

**INSTRUCTIONS FOR COMPLETION OF THE  
ANIMAL COMPONENT OF RESEARCH PROTOCOL  
MAIN BODY  
(ACORP INSTRUCTIONS)  
VERSION 4**

These instructions provide detailed guidance on completing the Main Body of the ACORP, and are referenced to the letters and numbers of the items in the ACORP. The PI may use the ACORP Main Body template provided in the RAMS application by using the link available in question 8 of section M.

Regulatory documents mentioned in the instructions are abbreviated as follows:

0730 – VA Handbook 0730 Security and Law Enforcement, August 11, 2000  
0730/2 – VA Handbook 0730/2 Security and Law Enforcement, May 27, 2010  
1108.01 – VHA Handbook 1108.01 Controlled Substances (Pharmacy Stock), November 16, 2010  
1200.07 – VHA Handbook 1200.07 Use of Animals in Research, November 23, 2011  
AAALAC FAQs – Association for Assessment and Accreditation of Laboratory Animal Care International FAQs, June 2011  
AVMA *Guidelines on Euthanasia* – The AVMA Guidelines on Euthanasia (formerly the 2000 Report of the AVMA Panel on Euthanasia), 2007  
AWAR – USDA Animal Welfare Act Regulations and Standards, Code of Federal Regulations, Title 9, Chapter 1)  
Form 7023 – USDA APHIS Form 7023  
Guide – Guide for the Care and Use of Laboratory Animals, 8<sup>th</sup> ed., 2011  
OLAW FAQs – Frequently Asked Questions – PHS Policy on Humane Care and Use of Laboratory Animals (<http://grants.nih.gov/grants/olaw/faqs.htm>)  
PHS Policy – Public Health Service Policy on Humane Care and Use of Laboratory Animals  
US Government Principles – US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training  
USDA APHIS Animal Care Policies – Animal Care Resource Guide Policies, USDA APHIS, March 25, 2011

**General Instructions:**

Answer each question by completing the table provided or entering the requested information at the ►. Enter “N/A” for any item that does not apply to this protocol. The sections of the form will expand as needed.  
To check an item, type “X” inside the ( ) provided.  
Define each abbreviation the first time it is used.  
ONLY include the individual appendices that are relevant to the protocol being submitted for review.

**Header for Every Page.** The header information for this appendix is pre-populated to match header for the Main Body of the ACORP to which it applies, to identify each page of this Appendix with that ACORP:

PI's last name: The PI's name is prepopulated  
Protocol No. Assigned by the IACUC – This is a unique identifier for each protocol that is automatically generated by the system to the protocol as a whole

Official Date of Approval – This is the date of final and unequivocal approval by the IACUC, as defined in the PHS Assurance, which determines the due dates of the first annual continuing review and the

triennial *de novo* review, as applicable. The IACUC committee will enter this date into the form when a decision is made.

**A. ACORP Status.**

1. Full Name of Principal Investigator – The Full name of the PI is prepopulated in the template that is generated from RAMS.
2. VA Station Name (City) and 3-Digit Station Number – Enter the name and 3-digit station number of the VA Station that will be responsible for overseeing the work to be performed on this protocol, regardless of the location where the work will be performed.
3. Protocol Title – The Protocol Title is prepopulated in the template that is generated from RAMS.
4. Animal Species covered by this ACORP – The IACUC must determine whether multiple species may be covered by any given ACORP. When very similar procedures are to be performed on more than one similar species (e.g., the same surgical procedure on mice and rats), it may be appropriate to document both species in a single ACORP. When different procedures are to be performed on very different species (e.g., surgery on mice and behavioral testing of cats), it is recommended that a separate ACORP be prepared for each species. In any case, it should always be clearly stated in the response to each item, which part of each response applies to which procedure and which species.
5. Funding Source(s). Based on the selection made in the RAMS application, the funding source is pre-selected. Check each source of funds that will support the work on this protocol. These determine the regulatory and reporting requirements that apply to the protocol.
6. Related Documentation – Identify here any related documentation that may be relevant for the IACUC to reference in the process of reviewing this protocol.
  - a. If this ACORP addresses work on a project that has already been submitted to the R&D Committee for review, identify the project:
    - (1) Title of project – enter the title of the project that has been submitted to the R&D Committee for review
    - (2) Date of R&D Committee approval – leave blank if the project has not yet been approved by the R&D Committee
  - b. Triennial review. *PHS Policy* (IV.C.5) requires a complete *de novo* review of animal use protocols at least once every three years. If this ACORP is being submitted for triennial review, summarize the work on the previously approved protocol that has already been completed, and report the numbers of approved animals that have been used. Describe any study results that have prompted changes in the protocol, and briefly summarize the changes that have been incorporated, to guide the reviewers to the details documented in other items of this ACORP.

For example, describe results that change the estimated success rate of the procedures, the estimated differences and/or variability of the data (which impact the numbers of animals needed), the hypotheses to be tested, or the specific procedures to be performed. The Triennial review can be started from the previously approved application. Open the previously approved application and expand the approved toolbar. Please select Create Event in the row corresponding to Triennial review. The PI will be re-directed to the

Research Study Application page and notice a new application with –Triennial review appended to the main study title will be created. The PI may click on the corresponding study ID link to open the Triennial review application, complete it and submit it for review.

- c. Any other relevant previously approved animal use protocol – If you would like the IACUC to be able to refer to any other approved animal use protocol when reviewing this one, identify the other protocol.

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):

Research – This includes all use of animals for a specific research study, including training of personnel to perform procedures required for the study, testing that is part of the experimental design, collection of specimens from donor animals, and breeding and colony management specifically for the purposes of the research study.

Teaching or Training – This includes all use of animals specifically for teaching or training purposes, as in workshops or for student laboratory exercises.

Testing – This includes all use of animals specifically for routine testing, as in safety studies required for FDA approval.

Breeding and colony management – This includes breeding and management of colonies being maintained as resources; breeding and colony management specifically to supply animals required for a research project should be designated “research”, above.

Holding protocol – Local policy may specify the use of holding protocols for animals transferred from expired or suspended protocols; There are no VA, PHS, or USDA regulatory requirements for such protocols.

Other – Describe the other type(s) of animal use covered by this protocol.

## **Proposal Overview**

- B. Description of Relevance and Harm/Benefit Analysis.** (*US Government Principles*, Principle II). Language that is informative to the general public is important because the ACORP serves to document the relevance of this work to the tax-paying public. A scientific abstract from a grant proposal is not appropriate. The IACUC is obligated to weigh the benefits to be gained from the work against potential concerns about animal welfare (*AAALAC FAQs*, C.3; *Guide*, p. 27), so it is important for the protocol to provide the information that the IACUC needs to assess this.
- C. Experimental Design** – Describe the procedures to be performed, how they fit into the sequence of events for each group of animals, and why they are necessary to the research objectives.
1. **Lay Summary.** Language that is informative to the lay reader is important for orienting the lay member(s) of the IACUC with regard to the conceptual design of the experiment.
  2. **Complete description of the proposed use of animals** (*PHS Policy*, IV.D.1.c.). Describe the proposed use of animals as it relates to the experimental design, providing sufficient detail and using language suitable for scientific colleagues who may not be experts in your discipline. For agricultural animals used in research, specify whether the research is biomedical or agricultural, and explain why (*Guide*, p. 32-33). VA policy requires that all procedures planned for the period of a VA award (even if that period is longer than three years) be included in the ACORP, even though the IACUC is required to perform a new review three years after the initial approval date (*PHS Policy*, IV.C.5).
    - a. **Summarize** the design of the experiment in terms of the sequence of events that animals in each group will experience, including which procedures and manipulations will be performed, and why these procedures and manipulations are necessary. Approval of this ACORP will apply specifically to the descriptions given here. Any differences from the procedures that were proposed in the grant proposal should be explained – for example, changes required by the IACUC must be designated as such (*PHS Policy*, IV.D.2), and the basis for other changes (such as new information obtained after submission of the grant) should be described. For complicated experimental designs, a flow chart, diagram, or table is strongly recommended to help the IACUC understand what is being proposed.
    - b. **Justify the group sizes and the numbers of animals requested.** (*US Government Principles*, Principle III) To show that the proposed work conforms to applicable US and VA requirements regarding numbers of animals used, describe how the number of animals needed for the experiments was estimated. Explain how this estimate is related to the experimental and control groups described in Item C.2.a, above, and how the optimal number of animals to be included in each group was estimated. The *Guide* (p. 25) states that whenever possible, the number of animals requested should be justified statistically. A power analysis is strongly encouraged to justify group sizes when appropriate.
    - c. **Describe each of the procedures** to be performed on any animal on this protocol. For each procedure, describe specifically what will be done, what the animals will be expected to experience if no special measures are taken to address potential pain or distress, and what measures are planned to address any potential pain or distress. Details are requested in separate appendices for antibody production (Appendix 2), surgical procedures (Appendix 5), and behavioral training (Appendix 6), so summary descriptions of those procedures are sufficient here. It is also sufficient to provide summary descriptions of any procedures that are detailed in SOPs approved by the IACUC, provided the SOPs are documented in Item Y. NOTE: Use Appendix 9 to document each of the procedures that involves a “departure” (as defined by OLAW) from a “must” or “should” standard in the *Guide*. Consult with the IACUC or Attending Veterinarian in case of questions about whether specific procedures represent “departures”.

- D. **Species.** Explain why the species indicated is/are particularly appropriate to the work proposed (*PHS Policy*, IV.D.1.b) Consider such characteristics as body size, availability of specific strains, breeds, or mutants, data from previous studies, and unique anatomic or physiologic features. Explain why these are important to the work proposed.

### Personnel

- E. **Qualifications and training --** Document the current qualifications of all personnel for performing the procedures for which they are to be responsible (*PHS Policy*, IV.C.1.f). If any personnel are not yet appropriately trained, plans for providing the additional required training will be requested in Item F.

1. PI – The PI is responsible for supervising all of the other personnel and ensuring that the use of animals is appropriate, and therefore must be qualified to work with research animals (1200.07, par. 8.m).

Name:

Animal research experience – Describe the PI's education, training, and other experience with animal research in general. A listing of academic degrees alone is not an adequate response.

Qualifications to perform specific procedures – complete the table to document the PI's experience with each of the specific procedures that the PI is to perform, in the species described in this ACORP. If the PI needs further training, enter "to be trained", and provide the details of the planned training in Item F, below. Qualifications to perform euthanasia will be requested in Item U.3, and need not be given here.

2. Other research personnel -- Provide the information requested for each additional member of the research staff who will be involved in the work with the animals on this protocol. Include members of the VMU staff who will be responsible for performing any of the experimental procedures on the animals on this protocol.

Name:

Animal research experience – Describe the individual's education, training, and other experience with animal research in general. A listing of academic degrees alone is not an adequate response.

Qualifications to perform specific procedures – complete the table to document the individual's experience with each of the specific procedures that he or she is to perform, in the species described in this ACORP. If the individual needs further training, enter "to be trained", and provide the details of the planned training in Item F, below. Qualifications to perform euthanasia will be requested in Item U.3, and need not be given here.

3. VMU animal care and veterinary support staff personnel – Provide the information requested for each member of the VMU animal care and veterinary staff who will perform support procedures in the animals on this protocol. "Support procedures" include special husbandry, pre-operative preparation, post-operative care, and other such services that go beyond routine husbandry but do not include performance of experimental procedures. Members of the VMU animal care staff who will perform specific experimental procedures on animals on this protocol should be documented as research personnel in item E.2, above.

Name:

Qualifications to perform specific procedures – complete the table to document the individual's qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, completion of special training). If the individual needs further training, enter "to be trained", and provide the details of the

planned training in Item F, below. Qualifications to perform euthanasia will be requested in Item U.3, and need not be given here.

4. Completion of required training (1200.07, par. 8.m(2)) – document that each of the research personnel involved in this protocol is current on his/her required training at the time of submission of this protocol by entering the most recent completion date for each course. If alternate training to the standard courses identified on the Collaborative Institutional Training Initiative (CITI) or AALAS Learning Library (ALL) websites has been approved by the CVMO, identify the course and the provider of the training (website, organization, instructor, etc.) as well as the completion date.

- F. **Training to be provided.** If any of the personnel in Item E need further training to perform the exact procedures assigned to them in the species addressed by this ACORP, describe the additional training that will be provided. Identify each procedure for which training is needed and describe the type of training that will be provided (e.g., classroom, seminar, workshop, observation of an experienced individual, and/or supervised practice). Then give the name, the qualifications, and the training experience of each trainer. If not applicable, enter “N/A”.
- G. **Occupational Health and Safety.** The institutional Occupational Health and Safety Program (OHSP) for protecting personnel involved in the use of animals in research (*PHS Policy*, IV.A.1.f, and *OLAW FAQs G.2*) should include not only training, but also on-going surveillance elements (1200.07, par. 10.c and Appendix C par. 4.a(2)).

Each individual included in Item E must be “enrolled” in an OHSP (1200.07, par. 10.a, and Appendix C par. 4.a). An “enrolled” individual must have the opportunity to participate fully in the Preventive Medicine Program (PMP) provided by the institution, but may elect to sign a waiver to decline participation in the optional components of such a program.

The frequency of interactions with the OHSP required for any individual depends on the risks relevant to that individual’s duties as well as the individual’s health status, and must be determined by OHSP personnel (1200.07, par. 10.c; *OLAW FAQs G.2*; and *Guide*, p. 17-19).

Non-routine OHSP measures include special vaccines, prophylactic measures (e.g., selegiline for MPTP or stable iodine for radioactive iodine), education, or additional health screening techniques. These may be required or may be optional but potentially beneficial to research, husbandry, or veterinary staff participating in or supporting some protocols. For field studies, identify any relevant zoonotic diseases, safety issues, or laws or regulations that apply, beyond those common to routine use of laboratory animals, and describe how these concerns will be addressed. Routine measures already included in the Occupational Health and Safety Program (e.g., vaccination for tetanus, rabies, and hepatitis B, and TB screening) need not be mentioned here.

### **Animals Requested**

- H. **Animals to be Used.** Complete the table, listing on a separate line each group of animals with any specific features that are required for the study. List each species on a separate line, and further subdivide the animals by any surgical alterations to be performed by the vendor (e.g., “ovariectomized rats”) and any other features such as strain, stock, genotype, breed, gender, age/size, and/or health status, if these are specified for the study. Combinations (“either” for gender) or ranges (for ages or sizes) may be entered if the study does not require specifics.

Identify the source from which animals on each line in the table will be obtained, giving the name of any commercial vendor or collaborator who will provide the animals, or specify that the animals will be obtained from a local breeding colony and identify the PI responsible for maintaining the colony.

Specify the least stringent health status acceptable for all of the animals on the same line of the table as follows:

- rodents and rabbits – “specific-pathogen-free (SPF)”, “gnotobiotic” (“germ-free” or “free of defined flora”), “conventional”, “feral”, or other description
- dogs, cats, pigs, and other “large animals” – “specific-pathogen-free (SPF)”, “conditioned”, “conventional”, “feral”, or other description.
- non-human primates – specify viral status (e.g., herpes B negative, SIV negative, etc.) and TB status.

- I. **Numbers of animals requested**, itemized by species, and categorized according to the pain and/or distress associated with the procedures to be performed (*USDA APHIS Animal Care Policy #11*). The total in each category, for each species, will be the total number approved by the IACUC for use over the life of the protocol. The USDA (*AWAR*, §2.36(b)(3-8)) and the VA (*1200.07*, par. 8.I(4)) require that the numbers of all animals actually used each year at a research facility be reported annually, categorized according to pain and/or distress. The columns shown here, breaking down the numbers by year, only show the projected estimates, which may differ from the actual usage. These estimates may be required by the local IACUC, but the yearly columns are otherwise shown only for the convenience of the PI.

Notes:

- For complex protocols involving many different procedures, list for each category the procedures that account for the assignment of animals to that category.
- Use a separate row in each table for each species and, within species, for each experimental group or combination of experimental groups that undergoes a different set of procedures. This should make it clear how the numbers shown in Item I relate to the animals described in Item C. It is not necessary to itemize by strain unless the different strains undergo different sets of procedures.
- No animal should be assigned to more than one USDA category. If several different procedures are to be performed in a single animal, the animal should be assigned to the category corresponding to the most painful/distressing procedure.
- If you have questions about the appropriate category assignments, please contact the Attending Veterinarian or IACUC Chair for assistance.
- Be sure to include all of the animals that will be used in connection with this protocol, including not only the actual study subjects, but also all additional individuals such as (but not exclusive to) breeders, tissue donors, and those generated in breeding colonies and culled because of unusable gender, genotype, or date of birth.

**USDA Category B:** Include in Category B all animals that will be bred or purchased exclusively for breeding, and that will not undergo any procedures other than those required by currently accepted standards of medical care. This includes breeders, and any young that may be culled because of unusable gender, genotype, or date of birth. If numbers cannot be determined exactly, estimate the maximum expected, as closely as possible. (Note: Animals that must undergo tail snips for genotyping must be assigned to category C, D, or E.)

**USDA Category C:** Include in Category C all animals that will only undergo procedures that involve no more than very brief or minor pain or distress, for which no pain relieving drugs are needed. Examples include observational studies, most intravenous and parenteral injections of non-irritating agents, most blood collections from peripheral vessels, and euthanasia for post-mortem collection of cells and/or tissues.

**USDA Category D:** Include in Category D all animals that will only undergo no more than procedures that are potentially painful or distressing, but for which the pain or distress is prevented or relieved by appropriate anesthetics, sedatives, analgesics, or other means (e.g., acupuncture). Examples include surgery performed under anesthesia (major or minor, survival or non-survival), tissue or organ collections or other painful procedures performed on living animals

under anesthesia (such as retro-orbital blood collection in rodents), prolonged restraint accompanied by tranquilizers or sedatives, and experiments with provisions for immediate euthanasia to effectively prevent pain and/or suffering in animals that are becoming sick. If an endpoint is defined such that the animals are likely to experience significant pain or distress, Category E is more appropriate.

**USDA Category E:** Include in Category E all animals that will undergo procedures in which pain or distress CANNOT be relieved. An important rule of thumb for deciding whether an animal should be assigned to Category E is to consider whether a human experiencing a comparable condition would be expected to seek relief. Examples include studies in which animals must be allowed to die without intervention (e.g. LD<sub>50</sub>, mortality as an end-point), studies that require endpoints that may be painful or stressful, studies that require withdrawal from addictive drugs (without palliative treatment), pain research, and studies that involve noxious stimuli that are not immediately escapable, food or water deprivation beyond that necessary for standard pre-surgical preparation, or paralysis or immobility in conscious animals.

**TOTALS:** Bring down totals for each species, over all groups and/or procedures in all categories, for each year (if using the yearly columns) and for the protocol as a whole.

- J. **Management of USDA Category D procedures.** For any protocol that includes Category D procedures, list each Category D procedure and provide the information requested in the table.

Describe how the animals will be monitored for evidence of pain or distress during the procedure and through post-procedure recovery, including the method(s) by which the animals will be monitored, the frequency with which they will be monitored, and how long post-procedure monitoring will continue.

The person(s) identified as responsible for monitoring must be documented in Item E, above.

Describe each method to be used to alleviate pain and/or distress during the procedure or the post-procedure recovery period, giving dose, route, and duration of effect of any analgesic, sedative, tranquilizer, or anesthetic to be administered, and providing comparable details for any other method(s) by which pain or distress will be alleviated

For any surgical procedure that you will describe in Appendix 5, only identify the procedure(s) in the "Procedure" column, and indicate "See Appendix 5 for details."

- K. **Justification of Category E procedures.** The USDA and the VA require each facility to include in the annual report of animal use the justification for each Category E procedure performed (*Form 7023, 1200.07* par. 8.I(4)). Indicate whether this protocol includes any Category E procedures. If so, identify each Category E procedure included and justify scientifically why the pain and/or distress cannot be relieved. For example, give the evidence that drugs available for relieving the anticipated pain or distress would make the results uninterpretable. If animals must be observed until natural death (e.g. in some studies of infectious disease or oncology), or if the endpoint that must be used otherwise allows the animals to experience more than very brief or slight pain or distress, you must explain why an alternate endpoint (such as moderate weight loss, clinical signs, tumor size, etc.) prior to death or the onset of pain or distress cannot be used. The justifications given here will be included in the annual reports submitted to the USDA and the VA. (If animals will undergo category D procedures as well as Category E procedures, give the details about the Category D procedures in Item J.)

### **Veterinary Care and Husbandry**

- L. **Veterinary Support.**

1. (*US Government Principles*, Principle VII). Identify and provide contact information for the

laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care. This may be a supervising Attending Veterinarian, or the veterinarian specifically assigned to the animals on this protocol.

2. Veterinary consultation during the planning of this protocol. VA Policy (1200.7, par. 8.f(2)(b)) requires that a laboratory animal veterinarian be consulted during the planning stages of every protocol, so that the veterinarian's recommendations can be incorporated into the ACORP before the protocol is submitted for IACUC review. To be valid, the most recent consultation must have occurred no more than 3 years before the protocol was submitted for IACUC review. As an alternative to a face-to-face meeting, the veterinarian may perform a pre-review of a draft of the ACORP and provide comments to the PI so that the ACORP can be revised prior to IACUC review. Document that this has been done by providing the information requested.

**M. Husbandry.** This item focuses on the equipment and services that will be required for the husbandry of the animals on this protocol. It may be useful to consult with the VMU staff for specifics on the housing and services that are available. The details and justifications for any special husbandry required will be requested in Appendix 6. Any "departures" from "should" or "must" standards in the *Guide* that are approved by the IACUC for this protocol must be documented as such in Appendix 9. Consult with the IACUC or Attending Veterinarian in case of questions about whether the husbandry required involves "departures".

1. Caging needs. To help the animal care staff plan, complete the table to describe the housing that will be needed for this protocol.
  - a. Species. Enter the species for which each type of housing is requested.
  - b. Type of housing. Enter any special features required. These may include features such as (but not limited to):

Gnotobiotic (germ-free or defined flora) isolation  
 Biohazard or other special hazard containment  
 Sterile microisolator caging, with filtered cage top  
 Non-sterile microisolator caging, with filtered cage top  
 Wire-bottom

If no special features are required, enter "standard (see SOP)" for housing according to any local SOP and enter the requested information about the SOP into the table in Item Y; or enter "standard, see below" and describe the standard housing below the table.

- c. Number of individuals to be housed in each housing unit. Give the range of numbers of individual animals that may be housed in any one housing unit. The *Guide* recommends that social animals be housed in stable pairs or groups unless they must be housed alone for experimental reasons or because of social incompatibility (*Guide*, p. 51 and 64). Provide the justification if any animals are to be housed singly (if species is not considered "social", then so note).
  - d. Indicate whether the housing described is consistent with the standards in the *Guide* and with *AWAR* (for USDA-regulated species). Document as a "departure" (in Appendix 9) any housing that is not consistent with the standards in the *Guide*.
  - e. Estimated maximum number of housing units needed at any one time. This allows the VMU staff to evaluate whether existing housing is sufficient to meet the needs of this protocol.
2. Enrichment. PHS Policy (IV.C.1) requires the IACUC to confirm that work involving animals is consistent with the requirements of the Animal Welfare Act. Enter here any special restrictions

or additions to standard enrichment that will be required. If no special modifications are required, and the enrichment will be provided according to a local SOP, enter “standard (see SOP)” in the table, and enter the information requested about the SOP into the table in Item Y. If the standard enrichment is not described in a SOP, enter “standard, see below” and describe it in the section below the table. Note that USDA requires an institutional exercise plan for dogs (AWAR, §3.8), and environmental enhancement to promote psychological well-being for nonhuman primates (AWAR, §3.81).

3. Customized routine husbandry. Enter here information or instructions that may be important for the animal husbandry staff to be aware of, in providing appropriate routine monitoring and care.

“Genetically modified animals” include specially in-bred strains as well as genetically engineered, transgenic, knock-in, or knock-out animals. Genetic modifications that are to be newly generated on or for this protocol may result in unexpected phenotypic changes, so the first generations of such animals should be carefully monitored.

“Devices” that extend chronically through the skin may include, but are not limited to, cannulae, acrylic implants, and catheters. Details about special care to be provided by the research staff will be requested in Appendix 6.

Other customized routine husbandry may include such features as special bedding material, alternate watering devices, or a modified schedule of bedding changes.

- N. **Housing Sites.** The IACUC is required to inspect semi-annually all sites where animals are housed (AWAR, §1.1 “study area” and §2.31(c)(2); *PHS Policy*, IV.B.2; *OLAW FAQs*, E.1; *1200.07*, par. 8f(1)(a)).

Housing on VA property. Include all locations on VA property where any animals on this protocol will be housed, regardless of whether they are purchased with VA funds, or used by personnel on official VA duty time.

Housing in non-VA facilities. Include all locations not on VA property where any animals on this protocol will be housed, regardless of whether they will be purchased with VA funds or used by personnel on official VA duty time. Be sure to consider affiliated institutions and contract facilities that purchase and house animals on your behalf to make custom antibodies or other biological products. Consult with your Attending Veterinarian or IACUC to determine which institutions must be entered. USDA policies and PHS policy clarifications may also be helpful.

For the status of AAALAC accreditation, enter one of the following (Consult your Attending Veterinarian or IACUC for the status of the non-VA facility.):

- “CFA” (Continued Full Accreditation)
- “DCA” (Deferred Accreditation)
- “PROB” (Probation)
- “RFA” (Restored Full Accreditation)
- “Other” – please explain

VA Policy ( *1200.07*, par. 7.e) requires that all facilities housing VA research animals be accredited by AAALAC. Under exceptional circumstances, a waiver may be requested in writing from the CRADO (Chief Research and Development Officer) or designee, through the CVMO (Chief Veterinary Medical Officer). See Appendix A of *1200.07* for information on how to contact the CVMO.

### **Special Features**

- O. **Antibody Production** – Include any animals on this protocol that will be used for the production of monoclonal or polyclonal antibodies (including for growing existing hybridoma cell lines).
- P. **Biosafety** – Include ALL substances that are to be administered, regardless of whether they are considered hazardous. These include, but are not limited to, chemicals, radioisotopes, infectious agents, biomaterials, prosthetic devices, and cells, tissues, or body fluids. Also include anesthetics, analgesics, antibiotics, etc., administered in connection with surgery or any other procedure on this protocol. Hazardous materials will be distinguished from nonhazardous materials in Appendix 3, “Biosafety”.
- Q. **Locations of procedures.** The IACUC is required to inspect semi-annually all locations where procedures are performed on animals (*OLAW FAQs E.1; 1200.07*, par. 8f(1)(a)). If any animals must be transported to or from any of these locations, the transportation must be in accordance with the *Guide*, the *AWAR*, and *PHS Policy*, in climate-controlled vehicles and sanitizable transport cages, as appropriate. Such transport must be discreet, such that hospital staff and patients are not aware of the transport, and are not exposed to allergens and/or body fluids from the transported animal(s).
- R. **Body Fluid, Tissue, and Device Collection.** Complete Appendix 2, 4, and/or 5, as appropriate for each collection, according to the columns checked in the table. Tail clipping performed for genotyping may be a surgical procedure (detailed in Appendix 5) or a non-surgical antemortem tissue collection (detailed in Appendix 4), depending on the age of the animal and the amount of tissue removed. Do not include tissue that is removed and discarded (e.g., ovaries removed in ovariectomy).
- S. **Surgery.** “Surgery” includes any major or minor, survival or non-survival surgical procedure.
- T. **Endpoint criteria.** *US Government Principles* (Principle VI) require that “animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.” The endpoint criteria specified should include both general criteria that apply to animals that get sick unrelated to any experimental procedures, and criteria specific to this protocol. Examples of appropriate criteria that should be considered include weight loss to less than a specified percentage of initial or expected body weight, anorexia for longer than a specified allowable duration, tumor size greater than a specified size or total tumor burden greater than a specified percentage of body weight, the presence of health problems refractory to medical intervention, and severe psychological disturbances. For genetically modified animals to be newly generated on or for this protocol, the possibility of unexpected phenotypic changes should be addressed in the endpoint criteria (*Guide*, p. 28-29). Other species-specific criteria should also be considered, and provisions should be made for addressing unexpected pain or distress. (The *Guide*, p. 5, requires “veterinary consultation ... when pain or distress is beyond the level anticipated” in the ACORP “or when interventional control is not possible’.)
- U. **Termination or removal from the protocol.** The disposition of each animal on this protocol must be specified. Transfer of animals to other protocols, and each method of euthanasia that may be used, must be specifically approved by the IACUC.

Check each method that may be used on this protocol and provide the specific information requested for each. If more than one version of any given method may be used (e.g., overdose by either of two different anesthetics), copy the relevant row of the table as needed for the additional versions.

For euthanasia by CO<sub>2</sub> narcosis, the *AVMA Guidelines on Euthanasia* (p. 9) require that death be verified and a secondary method of euthanasia be applied if the animal is not yet dead when it is removed from the chamber. Enter the method to be used to verify death, and enter a secondary physical method that will be used if necessary.

Indicate how each method of euthanasia is classified according to the recommendations of the latest *AVMA Guidelines on Euthanasia* (acceptable, conditionally acceptable, or unacceptable). If you are unsure of the classification of any of the methods of euthanasia that will be used on this protocol, contact your Attending Veterinarian or IACUC for guidance.

1. For each of the methods that is designated “Conditionally Acceptable” by the AVMA, describe the conditions specified by the *AVMA Guidelines on Euthanasia* and document how these conditions will be met.
2. For each of the methods that is designated “Unacceptable” by the AVMA, *PHS Policy* (IV.C.1.g) requires that the investigator give the scientific reason(s) that justify this deviation from the *AVMA Guidelines on Euthanasia*.
3. List all research personnel who will perform euthanasia on animals on this protocol, and do not include VMU animal care personnel who will perform routine euthanasia as a service. If VMU animal care personnel are to perform highly specialized euthanasia techniques specific to this protocol, it may be appropriate to include them as members of the research staff.

If any of the personnel require training on any of the methods they will be expected to perform, identify the individuals and explain how they will be trained before being permitted to perform the euthanasia procedure(s) without supervision.

4. Regardless of whether any animals are expected to die other than by euthanasia, provide instructions for the animal care staff in case an animal is found dead (including during any post-procedural recovery period).
  - a. Describe what should be done with the carcass. Include whether the carcass should be refrigerated or frozen for later examination by the research staff, and provide any special instructions to be followed for the protection of the staff, in the case of animals that have been treated with hazardous materials. If no special instructions are required, and the carcass may be handled according to a local SOP, enter “according to local SOP” and enter the information requested about the SOP in the table in Item Y.
  - b. Provide instructions about contacting a member of the PI’s staff in case an animal is found dead. If there is no need to contact the PI’s staff immediately, describe the routine notification procedures followed by the VMU. If those procedures are detailed in a local SOP, enter “according to local SOP” and enter the information requested about the SOP in the table in Item Y.

- V. **Special Procedures.** Special procedures include both special husbandry and other non-husbandry procedures that are required by the experimental design. Details about these procedures must be provided in SOP(s) or elsewhere in this ACORP (surgical procedures are documented in Appendix 5, and need not be included here). Refer to Appendix 6, “Special Husbandry and Procedures” for the level of detail and specific information that are required.

Examples of special husbandry include non-standard ...

methods for monitoring animal health,  
diets,  
caging,  
environmental conditions such as lighting cycles or temperatures,

enrichment, and  
means of identification.

Examples of other procedures include:

restraint practices such as chairing of non-human primates,  
application of noxious stimuli,  
forced exercise,  
behavioral conditioning,  
total body irradiation, and  
radiography or other imaging procedures.

For each special procedure detailed in an approved SOP, identify the SOP and enter the information requested about the SOP into the table in Item Y.

For each special procedure detailed in the main body of this ACORP, specify the Item(s) in which the details are given.

For each special procedure not detailed in an approved SOP or elsewhere in the main body of this ACORP, check "Appendix 6" in Item Y, and complete and attach Appendix 6, "Special Husbandry and Procedures".

- W. Consideration of Alternatives and Prevention of Unnecessary Duplication.** This item addresses minimizing the harm/benefit (*AAALAC FAQs*, C.3; *Guide*, p. 27) to be derived from this work by decreasing the potential for causing pain or distress in the research animals. *USDA APHIS Animal Care Policies* (#12) defines "alternatives or alternative methods ... as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research", and addresses the requirement for a written narrative about how alternatives to painful and distressful procedures were considered (AWAR, §2.31(d)(1)(ii)). VA Policy (1200.07, par. 8.f(2)(a)3) requires that the IACUC review documentation that alternatives have been considered for each of the potentially painful or distressing animal procedures proposed. Complete items W.1 through W.5 below, keeping copies of computer database search results in your files to demonstrate your compliance with the law if regulatory authorities or the IACUC should choose to audit your project.
1. The AWAR (§2.31(d)(1)(ii-iii)) require investigators to consider less painful or less stressful alternatives to each potentially painful or distressing procedure, and provide assurance that proposed research does not unnecessarily duplicate previous work. Perform one or more database searches to meet these mandates unless compelling justifications can be made without doing so. You must provide complete information in the first five columns of the table to comply with *USDA APHIS Animal Care Policies* (#12).
  2. Replacement refers to the use of non-animal systems (for example, computer, mechanical, or chemical models) or *in vitro* techniques instead of animals, use of non-mammalian species instead of mammalian species, and use of less-sentient mammals for more-sentient mammals.
  3. Reduction refers to the use of smaller numbers of animals to obtain scientifically valid results. This is typically achieved by optimizing the experimental design – for example, by minimizing the number of control groups needed, by minimizing uncontrolled variability by collecting paired data, by maximizing the amount of data collected from each animal, and/or by using more sophisticated measuring techniques or more sensitive equipment to improve precision so as to minimize the group sizes needed.
  4. Refinement refers to use of approaches that lessen or eliminate pain or distress in the animals that are used. This includes (1) choosing procedures that prevent or relieve pain or distress

likely to be associated with the experimental design, (2) setting the earliest possible endpoints for the experiments, (3) appropriate use of analgesics, anesthetics, and tranquilizers, including selection of better agents (more effective, with fewer or less severe potential side effects) as they become available, (4) improving post-surgical care with new technology as it becomes available, and (5) special husbandry such as providing softened food after procedures likely to cause discomfort with swallowing, soft bedding, easier access to food, or environmental enrichment, as appropriate.

5. The proposed research cannot be approved if it unnecessarily duplicates previous work.

**X. Other Regulatory Considerations.**

1. **Controlled drugs** – Include in Appendix 3 all controlled substances to be administered on this protocol, and check “Appendix 3” in Item Y, below.
  - a. VA policy (1108.01, par. 20.e; 0730, par. 6.b) requires all drugs classified as controlled substances by the DEA to be stored routinely under double lock, and be accessible only to authorized personnel. If any substances will NOT be stored this way, an explanation must be given of how they will be stored and secured, and why this is necessary.
  - b. VA policy (1108.01, par. 20.a) requires that all controlled substances that are used on VA property must be ordered through and received by the local VA pharmacy prior to issue for research use. This means for example, that VA policy does not permit any controlled substances obtained through an affiliate institution to be brought onto VA property to be administered to animals in the VMU.

If the controlled substances will only be used at non-VA locations, 1108.01 states that, “the local Chief of Pharmacy Services must be consulted to determine whether controlled substances are to be obtained through the VA pharmacy.”

If any controlled substances that are to be used on VA property will NOT be procured through the local VA pharmacy, please explain how they will be procured and why this is necessary.

2. **Human patient care equipment or procedural areas.** Human patient care equipment or procedural areas may be used for animal studies only if approved by the officials responsible for the patient care equipment and space, and if documented in Appendix 7.
3. **Explosive agents.** These include explosive anesthetics and any other potentially explosive agents to be used in the work with the animals on this protocol. Each of these must be documented in Appendix 8 as well as in Appendix 3.

- Y. Summary of Attachments.** The attachments that are required depend on the protocol, and on local policies. This section summarizes the documents that apply to this protocol.

**Appendices.** Each Appendix that is required according to the responses given in the Items above must be completed and attached to this protocol. Do not attach blank appendices that are not applicable to this ACORP. Check with your IACUC as to whether completion of Appendix 1, “Additional Local Information”, is a local requirement.

Appendix 1, “Additional Local Information”

Appendix 2, “Antibody Production” (see Item O)

Appendix 3, “Biosafety” (see Items P, X.1 and X.3)

Appendix 4, “Ante-mortem Specimen Collection” (see Item R)

Appendix 5, “Surgery” (see Item S)

- Appendix 6, “Special Husbandry and Procedures” (see Item V)
- Appendix 7, “Use of Patient Care Equipment or Areas for Animal Studies” (see Item X.2)
- Appendix 8, “Use of Explosive Agent(s) within the VMU or in Animals” (see Item X.3)
- Appendix 9, “Departures from “Must” and “Should” Standards in the *Guide*”

**Standard Operating Procedures (SOPs).** Each of the SOPs referred to elsewhere in this ACORP must be listed in the table provided, including the following information:

Item. Identify the Item (by letters and numbers, as applicable) in which the SOP is referenced. Items that commonly refer to SOPs are shown, as prompts. Additional lines may be added to the table as needed.

SOP Title and ID. Identify each SOP by its title and any local identifier (such as an ID number). Use a separate line for each SOP.

Approval Date. Enter the date of the most recent IACUC approval of the SOP. 1200.07 (par. 7.c) requires that each SOP be reviewed and approved by the IACUC at least annually to remain in effect.

The full text of each of the approved and dated SOPs referenced in the ACORP must be uploaded onto the JIT management website when the ACORP is submitted for Just-in-Time processing before VA funding support is released. Please see your local research administrators for instructions regarding whether to attach copies of the SOPs when submitting the ACORP for local IACUC review.

- Z. **Certifications.** The typed names and dated signatures certify agreement with the terms of the ACORP, and are required as shown below for the Main Body and each of the attached Appendices for any ACORP that is to be submitted to VA Central Office for Just-In-Time approval prior to release of VA funding to support the work. Do not include signatures for any Appendices that do not apply, and which therefore should not be attached.
1. **Main Body of the ACORP.** The Principal Investigator(s) must certify the accuracy of the information presented in the ACORP, and the agreement to perform the work as described. The IACUC Chair and the Attending Veterinarian must sign to certify review and approval of the protocol. All signatures should appear on the final version of the ACORP, which incorporates all changes required by the IACUC for approval.
  2. **Appendix 2. Antibody Production.** No signatures required.
  3. **Appendix 3. Biosafety.** The Principal Investigator(s) and the IACUC officials must certify their agreement to ensure that personnel will be informed of the risks involved and provided with the SOPs and training needed to minimize their risks of exposure to hazardous agents. The Biosafety Official and the Radiation Safety Official must certify that the hazardous agents that are included on this protocol are correctly identified, and that their use has been approved by the relevant oversight committees or officials.
  4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.
  5. **Appendix 5. Surgery.** The Principal Investigator(s) must certify the accuracy of the description of the surgical procedures described in Appendix 5, and the agreement to perform and document the work as described.
  6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.
  7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.** The Principal

Investigator(s) must certify the accuracy of the information presented in Appendix 7, and the agreement to perform the work as described. The officials responsible for the use of the patient care equipment and/or areas for these studies must sign to verify their approval.

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.** The Principal Investigator(s) must certify the accuracy of the information presented in Appendix 8, and the agreement to perform the work as described. The officials responsible for overseeing the use of explosive agent(s) in this protocol must sign to verify their approval.
9. **Departures from “Must” and “Should” Standards in the *Guide*.** No signatures required.