The minimum period for concordance review and generation of a memo to the NIH is 2 days; in certain special cases concordance reviews may be performed within 24 hours, but this should be the exception! The OAWA/IACUC do not have the staff for ‘regular’ sudden requirements of review and memo generation.

** You can view the entire text discussion of this topic in the online publication at: http://grants.nih.gov/grants/olaw/GuideBook.pdf
ANESTHESIA RECOVERY

- All animals recovering from anesthesia must be attended until they have recovered their swallowing reflexes.
- In most species, this is usually indicated by the animal moving purposely. All animals should be kept warm during the recovery period.
- While heat lamps have been used, there is a significant risk of heat injury if not properly attended.
- If using a heating pad, place the animals in a cage that is 1/2 on and 1/2 off of a heating pad.
- Whenever a heat source is used, a thermometer should be placed at the animal's level to monitor actual heat levels.
- Animals will overheat and can 'sun burn.'
- Animals which have had any blood/fluid loss during surgery should be provided with fluid or blood replacement during surgery and/or the anesthesia recovery period.
- In small rodents, this is best accomplished via the intraperitoneal or subcutaneous route.
- A warmed cc or 2 of sterile saline given SQ is always a good idea in small mammals.

REPORTING NEONATES

The Duke IACUC Annual Progress Report requests animal numbers for adult animals, and for neonatal animals used in experiments. Although the PHS Policy does not explicitly require a mechanism to track animal usage by investigators, it does require that proposals specify a rationale for the approximate number of animals to be used and be limited to the appropriate number necessary to obtain valid results. This implicitly requires that institutions establish mechanisms to document and monitor numbers of animals acquired and used, including any animals that are euthanatized because they are not needed. Monitoring should not exclude the disposition of animals inadvertently or necessarily produced in excess of the number needed or which do not meet criteria (e.g., genetic) established for the specific study proposal. Institutions have adopted a variety of administrative, electronic, and manual mechanisms to meet institutional needs and PHS Policy requirements. [A7]

Test Your Knowledge

Toe clipping in mice must be performed prior to ____ days of age?
A. 12
B. 21
C. 15
D. 18

2. Anesthesia must be used when performing tail clipping in mice over ____ days of age.
A. 12
B. 20
C. 21
D. 18

3. Duke Institutional Animal Care and Use (IACUC) meetings are held:
A. As needed
B. Monthly
C. Weekly
D. Every six months

4. Mice can be identified by using
A. Ear tags
B. Tattoos
C. Micro chips
D. All of the above

5. All individuals who have contact with or will be participating in the use of animals are required to complete Animal Handlers Parts I and II training prior to receiving approval on a protocol.
True
False

6. Euthanasia via CO2 must be ensured by a secondary method.
True
False

7. CO2 is an acceptable form of euthanasia for mice aged 0-14 days.
True
False

8. A rodent protocol using CO2 as a method of euthanasia annual review will not be approved until all individuals listed on the protocol have completed CO2 training.
True
False

Answers: 1-A; 2-B; 3-B ; 4-D; 5-True; 6-True ; 7-False; 8-True
Questions? Email Bill Wade (w.wade@duke.edu) for explanations.
QUESTIONS, CONCERNS OR COMPLAINTS ABOUT THE CARE, USE AND/OR WELFARE OF RESEARCH ANIMALS?

Any individual who has concerns related to the use of animals in biomedical research at Duke University is encouraged to voice those concerns. Please contact the Duke Animal Welfare Hotline (919.684.3535) or E-Mail the Duke IACUC at IACUC@duke.edu

Duke University will not tolerate any misuse or neglect of animals nor will the institution accept reprisal against an individual who has come forward with concerns or allegations of wrongdoing involving the care and use of animals. Such reprisal is prohibited by federal law (USDA Regulations & the 9th Code of Federal Regulations). Individuals who feel that action has been taken against them because they reported an apparent violation of animal care and use requirements, should present their case to one of the individuals listed below:

Laura Hale, MD, PhD
Chair
Institutional Animal Care and Use Committee
919.684.6720

Ron Banks, DVM, Dipl. ACVPM, Dipl. ACLAM, CPIA
Director
Office of Animal Welfare Assurance
919.684.4744

John Norton, DVM, PhD, Dipl. ABT, Dipl. ACLAM
Director
Division of Laboratory Animal resources
919.684.4204

USING RECOMBINANT DNA (rDNA) IN ANIMALS?

New IBC FORM!

Researchers who create or handle recombinant materials at Duke must comply with the NIH Guidelines for Research Involving Recombinant DNA Molecules, including the creation or cross-breeding of transgenic animal strains, and the insertion of rDNA (plasmids, viral vectors, RNA produced from DNA, siRNA, miRNA, shRNA, or synthetic DNA or RNA) into animals or cells transferred into animals.

All animal rDNA work must be registered with the Duke Institutional Biosafety Committee (IBC). The IBC has recently updated the Recombinant DNA Registration Form to assist researchers who perform work with rDNA.

The new form is found on the IBC website under “IBC Forms”: http://www.safety.duke.edu/BioSafety/ibc.htm, and must be used when submitting rDNA work for review by the IBC. Questions? Contact Biological Safety, 684-8822

VEHICLE INSPECTION

Do you use a privately owned vehicle to transport animals under an approved protocol? If so, there are requirements for ensuring that the vehicle is appropriate for the transport of the species in question. Vehicles must be inspected prior to approval of the protocol and twice a year afterwards, for as long as the vehicle is used. Semiannual inspections are conducted during the IACUC site visits as required by federal guidelines.

All vehicles must meet basic requirements to include climate control, ease of cleaning or sanitation and security of cargo (cages, containers). In addition to having the vehicle inspected by the IACUC there are other considerations pertaining to the use of a privately owned vehicle for animal transport. State regulations may require that permits be in place for transporting animals across state lines.

If you have questions about the use of a privately owned vehicle, please contact Bill Wade at the OAWA at 668.6722 or w.wade@duke.edu

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Director
Division of Laboratory Animal resources
919.684.4204

ANNUAL REFRESHER TRAINING (ART)

ART made be found at the OESO On-Line Learning Web Site. Go to http://safety.duke.edu. Click ‘ON-LINE TRAINING’ on the left margin, and select Annual Refresher Training.

ART Brown Bag seminars are scheduled early in the calendar year. These are an alternative to the web module. Attendees receive credit for the annual refresher training.

Advantage of the ART Brown Bag Seminar? No quiz

Advantage of the Web version of ART? Completed at your convenience.
**CO₂ Euthanasia**

**FACT 1:** Performing CO₂ euthanasia properly, is a critically important activity.

**FACT 2:** Performing CO₂ is only effective in certain ages of rodents!

**FACT 3:** Failure to perform CO₂ properly requires a federal report to the NIH.

How to keep compliant? **Review the Summary of Guidelines for CO₂ Euthanasia:**

- **Gestational age 0 to gestational age 14:**
  - Euthanasia of the mother; or
  - Removal of the uterus/fetus
- **Gestational age 15 to birth:**
  - Skillful injection of chemical anesthetics; or
  - Decapitation with surgical scissors; or
  - Cervical dislocation
- **Birth to 14 days of age:**
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical dislocation.
- **15 days of age through weaning:**
  - CO₂ euthanasia (with extreme caution); or
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical Dislocation.
- **Post weaning through adulthood:**
  - CO₂ euthanasia (with extreme caution); or
  - Overdose of chemical anesthetics; or
  - Decapitation; or
  - Cervical Dislocation.

**Caution:** Animals younger than weaning may not respond to CO₂ as full grown adults do, some are resistant to CO₂ euthanasia for up to 30 days of age. **ALWAYS USE A SECONDARY METHOD OF EUTHANASIA!**

For more information on methods of euthanasia, consult:


**UPCOMING ANIMAL PROGRAM TRAINING OPPORTUNITIES**

The Office of Animal Welfare Assurance (OAWA) and the Division of Laboratory Animal Resources (DLAR), support of the Duke Animal Care and Use Program (ACUP) by providing training for principal investigators and research staff who conduct work with animal models.

Training opportunities available free of charge to Duke research staff include:

- **Brown Bag Seminars** – provides continuing education for the Duke research community through topical and timely seminars. AALAS CE credit approved.

- **OESO on-line training** – Animal Handlers Parts I & II are required for all new research staff listed on an animal model protocol. Animal Handler Part III (Annual Refresher Training), CO₂ Euthanasia Training, Primate B-Virus Training, and several other training courses are available for review.

- **AALAS Learning Library** – The Duke animal care & use program maintains on-line accounts for all Duke animal users. This on-line training resource is available through the American Association for Laboratory Animal Science.

- **Animal Tracks** – The animal program’s monthly publication contains new and pertinent information on animal care and use, research methodologies and regulatory updates.

- **Individual and lab training** – OAWA and DLAR staff are available to provide individual and lab staff training on a variety of subjects and procedures. To include handling and restraint, euthanasia, injection and withdrawal techniques, aseptic surgery procedures, consultation on anesthesia equipment, etc.

Contact Bill Wade at phone number 668.6722 or Email: w.wade@duke.edu or Michelle Calkins at phone number 681.1831 or Email at michelle.calkins@duke.edu for training assistance.
Duke ACUP’s Brown Bag Seminar

Thursday, April 15th, 2010
Noon – 1 p.m.
Bryan Building Auditorium

Christine Reaves
Strategic Account Manager
Harlan Laboratories

Will be presenting:

**“The C57BL/6 mouse: Everything you should know about the most widely used mouse in research.”**

**“Immunodeficient research models: How to achieve faster tumor take rates, increase efficiency and control research variables.”**

The session will be held in the Bryan Research Building Auditorium, located on the ground floor.

Refreshments will be provided

Please plan on arriving prior to noon in order to get refreshments, sign in, and be seated.

This session will count for 1 CEU of AALAS In-house Training Credit