## DoD Animal Use Protocol Format

### Requirements

All DoD intramural animal use protocols must use the format shown in this appendix. This protocol format includes requirements of the Animal Welfare Act Regulations, the Guide, and other applicable Federal regulations and DoD directives. Protocol cover sheets are not mandatory and can vary by organization and IACUC needs and policies. Below is only a sample version. A signed PI assurances page can provide appropriate attestation that the Principal Investigator has completed the proper consultation during the protocol development process.

### Protocol cover sheet

Before the protocol is submitted for Institutional Animal Care and Use Committee (IACUC) review, it is recommended that certain signatures are present on protocol cover sheet. They include those of the Principal Investigator (PI); either the department or division chief or the scientific review committee chairperson, the consulting veterinarian, the individual performing the statistical review (only for protocols that collect and analyze data) and the medical training reviewer (only for protocols that train medical procedures). Site IACUCs may require more or less signatures on a cover sheet before submission based upon their internal policies and reviewing procedures.

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I. Name of Facility

II. Proposal Number

III. Title

IV. Principal Investigator(s)/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD)

V. Scientific/Division or Department Chief Review/Phone/E-mail

a. Printed Name (First Name, MI, Last Name);Title; Division

b. Signature; Date (YYYYMMDD)

VI. Medical Training Review/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD)

VII. Statistical Review/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD)

VIII. Consulting Veterinarian/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name}; Title; Division

b. Signature; Date (YYYYMMDD)

**Sample Protocol Cover Sheet**

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*a. Scientific/division/department review.* This review verifies that the animal use proposal received appropriate scientific peer review and is consistent with good scientific practices.

*b. Medical training review.* This review verifies that the animal use proposal received approval from the immediate medical chain of authority and is consistent with good training practices for the defined medical procedures training.

*c. Consulting veterinarian.* The Animal Welfare Act Regulations require that a qualified veterinarian must be consulted in the planning of procedures/manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics. It is a recommended practice for a consulting veterinarian to review any animal-use protocol before IACUC submission.

*d. Statistical review.* A person knowledgeable in biostatistics is required to review all proposals to ensure 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. This review is not required for protocols whose aims solely involve training personnel.

## DoD animal use protocol outline

*a.* The outline format shown below is designed to be used with several word-processing programs on a personal computer as a "fill-in-the-blank" type of document. DoD site IACUCs may create slight variations to the format but must include all pertinent information. If removing information from the format, please consult your appropriate DoD component oversight office listed in DHA-MSR 6025.02. Each paragraph and subparagraph in the format must have a response. Title headings do not require a response. Portions of the protocol format that are not applicable should be marked "N/A." There are no space limitations for the responses. Pertinent standing operating procedures or similar documents that are readily available to the IACUC may be referenced to assist in the description of specific procedures. Each IACUC might require additional information in the protocol submission process.

PROTOCOL TITLE
PRINCIPAL INVESTIGATOR(S)
CO-INVESTIGA TOR(S)
I. NON-TECHNICAL SYNOPSIS
II. BACKGROUND
II.1. Background
II.2. Literature Search for Duplication
II.1.1. Literature Source(s) Searched
II.1.2. Date of Search
II.1.3. Period of Search
II.1.4. Key Words of Search
II.1.5. Results of Search
III. OBJECTIVE/HYPOTHESIS
IV. MILITARY RELEVANCE
V. MATERIALS AND METHODS
V.1. Experimental Design and General Procedures
V.1.1. Experiment 1
V.1.2. Experiment 2
V.2. Data Analysis
V.3. Laboratory Animals Required and Justification
V.3.1. Non-animal Alternatives Considered
V.3.2. Animal Model and Species Justification
V.3.3. Laboratory Animals
V.3.3.1. Genus and Species
V.3.3.2. Strain/Stock
V.3.3.3. Source/Vendor
V.3.3.4. Age
V.3.3.5. Weight
V.3.3.6. Sex
V.3.3.7. Special Considerations
V.3.4. Number of Animals Required (By Species)
V.3.5. Refinement, Reduction, Replacement
V.3.5.1. Refinement
V.3.5.2. Reduction
V.3.5.3. Replacement
V.4. Technical Methods
V.4.1. Pain/Distress Assessment
V.4.1.1. APHIS Form 7023 Information (See veterinarian for assistance)
V.4.1.1.1. Number of animals
V.4.1.1.1.1. Column B: _(Animal#)
V.4.1.1.1.2. Column C: _(Animal#)
V.4.1.1.1.3. Column D: _(Animal#)
V.4.1.1.1.4. Column E: _(Animal#)
V.4.1.2. Pain Relief/Prevention
V.4.1.2.1. Anesthesia/Analgesia, tranquilization
V.4.1.2.2. Pre- and Post-procedural Provisions
V.4.1.2.3. Paralytics
V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures
V.4.1.3.1. Sources Searched
V.4.1.3.2. Date of Search
V.4.1.3.3. Period of Search
V.4.1.3.4. Key Words of Search
V.4.1.3.5. Results of Search
V.4.1.4. Unalleviated Painful/Distressful Procedure Justification
V.4.2. Prolonged Restraint
V.4.3. Surgery

V.4.3.1. Pre-surgical Provisions
V.4.3.2. Procedure
V.4.3.3. Post-surgical Provisions
V.4.3.4. Location
V.4.3.5. Surgeon
V.4.3.6. Multiple Major Survival Operative Procedures
V.4.3.6.1. Procedures
V.4.3.6.2. Scientific Justification
V.4.4. Animal Manipulations
V.4.4.1. Injections
V.4.4.2. Biosamples
V.4.4.3. Adjuvants
V.4.4.4. Monoclonal or Polyclonal Antibody (MAbs) Production
V.4.4.5. Animal Identification
V.4.4.6. Behavioral Studies
V.4.4.7. Other Procedures
V.4.4.8. Tissue Sharing
V.4.5. Study Endpoint
V.4.6. Euthanasia
V.5. Veterinary Care
V.5.1. Husbandry Considerations
V.5.1.1. Study Room
V.5.1.2. Special Husbandry Provisions
V.5.1.3. Exceptions
V.5.2. Veterinary Medical Care
V.5.2.1. Routine Veterinary Medical Care
V.5.2.2. Emergency Veterinary Medical Care
V.5.3. Environmental Enrichment
V.5.3.1. Enrichment Strategy
V.5.3.2. Enrichment Restriction
VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING
VII. BIOHAZARD/SAFETY:
VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the Principal Investigator unless directed by the IACUC.

IX. ASSURANCES: The law specifically requires several written assurances from the Principal Investigator. Please read and sign the assurances page as indicated.

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. Veterinary Consultation: I assure I have consulted with a qualified veterinarian during the creation of this protocol and assure that all veterinary care and veterinary support planning has been initiated.

C. Duplication of Effort: When applicable, I have made every effort to ensure that this protocol is not an unnecessary duplication of previous research experiments.

D. Statistical Assurance: When applicable, 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.

E. 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.

F. 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.

G. 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 animals used. 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 animal use activities.

H. Scientific Review: I verify this proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific practice.

I. Medical Training Review: I verify I have consulted with the immediate medical chain of command regarding the necessity to use animals for training the medical techniques outlined in this protocol and have thoroughly considered and/or used alternative modalities before the use of animals . I will also use the minimum number of animals necessary to complete training objectives. (applicable to medical training protocols)

J. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the procedures being conducted have 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 or procedures, which may potentially cause more than momentary or slight pain or distress to animals (Category D or E). This potential pain and/or distress WILL or 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/adequate to accomplish the objectives of this proposed activity.

Signature Date {YYYYMMMDD)

(PRINT) First Name, Ml, Last Name of Principal Investigator

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**DOD Animal Use Protocol Format**

*a.* Some information may be added to the format to meet local IACUC needs. However, all labeled paragraphs and subparagraphs will remain in the same relative order. The added information will be similar or complementary to the information requested. Other types of requirements specific to a given Service, command, or locale (such as budgeting information, local coordinating requirements, or specific proposal review requirements, and so forth) can be added by placing them in front or behind the standard format.

## Protocol format

The format shown below is the same protocol format previously outlined. Explanations have been added to aid in completing the protocol proposal.

PROTOCOL TITLE: Title must include species of animal(s) used in research.

PRINCIPAL INVESTIGATOR
CO-INVESTIGATOR(S)

I. NON-TECHNICAL SYNOPSIS: Provide a brief, narrative description of the proposal that is easily understood by a high school graduate. Include animal use in your description.

II. BACKGROUND

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 will be provided.

II.2. Literature Search for Duplication: This search must be performed for research protocols to prevent unnecessary duplication of previous experiments. A search using multiple relevant research databases should be performed; requirements for database search criteria are at the discretion of the IACUC. This literature search is not required for training, testing, validation protocols, or protocols of repetitive nature.

II.1.1. Literature Source(s) Searched

II.1.2. Date of Search

II.1.3. Period of Search

II.1.4. Key Words of Search

II.1.5. Results of Search: Provide a narrative description of the results of the literature search.

Ill. OBJECTIVE/HYPOTHESIS: State the objective of this protocol or the hypothesis to be tested.

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

V. MATERIALS AND METHODS

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 which may include a summary table of the experimental groups as applicable. Succinctly outline the formal scientific plan and direction of experimentation or other proposed activity. 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. 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 objectives yet be sufficient to yield meaningful results (when data is collected). The minimum number includes animals necessary for control or technique development, and so forth. Inclusion of a summary table or flow chart showing the distribution of animals by experimental group is highly recommended for research protocols. The total number of animals required for the study is listed in section V.3.4.

V.1.1. Experiment 1

V.1.2. Experiment 2

V.2. Data Analysis: If applicable, 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. 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. Be certain to include animals necessary for controls or technique development, and so forth. This section is only required when data is collected and scientifically analyzed; for example, data analysis is not applicable to training protocols.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: State all non-animal alternatives (for example, computer modeling, in vitro cell culture work, cadavers, simulation etc.) that were considered. Explain why animals are needed. Explain if non-animal alternatives are utilized in addition to animal models.

V.3.2. Animal Model and Species Justification: Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model? If less sentient (invertebrate versus vertebrate) animal models were considered but not chosen, explain **why.**

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species

V.3.3.2. Strain/Stock: If inbred or specialized animals are required, use proper terminology. (See the attending veterinarian for assistance.)

V.3.3.3. Source Vendor: Provide a preferred source for the animals. 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. (See a staff veterinarian for assistance.)

V.3.3.4. Age: Age could also reference "adult" vs "neonate" or "age commensurate with weight."

V.3.3.5. Weight: range of acceptable weight during protocol activity or weight commensurate with age.

V.3.3.6. Sex M, F, M and F

V.3.3.7. Special Considerations: List specialized requirements for animals here (for example, simian immunodeficiency virus or herpes antibody free, *Pasteurella* free, vaccination status, and so forth).

V.3.4. Number of Animals Required (By Species): The number of animals stated here must correspond exactly to that described in section V.1. If, during the completion of the protocol, additional animals are needed owing to technical or likely unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval of additional animals.

V.3.5. Refinement, Reduction, Replacement (3 Rs): Investigators are required to consider the 3 Rs when preparing an animal use protocol. In the paragraphs below, describe all provisions in this protocol that refine, reduce, or replace the use of animals. Discuss what provisions were considered and why they were not chosen. If N/A is used, explain why.

V.3.5.1. Refinement: Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing refinements, list refinement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.2. Reduction: 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 would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.3. Replacement: Procedures or measures that eliminate the use of animals. Examples of replacements include but are not limited to the use of non-animal models or less sentient animal species. In addition to listing replacements that will be used, also list replacement alternatives that would allow you to meet your proposal objectives and were considered but not adopted. Explain why they were not adopted.

V.4. Technical Methods: This information must be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure, obtain a clear understanding of what is to be done and how the animals will be handled, and 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: The law defines 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 a qualified veterinarian. Explain if more than momentary pain and distress is anticipated and the methods used to detect pain and distress and the frequency of those observations.

V.4.1.1. APHIS Form 7023 Information: (See your attending veterinarian for assistance.) The protocol must contain an estimate of the number of animals that will be counted in columns C, D, and E of the APHIS Form 7023, Annual Report of Research Facility. Columns C, D and E represent specific pain categories.(See below paragraphs, V.4.1.1.1.1.-V.4.1.1.1.1.4.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for one 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.

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column B: (animal #)
Examples of activities include animals used solely for breeding, holding, or conditioning purposes.

V.4.1.1.1.2. Column C: (animal #)
Examples of research procedures/manipulations that would require an animal to be placed in Column C are studies involving not more than slight or momentary pain and/or distress in a human being to which that procedure is applied.

V.4.1.1.1.3. Column D: (animal #)
Examples of procedures/manipulations that would require an animal to be placed in Column D are procedures where anesthesia or analgesia will be administered to avoid or effectively relieve pain or distress. General anesthesia given for surgical procedures, or the use of analgesia or anti-inflammatory agents are examples of research manipulations in this category.

V.4.1.1.1.4. Column E: (animal #)
Examples of procedures/manipulations that would require an animal to be placed in Column E are procedures 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.

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to effectively relieve or prevent pain or distress if the study will cause more than slight or momentary pain or distress. If pain/distress relief/prevention is planned, specify agents to be used and when these agents will be given (preemptive or post-procedural). Provide agent, dosage, and frequency and duration of administration.

V.4.1.2.2. Pre- and Post-procedural Provisions: Describe the provisions for both pre- and post-procedural care, including provisions for post-procedural observations, frequency, and duration of observations. (Information concerning pre- and post-surgical care should be listed in paragraphs V.4.3.1 and V.4.3.3). If analgesics are used for pain/distress relief, provide the frequency and duration of administration, observational criteria utilized to determine if animals are experiencing pain or distress pre and post-procedure, 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 that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent and adequate vital signs.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: Respond N/A if the animals will experience not more than momentary or slight pain or distress and are placed in column C of APHIS Form 7023. (See paragraph V.4.1.3.5. and V.4.1.1.)

V.4.1.3.1. Source(s) Searched: Examples include AGRICOLA, CAAT, BIOSIS, Altweb, etc.

V.4.1.3.2. Date of Search

V.4.1.3.3. Period of Search

V.4.1.3.4. Key Words of Search: Examples are pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful procedure, etc.

V.4.1.3.5. Results of Search: Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Act specifically states that the P.I. **MUST** provide a narrative description of the methods and sources, e.g., the Altweb (Johns Hopkins Center for Alternatives to Animal Testing), MEDLINE, Life Sciences Abstracts, AGRICOLA, and BIOSIS) that he/she used to determine that alternatives to the painful procedure were not available. Discuss alternatives (those that would meet your proposals objectives) considered but not chosen. The alternatives literature search **MUST** be performed even when animals are placed in Column D (the pain or distress is alleviated through the use of analgesics or anesthetics) and Column E.

V.4.1.4. Unalleviated Painful/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.

V.4.2. Prolonged Restraint: Describe (period of restraint, method, and timing of animal observations, habituation/training of animal to restraint device) and justify In detail any prolonged restraint greater than 12 hours for nonhuman primates or in accordance with IACUC policy for other species. Examples of restraint methods are primate chairs, restraint boards, metabolism cages, etc. This section is not intended for short-term actions such as rabbit restraint for bleeding, and so forth.

V.4.3. Surgery: 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.

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 utilized to determine if animals are experiencing pain/distress, and the location for the pre-surgical care.

V.4.3.2. Procedure: Describe in detail any surgical procedures planned.

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 utilized to determine if animals are experiencing pain/distress, and the location for the post-surgical care.

V.4.3.4. Location: Give the location/room number for the proposed surgical procedure.

V.4.3.5. Surgeon:

V.4.3.6. Multiple Major Survival Operative Procedures: The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures:

V.4.3.6.2. 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 furnished to the IACUC to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections: Information must include route of injection, dosage, frequency, duration, maximum volume injected per site, needle size or range, anatomic injection site and if non-pharmaceutical grade substances are used, please provide assurances regarding purity, osmolarity, pH, pyrogenicity, and sterility and the methods used to determine the safety of non-pharmaceutical grade substances. Also discuss any vehicles, solvents, or diluents used to formulate the product(s) to be administered.

V.4.4.2. Biosamples: Examples include cerebrospinal fluid taps, blood sampling, and biopsies. List volumes taken, sampling site, frequency of sampling, needle size, and method of sampling. Procedures performed for biosamples obtained during a necropsy need not be described here.

V.4.2.3. 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 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.4.2.4. Monoclonal or Polyclonal Antibody (MAbs) Production: Provide a scientific justification for in vivo MAbs or PAbs production. What in vivo methods of MAbs or PAbs were considered but not used? For in vivo MAbs or PAbs 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(s) and veterinary medical treatment) in the event of an adverse reaction.

V.4.2.5. Animal Identification: Describe the method of animal identification used in this study. Examples include: microchips, tattoos, ear tags, and cage cards.

V.4.2.6. Behavioral Studies: Fully describe the use of aversive stimuli, food or water restriction, light/dark cycle, reverse light cycle, and so forth, that would affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria (for example, weight loss or state of hydration) for temporary or permanent removal of the animal from the study. Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.2.7. Other Procedures: Describe all procedures which have not been explained in other sections of this proposal that will be performed while conducting this research. Examples include electrocardiograms, radiology, and aerosol exposure.

V.4.2.8. Tissue Sharing: List what tissues 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, 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 (death as an endpoint) 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: If requested, the attending veterinarian of the facility will assist PIs with preparing this section.

V.5.1. Husbandry Considerations: Federal 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 include micro-isolators, metabolic cages, food and water restriction.

V.5.1.3. Exceptions: Describe any deviations/exceptions to The Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act regulations, or IACUC policy that have an impact on animal housing space, watering, feeding, enrichment, environmental conditions. sanitation, the use of non-pharmaceutical grade drugs, etc. Deviations/exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

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(s) and veterinary medical treatment) in the event of debilitating illness or an adverse reaction. An animal health scoring chart may be necessary.

V.5.2.2. Emergency Veterinary Medical Care: Describe emergency veterinary medical care.

V.5.3. Environment Enrichment

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

V.5.3.2. Enrichment Restriction: Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of social species such as rodents, pigs, 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 and training by procedure of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures, and/or manipulations described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Contact the attending veterinarian for assistance with this requirement (please see endnotes below).

VII.BIOHAZARD/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal (for example, viral agents, toxins, radioisotopes, oncogenic viruses, and chemical carcinogens). 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, and pain assessment criteria may be included at the discretion of the P.I. unless directed by the IACUC.

**Personnel qualifications and training.**

a. Study Personnel Qualifications must be included in section VI of the protocol description. The table format is the preferred but not required method for ease of reviewing this section. A table is recommended to contain at the following four column headings to gather an understanding who is performing the procedures and what makes them qualified and appropriately trained to safely and appropriately execute the activities:

(1) Name of the person(s) performing protocol activities.
(2) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a mouse). Itemize each activity being performed in the protocol. List per species if there are multiple species in the protocol. If more than one individual is performing the activity, list each individual separately.
(3) Qualifications and experience of the person performing the activity (for example, assistant laboratory animal technician (ALAT), three years of experience).
(4) Training of the person performing the activity (for example, Canine Procedures Workshop, Mar 2019 or Rodent Hands-on training, Jan 202.