Specific information requested in the following animal-use protocol template is a result of requirements of the Animal Welfare Regulations (AWR), the Guide for the Care and Use of Laboratory Animals, and other applicable Federal regulations and DOD directives.

This document is intended to be an aid in the preparation of an U.S. Air Force DOD–sponsored animal use proposal. It is a companion document to an identical protocol template that does not have the written explanations and instructions provided for individual paragraphs (ref. animal-use protocol template in AR 40-33 / AFMAN 40-401(I), Appendix C). Either template may be used to format DOD-sponsored animal research proposals; however, unless highly experienced in using the template without the provided instructions, it is preferable to enter the requested information directly into this document. If desired, explanations and instructions in red may then be blocked out and deleted. Use of a word processor makes completion of this template a “fill-in-the-blanks” exercise. Please provide all response entries in the following font: Arial, Regular, 12, Black. With the exception of title headings, each paragraph and subparagraph in the following template must have a response. Portions of the template that are not applicable to your particular protocol, i.e., no surgery or no prolonged restraint, should be marked “N/A”. There are no space limitations for responses to the requested information.

Pertinent standing operating procedures or similar documents, that are readily available to your IACUC, may be referenced to assist in the description of specific procedures. It is critical that only animal studies or procedures documented in an IACUC–approved protocol be performed at your facility. Additionally, Principal Investigators, or other delegated research personnel, should maintain accurate experimental records and be able to provide an audit trail of animal expenditures and use that correlates to their approved protocol.

Any requirements specific to your organization (such as budgetary information, local coordinating requirements, specific scientific review requirements, etc.) may be added to this standard protocol template. Adding information within the template is acceptable in order to meet institutional needs as long as the standard format is maintained. In other words, all of the labeled paragraphs and subparagraphs must remain in the same relative order with the added information being similar or complementary to the information requested. It is important to note that this standardized protocol template does not in any way prohibit local organizations from using any (or all) of their current animal use protocol. However, regulations mandate that all of the requested information in the following template be answered in the specific order listed.
INTELLECTUAL PROPERTY STATEMENT

Research protocols drafted by Government scientists include intellectual property (e.g., concepts, ideas, experimental approaches, etc.), some of which is innovative or original and therefore considered proprietary to the investigators and/or the sponsoring agency. All Government and non-Government personnel handling this protocol shall exercise EXTREME CARE, to ensure that the information contained herein is NOT DUPLICATED OR DISCLOSED, in whole or part, for any purpose other than to evaluate the protocol, without the written permission of the principal investigator or the sponsoring agency.
I. NAME OF FACILITY: 

II. PROTOCOL NUMBER: Leave blank
Protocol accession number will be assigned by the USAF Surgeon General Research Oversight Committee (SGROC) administrative support personnel.

III. PROTOCOL TITLE
Title must include the common name, genus and species of the animal(s) used in research.
Example: Evaluation of Suture Patterns in the Laboratory Mouse (Mus musculus).

IV. PRINCIPAL INVESTIGATOR
Signature block and signature is required – Include Title, Department / Division, Phone, Fax and E-mail address.

V. SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.
Signature block and signature of Department / Division Chief, or Scientific Review Committee chairperson, is required – Include Title, Department / Division, Phone, Fax and E-mail address.

VI. STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal and ensures that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.
Signature block and signature of the statistical reviewer is required – Include Title, Department / Division, Phone, Fax and E-mail address.

VII. ATTENDING VETERINARIAN: In accordance with Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.
Signature block and signature of the Attending Veterinarian is required – Include title, Department / Division, Phone, Fax and E-mail address.

VIII. IACUC APPROVAL
Provide written documentation of protocol approval, in the form of an IACUC approval letter on institutional letterhead, signed and dated by either the IACUC chairperson or the IACUC administrator. An IACUC approval letter is required from the facility where the animal research is planned, and must include, if applicable, any subcontracted facilities.

The completed signature coordination pages, along with the IACUC approval letter requested in VIII. IACUC APPROVAL, may be faxed or mailed to: Chief, Animal Use Programs, Division of Biomedical Research and Regulatory Compliance (SGRC), HQ, USAF, Office of the Surgeon General, 5201 Leesburg Pike, Suite 1400, Falls Church, VA. 22041. Phone: 703-681-4132 / Fax: 703-681-4518.
(Start a new page here)

PROTOCOL TITLE
Title must include the common name, genus and species, of the animal(s) used in research.
Example: Evaluation of Suture Patterns in the Laboratory Mouse (Mus musculus).

PRINCIPAL INVESTIGATOR
Signature block and signature is required – Include Title, Department / Division, Phone, Fax and E-mail address.

CO-INVESTIGATOR(S)
Signature block and signature required – Include Title, Department / Division, Phone, Fax and E-mail address.

I. NON-TECHNICAL SYNOPSIS
Provide a brief narrative description of the proposal that is easily understood by a high school graduate.
Avoid the use of technical jargon or medical terms that a layperson would not be familiar with. If using a technical term cannot be avoided, include a brief definition or explanation. Include animal use in your description. (NOTE: This information may be used to complete the DOD Annual Report to Congress.)

II. BACKGROUND
No response is required under this section title.

II.1. Background
Include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited, and a description of the general approach should be provided.

II.2. Literature Search for Duplication
No response is required under this section title. This search must be performed to prevent unnecessary duplication of previous experiments.

II.2.1. Literature Sources Searched
List the databases searched for unnecessary duplication. A search of the Biomedical Research Database (BRD) is required (http://www.scitechweb.com/acau/brd/). In addition, a search of the Computer Retrieval of Information of Scientific Projects (CRISP) database (http://www.crisp.cit.nih.gov/) or the Federal Research in Progress (FEDRIP) database (http://grc.ntis.gov) is also required. Additional searches in databases such as PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed), or those specific to the area of proposed research, are highly recommended.

II.2.2. Date of Search
List the date(s) literature searches were performed.

II.2.3. Period of Search
List the time period(s) covered by the searches.

II.2.4. Key Words of Search
List key words used to perform the searches.

II.2.5. Results of Search
Provide a narrative description of the results of the literature search.
III. OBJECTIVE / HYPOTHESIS
State the objective(s) of this protocol or the hypothesis to be accepted or rejected. (NOTE: This information will be used to complete the DOD Annual Report to Congress.)

IV. MILITARY RELEVANCE
Provide a brief and succinct military justification for the research with regard to military needs and mission requirements. If applicable, state the Science and Technology Objective(s) (STO) that this work supports and the funding agency.

V. MATERIALS AND METHODS
No response is required under this section title.

V.1. Experimental Design and General Procedures
This section includes an explanation of experimental design. Technical methodology need not be described in this section; rather, it should be described under paragraph V.4, Technical Methods. Provide a complete description of the proposed use of animals to include a summary table of the experimental groups. Succinctly outline the formal scientific plan and direction of experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design of each separate experiment in sub-parts to this section (e.g. Experiment 1, Experiment 2, and so forth). The length and detail required in this section depends largely on the complexity of the study. A clearly understandable description of the numbers of animals and their distribution into experimental groups is essential. The number requested must equal the minimum number required to complete the study yet be sufficient to yield meaningful results. The minimum number should include animals necessary for controls and/or technique development.

The total number of animals required for the study is listed in section V.3.4. Number of Animals Required (by Species). It is critical that reviewers of this protocol are clearly able to follow the reasoning and calculations for the number of animals required, and can verify that the experimental design clearly supports the number of animals requested.

V.1.1. Experiment 1

V.1.2. Experiment 2

V.2. Data Analysis
List the statistical test(s) planned or describe the strategy intended to evaluate the data. Describe the statistical methodology used to determine group size and total number of animals required. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, estimated effect size and expected variability.

V.3. Laboratory Animals Required and Justification
No response is required under this section title.

V.3.1. Non-animal Alternatives Considered
DOD policy requires that alternatives to the use of animals be thoroughly investigated prior to submission of any proposal involving animals. Provide scientific justification for the proposed use of animals in this study. List all non-animal alternatives that were considered (e.g., computer modeling, cell cultures, etc.), and explain why these alternatives cannot be used to meet the research objectives.
V.3.2. Animal Model and Species Justification
Provide scientific justification for selection of the proposed animal model -- what physiological and morphological characteristics does this animal possess that make it the best possible model for this research proposal? If less sentient animal models were considered (e.g., invertebrate vs. vertebrate, mice vs. rabbits, pigs vs. nonhuman primates, etc.) but not chosen, explain why.

V.3.3. Laboratory Animals
No response is required under this section title.

V.3.3.1. Genus / Species

V.3.3.2. Strain / Stock
Specify any strain, stock, breed, or other specialized animal requirements using appropriate terminology – if assistance is needed, see the attending veterinarian.

V.3.3.3. Source / Vendor
Provide a preferred source for the animals and enter the source/vendors USDA license number (or exception to policy number), if available. Animals will be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with Code of Federal Regulations, Title 9, Animals and Animal Products, Chapter 1, Subchapter A, Animal Welfare, Parts 1, 2, and 3. Procurement of animals from non-USDA licensed sources requires an exception to policy – if assistance is needed, see the attending veterinarian.

V.3.3.4. Age
Age range is acceptable.

V.3.3.5. Weight
Weight range is acceptable.

V.3.3.6. Sex
Specify either male or female, but not “either”. If both sexes will be used, specify animal numbers for each.

V.3.3.7. Special Considerations
Special requirements for the research animals, if any, should be reflected here, (e.g., Specific Pathogen Free (SPF) or Viral Antibody Free (VAF), Pasteurella free, SIV/SRV/STLV free, etc.).

V.3.4. Number of Animals Required (by Species)
All that is required in this section is the total number of animals proposed for the study. The number of animals requested here should match exactly those described in V.1.Experimental Design & General Procedures. Keep in mind the number requested should be the minimum numbers necessary to complete the study, but must be sufficient to yield meaningful results. If too few animals are requested, and statistical significance is not achieved, the animals will have been misused. Be certain to include animals necessary for controls or technique development. If additional animals are needed due to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval for additional animals. List only the type of animal(s) and the number(s) required.

**Example:**
(a) Mice 320
(b) Rats 250
(c) Guinea pigs 175
V.3.5. Refinement, Reduction, Replacement (3 R’s):
No response is required under this section title. The DOD is often required to provide specific examples of its alternatives initiatives. Does this protocol have any provisions that would qualify it to be identified as one that refines, reduces or replaces (3 R’s) the use of animals? For example, does your study use statistical tests that require fewer animals, e.g., a modified LD50 test like Thompson & Weil, or are you using cell cultures, computer modeling or any other technique that will influence the numbers of animals required? Are you using animals lower on the phylogenetic scale? Please provide a short description of the features that you feel qualify the study as one that employs any of the “3 R’s”. In addition, discuss what provisions were considered and not adopted and why they were not adopted. If “N/A” is used, explain why.

V.3.5.1. Refinement
Include any procedures or measures taken to eliminate or minimize pain or distress in the animals or used to enhance their well-being. Examples of refinement include, but are not limited to, the use of anesthetics or analgesics to decrease pain or distress, the use of remote telemetry to decrease the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing the refinements that will be used, also list the refinement alternatives that were considered but not adopted, and explain why they were not adopted.

V.3.5.2. Reduction
Include any procedures or measures taken to reduce the number of animals used. Examples of reduction include, but are not limited to, the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages. In addition to listing reductions that will be used, list reduction alternatives that were considered but not adopted, and explain why they were not adopted.

V.3.5.3. Replacement
Include any procedures or measures taken that eliminate the use of animals. Examples of replacements include, but are not limited to, the use of non-animal models, such as cell cultures, or the use of a less sentient animal species. In addition to listing replacements that will be used, list replacement alternatives that were considered but not adopted, and explain why they were not adopted.

V.4. Technical Methods
No response is required under this section title. Information must be presented in sufficient detail, documented or referenced, so that reviewers of this protocol may obtain a clear understanding of what is to be done and how the animals will be handled. Clarity is essential in order for reviewers to make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DOD regulations, guidelines, and Federal law.

V.4.1. Pain / Distress Assessment
No response is required under this section title. The Animal Welfare Regulations define a painful procedure as one that would “reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures.” If a procedure may involve pain or distress, even if relieved by anesthetics or analgesics, the P.I. must consult with the attending veterinarian.

V.4.1.1. APHIS Form 7023 Information
No response is required under this section title. (The attending veterinarian should be able to provide assistance in completing this section of the proposal). This proposal must contain an estimate of the number of animals that will be counted in columns C, D, and E of APHIS Form 7023, Annual Report of Research Facility. Columns C, D, and E represent specific pain categories. (See paragraphs, V.4.1.1.1-3.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for a protocol to have animals listed in several columns. For
instance, control animals may be placed in Column C while experimental animals may be placed in Column D, depending upon the nature of the protocol. Reflect use of more than one species of animals in a duplicate table. The total numbers reflected in these three columns will add up to the number of animals requested for the entire protocol in paragraph V.3.4. Number of Animals Required (by Species).

V.4.1.1.1. Number of Animals
No response is required under this section title.

V.4.1.1.1.1. Column C …………____ (# of animals)
Examples of research procedures or manipulations that would require an animal to be placed in Column C are those involving not more than slight or momentary pain or distress in a human being to which that procedure is applied.

V.4.1.1.1.2. Column D …………____ (# of animals)
Examples of research procedures or manipulations that would require an animal to be placed in Column D are those where anesthetics or analgesics will be administered to avoid or effectively relieve pain or distress. General anesthetics given for surgical procedures, or the use of analgesics or anti-inflammatory agents, are examples of this category.

V.4.1.1.1.3. Column E …………____ (# of animals)
Examples of research procedures or manipulations that would require an animal to be placed in Column E are those in which alleviation of pain or distress are contraindicated for a scientifically justifiable reason such as the experimental results are likely to be confounded if drugs relieving pain or distress were administered. Detailed justification for putting animals into this category is required below in paragraph V.4.1.4. Unalleviated Painful or Distressful Procedure Justification.

V.4.1.2. Pain Relief / Prevention
No response is required under this section title. The attending veterinarian should be able to provide assistance in completing this section of the proposal.

V.4.1.2.1. Anesthesia / Analgesia / Tranquilization
If applicable, describe the method or strategy planned to effectively alleviate pain or distress. If pain or distress alleviation is planned, specify the agent(s) to be used, when they will be given (pre-emptive or post-procedural), and by whom they will be given. Provide agent, dosage, route, frequency, injection site, needle size, and so forth.

V.4.1.2.2. Pre- and Post-procedural Provisions
Describe the provisions for both pre- and post-procedural care, including provisions for clinical observations and frequency of these observations. Information concerning pre- and post-surgical care should be listed below in paragraphs V.4.3.1 and V.4.3.3. If analgesics are used for pain or distress relief, provide the frequency of administration, observational criteria used to determine if animals are experiencing pain or distress (pain assessment parameters), and the location for the post-procedural care.

V.4.1.2.3. Paralytics
The use of paralytic agents without anesthesia is prohibited. Describe the monitoring method(s) that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent(s).

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures
Respond “N/A” if animals will not experience more than momentary or slight pain or distress and are placed in Column C of APHIS Form 7023 (see paragraph V.4.1.1. APHIS Form 7023 Information). The
alternatives literature search MUST be performed even if animals are placed in Column D and the pain or distress is alleviated through the use of analgesics or anesthetics. Where Federal law requires specific testing procedures to be performed, state the applicable Code of Federal Regulations (CFR), or other applicable legal guidance, that requires this testing.

V.4.1.3.1. Sources Searched
Examples of databases that may be used to complete the alternatives literature search includes: Johns Hopkins Center for Alternatives to Animal Testing (Altweb), AGRICOLA, PubMed, BIOSIS, and so forth. Most databases may be accessed at http://www.nal.usda.gov/awic/databases/database.htm.

V.4.1.3.2. Date of Search
List the date(s) literature searches were performed.

V.4.1.3.3. Period of Search
List the time period(s) covered by the searches.

V.4.1.3.4. Key Words of Search
List key words used to perform the searches. Examples of key words includes: pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful experimental procedure(s), and so forth.

V.4.1.3.5. Results of Search
Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Regulations specifically state that the P.I. must provide a narrative description of the methods and sources that he/she used to determine that alternatives to the painful or distressful procedure(s), including those in which pain or distress is alleviated, were not available. In addition, include a discussion of what alternatives were considered but not adopted, and explain why they were not adopted.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification
Procedures that cause more than slight or momentary pain or distress that is not alleviated through the effective use of anesthetics or analgesics must be justified on a scientific basis in writing by the P.I. This paragraph must be completed if there are ANY animals in this protocol that will experience unalleviated pain or distress and are placed in Column E of APHIS Form 7023 (see paragraph V.4.1.1.APHIS Form 7023 Information).

V.4.2. Prolonged Restraint
Describe and justify in detail any prolonged restraint (greater than twelve hours for nonhuman primates or in accordance with IACUC policy for other species) intended for use during the study, e.g., primate chairs, restraint boards, metabolism cages, etc. The description should specify who will be performing the restraint procedures on the animals and for how long. Also describe the planned procedures for habituation or training of animals to the device prior to the prolonged restraint. This section is not intended for short-term actions such as rodent restraint for bleeding, etc.

V.4.3. Surgery
No response is required under this section title. Major survival operative procedures on non-rodent species will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions. Non-survival operative procedures do not require a dedicated facility, but they should be performed using surgical gloves, mask, and clean instruments. Additionally, the surgical site should be clipped and cleaned prior to surgery. Major survival rodent surgery does not require a dedicated facility but it must be performed using aseptic technique; that is, aseptic patient preparation, surgical gloves, mask, and sterile instruments. A major operative procedure is defined as a procedure that “penetrates and exposes a body cavity, or causes substantial or permanent impairment of physical or physiological function”.

USAF Protocol Template
Date Modified 1Feb2004
V.4.3.1. Pre-surgical Provisions
Describe the provisions for pre-surgical care, including provisions for pre-surgical observations and frequency of pre-surgical observations. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria used to determine if animals are experiencing pain or distress (pain assessment parameters), and the location for the pre-surgical care.

V.4.3.2. Procedure(s)
Describe in detail any surgical procedures planned including approach, surgical manipulation(s), and closure (layers, materials, patterns, and so forth.). Each procedure should be described separately and in sufficient detail that someone with appropriate surgical training could repeat it with minimal variation.

V.4.3.3. Post-surgical Provisions
Describe the provisions for post-surgical care, including provisions for post-surgical observations, frequency of post-surgical observations and criteria for early euthanasia owing to surgical complications or pain that cannot be relieved. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria used to determine if animals are experiencing pain or distress (pain assessment parameters), and the location for the post-surgical care.

V.4.3.4. Location
List the location (Building & Room #) of the proposed surgical procedure(s).

V.4.3.5. Surgeon
List the name(s) of the surgeon(s) completing the procedure(s) described above in V.4.3.2.Procedure.

V.4.3.6. Multiple Major Survival Operative Procedures
No response is required under this section title. The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures
If applicable, and not previously described in V.4.3.2.Procedure(s), describe in detail any multiple major survival operative procedure(s) planned including approach, surgical manipulation(s), and closure (layers, materials, patterns, and so forth.). Each procedure should be described separately and in sufficient detail that someone with appropriate surgical training could repeat it with minimal variation.

V.4.3.6.2 Scientific Justification
If applicable, provide scientific justification for performing multiple major survival operative procedures on the same animal. Cost savings alone is not considered a basis for scientific justification.

V.4.4. Animal Manipulations
Describe any injections, sampling procedures, or other manipulations of the animals necessary for the study. A reference or SOP may be provided in order to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections
Provide any other additional injection information, not previously provided in V.4.1.2.1. Anesthesia / Analgesia / Tranquilization, including agent, dosage, route, frequency, injection site, needle size, and so forth.

V.4.4.2. Biosamples
Describe any planned biosample collection procedures. Examples of biosamples include, but are not limited to, cerebrospinal fluid taps, blood sampling, and biopsies. List volume collected, sampling site,
frequency of sampling, needle size, and method of sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

V.4.4.3. Adjuvants
List any adjuvants used and the plan for their use. Provide a scientific justification for the use of Complete Freund's adjuvant (CFA) and discuss why other less reactive adjuvants cannot be used. Provide dosages, volumes, route, number of injection sites, and injection site locations. Specify frequency and method of injection site monitoring and include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.4. Monoclonal Antibody (MAb) Production
Provide a scientific justification for in vivo MAb production. What in vitro methods of MAb production were considered but not used? For in vivo MAb production, specify the priming agent, animal monitoring frequency, number and frequency of abdominal taps, and fluid replacement therapy. Include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.5. Animal Identification
Describe the method of animal identification used in this study. Examples include microchips, tattoos, ear tags, and cage cards.

V.4.4.6. Behavioral Studies
Fully describe the use of aversive stimuli, food or water restriction, and so forth, which could affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria for temporary or permanent removal of the animal from the study (for example, weight loss or state of hydration). Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.4.7. Other Procedures
Describe all procedures that have not been explained in other sections of this proposal that will be performed while conducting this research such as electrocardiograms, radiology, aerosol exposure, etc.

V.4.4.8. Tissue Sharing
If applicable, list any tissues that will be shared, with whom, and for what purpose.

V.4.5. Study Endpoint
State the projected study endpoint for the animals (for example, recovery and return to issue pool, euthanasia, or death without early euthanasia). Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P. I. must ensure that unnecessary pain or distress is prevented by carefully considering “When is the experimental question answered?” so that the animals can be expeditiously removed from the study. Define specific criteria that will be used to determine study endpoint (for example, percentage of body weight loss, loss of locomotion and significant lowering of body temperature, decreased food or water consumption, and decreased activity). Specifically address and scientifically justify any proposal in which critically ill or moribund animals are allowed to die as a result of the experimental procedures without the benefits of veterinary medical treatment or early euthanasia. Explain the plan for the disposition of surviving animals or animals removed from the study prior to its completion.

V.4.6. Euthanasia
If applicable, discuss the euthanasia method. The Animal Welfare Act defines euthanasia as “humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death.” The current American Veterinary Medical
Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. If requested, the attending veterinarian will assist in selecting the best method for euthanasia.

V.5. Veterinary Care

No response is required under this section title. The attending veterinarian should be able to provide assistance in completing this section of the proposal.

V.5.1. Husbandry Considerations

Federal Animal Welfare Regulations require that animal housing and living conditions must be appropriate to their species and contribute to their health and comfort. Briefly describe animal husbandry to include routine animal observations, caging methods, feed and water provisions, environmental parameters, sanitation schedules, and light cycles.

V.5.1.1. Study Room
Where will the experimental procedure be conducted? Will the animal be housed in this room for more than 12 hours?

V.5.1.2. Special Husbandry Provisions
Examples included micro-isolators, metabolic cages, food and water restriction, etc.

V.5.1.3. Exceptions
Describe any deviations or exceptions to The Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations, or IACUC policy that have an impact on animal housing space, feeding, and sanitation. Deviations or exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

No response is required under this section title.

V.5.2.1. Routine Veterinary Medical Care
Describe the routine veterinary medical care. State if the animals will be observed daily or more frequently. Indicate what will happen if the animal becomes ill or debilitated during the study and requires evaluation. List the criteria used for health evaluation while the animals are on study (for example, weight loss, ruffled fur, dehydration, decreased activity, and hunched body position). Include a response plan (for example, alternative early endpoint and veterinary medical treatment) in the event of debilitating illness or an adverse reaction.

V.5.2.2. Emergency Veterinary Medical Care
Describe the provisions for emergency veterinary medical care.

V.5.3. Environmental Enrichment

No response is required under this section title.

V.5.3.1. Enrichment Strategy
Discuss any enrichment provided to animal species listed in this protocol.

V.5.3.2. Enrichment Restrictions
If applicable, provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of nonhuman primates and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC.
VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING
List the names, qualifications, including any educational degrees, training and experience, by procedure, of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures (e.g., surgery, euthanasia, pre- and post-operative care, etc.), and/or manipulations (e.g., injections, phlebotomy, restraint, etc.) described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Training should include required institutional courses as described in the Animal Welfare Regulations (9 CFR paragraph 2.32(c)). The attending veterinarian should be able to provide assistance in completing this section of the proposal.

NOTE: A “Study Personnel Qualifications / Training Table” must be included under this section and contain the following four column headings:

1) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a dog).
2) Name of the person performing the activity.
3) Qualifications of the person performing the activity (for example, assistant laboratory animal technician (ALAT), 2 years experience).
4) Training of the person performing the activity (for example, Canine Procedures Workshop, 1999).

Itemize each activity being performed in the protocol and list by species if there are multiple species in the protocol. If more than one individual is performing the activity, list each individual separately.

VII. BIOHAZARDS/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal, e.g., viral agents, toxins, radioisotopes, oncogenic viruses, chemical carcinogens, allergens, zoonotic diseases, physical hazards, etc. Describe safety precautions and programs designed to protect personnel from biohazards associated with this research and any surveillance procedures in place to monitor potential exposures.

VIII. ENCLOSURES
Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, pain assessment criteria, etc., may be included at the discretion of the P.I.

IX. ASSURANCES
The Federal Animal Welfare Regulations specifically requires several written assurances from the P.I. Please read and sign the assurances as indicated.

PROTOCOL TITLE: Enter protocol title.

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.
C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard / Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely, "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

(Principal Investigator Printed Name)          (Principal Investigator Signature)          (Date)

H. Painful Procedure(s): (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL

WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(Principal Investigator Printed Name)          (Principal Investigator Signature)          (Date)

Information requested above in Section IX. Assurances may be faxed or mailed to: Chief, Animal Use Programs, Division of Biomedical Research and Regulatory Compliance (SGRC), HQ, USAF, Office of the Surgeon General, 5201 Leesburg Pike, Suite 1400, Falls Church, VA. 22041. Phone: 703-681-4132 / Fax: 703-681-4518.
X. ACCREDITATION: For each of the following items, if applicable, provide the requested information for each facility where animal research will be conducted. Information may be faxed or sent to the address noted below.

1. U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Animal Care Inspection Report: A copy of your institute’s most recent USDA Facility Inspection Report, for any and all facilities where animal research is performed, including any subcontracted facilities.

2. A copy of your institute’s most recent Association for Assessment and Accreditation of Laboratory Animal Care, (AAALAC) “Accreditation Letter” indicating current accreditation status (e.g. full, provisional, probationary, or revoked). Alternatively, you may request that an “Accreditation Letter” be sent directly from AAALAC. The letter may be faxed or sent directly to the address noted below.


4. In the event that items #2 and #3 above do not apply to your institution, please provide a statement, signed by the Institutional Official on institutional letterhead, that the care and use of animals will be performed in accordance with the National Research Council’s 1996 “Guide for the Care and Use of Laboratory Animals” and applicable Federal regulations.

Information requested above in Section X. Accreditation may be faxed or mailed to: Chief, Animal Use Programs, Division of Biomedical Research and Regulatory Compliance (SGRC), HQ, USAF, Office of the Surgeon General, 5201 Leesburg Pike, Suite 1400, Falls Church, VA. 22041. Phone: 703-681-4132 / Fax: 703-681-4518.

Useful Links:
The DOD Directive 3216.1, dated 17 April, 1995, and AR 40-33 / AFMAN (I), dated 1 December 2003, provides policy and requirements for the use of animals in DOD-sponsored research. These requirements may differ from those of other funding agencies. These documents are available online at one of the following addresses:

Air Force Medical Service (AFMS) Knowledge Exchange website: https://kx.afms.mil
(If link does not work, copy and paste the link into the address box on your internet browser then press enter. A user name and password may then be obtained by joining).

(User name and password may be obtained by joining Knowledge Exchange on the AFMS Knowledge Exchange website above).

Animal Welfare Act and Regulations:  

United States Department of Agriculture Animal Care website:  
http://www.aphis.usda.gov/ac/

United States Department of Agriculture Animal Care Policies:  
http://www.aphis.usda.gov/ac/polmanpdf.html

Guide for the Care and Use of Laboratory Animals:  
http://www.aaalac.org/guide.htm

Office of Laboratory Animal Welfare:  
http://grants.nih.gov/grants/olaw/olaw.htm

Public Health Service Policy on Humane Care and Use of Laboratory Animals:  
http://grants.nih.gov/grants/olaw/references/phspol.htm

2000 Report of the AVMA Panel on Euthanasia  
http://www.avma.org/resources/euthanasia.pdf

Animal Welfare Information Center (AWIC):  
http://www.nal.usda.gov/awic/

USDA / National Agricultural Library / AWIC Literature Search Databases:  

USDA / National Agricultural Library / AWIC Alternatives and Searches:  

National Institutes of Health (NIH) Laboratory Animal Medicine Drug Formulary:  

Washington State University Laboratory Animal Medicine Drug Formulary:  
http://campusvet.wsu.edu/infofac/drugdoses.htm

AAALAC – Association for the Assessment and Accreditation of Laboratory Animal Care, International:  
http://www.aaalac.org/

AALAS IACUC Resources – online informational resources for members and staff of Institutional Animal Care and Use Committees:  
http://www.iacuc.org