# 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.

Always use the most recent version of the Main Body of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

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, 8th 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 I 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.
Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.
ONLY include the individual appendices that are relevant to the protocol being submitted for review.

**Header for Every Page**

Enter in the header, to identify each page of the ACORP:

PI's last name

Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record

Official Date of Approval – 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

A. **ACORP Status.**

1. Full Name of Principal Investigator -- For protocols to be submitted for secondary Just-In-Time review, the Principal Investigator on the ACORP must be the Principal Investigator of the grant proposal to be funded.

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 – For protocols to be submitted in support of a grant proposal, funding agencies typically require the title of the protocol to be the same as the title of the grant proposal.

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). 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.

   Department of Veterans Affairs -- This includes salary support for personnel involved in this research on VA official duty time, as well as specific project funding.

   United States Public Health Service (e.g. National Institutes of Health).

   Private or Charitable Foundation – Identify the Foundation:

   University Intramural Funds – Identify the University and Funding Component (e.g., give the name of the Department, Center, or Office providing the funding):

   Private Company – Identify the Company:

   Other – Identify Other Source(s):

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.

   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.l(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. $LD_{50}$, 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.l(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 $CO_2$ 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 (1*200.07,* 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, <u>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.</u> 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 <u>final</u> 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.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 1 – ADDITIONAL LOCAL INFORMATION
# (ACORP APP. 1 INSTRUCTIONS)
**VERSION 4**

This appendix is provided for use by the local IACUC, at its discretion, to collect any additional information that it requires.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the 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
- Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record to the protocol as a whole
- Official Date of Approval – 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 may provide instructions here for completion of this Appendix. Please consult with the IACUC for additional information.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 2 – ANTIBODY PRODUCTION
# (ACORP APP. 2 INSTRUCTIONS)
**VERSION 4**

These instructions provide detailed guidance on completing Appendix 2 of the ACORP, and are referenced to the numbers of the items in Appendix 2. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 2 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

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.

Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the 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

Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record to the protocol as a whole

Official Date of Approval – 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

1. **Immunization.** Immunization of animals is essential to the production of polyclonal antibodies, and is the first step in creating new hybridomas for production of monoclonal antibodies. Document the immunization protocol for any animal that will be immunized on this protocol. Describe any priming injection by entering a negative number for the immunization day (the number of days before the first injection of antigen), "N/A" for the antigen, and the description of the primer in the column for the adjuvant. For each primer, antigen, and adjuvant that is expected to cause pain or distress in the animals, explain why it is necessary to use this agent in this protocol. Each primer, antigen, and adjuvant administered to the animals should also be documented in Appendix 3.

2. **Survival Blood Collection.** Collection of blood from animals that are to survive the procedure requires consideration of the volumes of blood that can be safely removed, the possibility of volume replacement, and appropriate administration of anesthetics, tranquilizers, and/or analgesics. This blood collection should also be documented in Item R of the ACORP.

3. **Terminal Blood Collection.** Collection of blood by exsanguination requires appropriate management of the euthanasia, which should be described in Item U of the ACORP. This blood collection should also be documented in Item R of the ACORP.

4. **Harvesting Feeder Cells.** If cells must be harvested from donor animals to support the growth of hybridoma colonies for the antibody production on this protocol, the procedures performed on the donor animals must be described. The harvesting of feeder cells should also be documented in Item R of the ACORP, and the number of animals to be used for this must be included in Item I of the ACORP.

5. **Expansion of Hybridoma Cell Line(s) *in vivo*.** Use of animals to expand hybridoma cell lines so that antibody can be harvested from ascites fluid requires consideration of the effects of the injection and growth of the hybridoma cell line(s), and of the abdominal taps to be performed, on the host animals. See guidelines for monoclonal antibody production in "Working with the VA IACUC" (www.citiprogram.org). The animals used for this should be included in Item I of the ACORP, the priming agent and the hybridoma cells documented in Appendix 3, and the collection of ascites fluid included in Item R of the ACORP.

If any of the procedures described in this Appendix represent "departures" from the standards in the *Guide*, be sure to include those "departures" in Appendix 9. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 3 – BIOSAFETY
# (ACORP APP. 3 INSTRUCTIONS)
**VERSION 4**

These instructions provide detailed guidance on completing Appendix 3 of the ACORP, and are referenced to the numbers of the items in the appendix. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of this Appendix, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

Regulatory documents mentioned in the instructions are abbreviated as follows:

*Guide* – Guide for the Care and Use of Laboratory Animals, 8th 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)

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.
- Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the 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
- Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record to the protocol as a whole
- Official Date of Approval – 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

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Include ALL materials administered to animals on this protocol, such as, but not limited to, radioisotopes, chemicals, drugs (standard clinical agents as well as test agents, and all controlled substances listed in Item X.1 of the main body of the ACORP), infectious agents, biomaterials, prosthetic devices, and cells, tissues, or body fluids. For each material, enter "X" in the ( ) for each of the descriptions that applies to the nature of the material. Some materials may fall into more than one category, and will be addressed in more than one corresponding Item below.

2. **Summary of How Materials will be Administered.** Provide the details of how each of the materials will be administered and what the effects of the administration are expected to be. Specify where in the ACORP further details about the administration of each material are provided, indicating whether the details are in the Main Body of the ACORP or an Appendix (enter

the Appendix number), and entering the letter or number of the Item. Indicate whether the animals will be anesthetized, sedated, or chemically tranquilized for the administration of each material.

OLAW requires that only pharmaceutical grade compounds be administered to animals unless the use of non-pharmaceutical grade compounds is justified by scientific necessity and the lack of availability of an acceptable veterinary or human pharmaceutical grade compound (*OLAW FAQs*, F.4). Complete listings of the compounds approved by the FDA for administration to humans or animals are available on-line: http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm for humans, and http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847 for animals. Mark with a * each material, diluent, or vehicle to be administered to the animals on this protocol that is not pharmaceutical grade. For each of these, provide the justification for using a non-pharmaceutical grade compound, and describe how it will be ensured that the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, formulation, and pharmacokinetics of the material will be suitable for use in the animals (*Guide*, p. 31). Note that OLAW specifically advises that cost-savings alone do not adequately justify the use of non-pharmaceutical grade compounds in animals.

3. **Anesthesia, Sedation, or Tranquilization.** Anesthesia, sedation, or tranquilization may be important to ensure the safety of the personnel when administering hazardous materials. Complete 3.a. and 3.b. below:

   a. For each material to be administered with the animals under anesthesia, sedation, or chemical tranquilization -- identify the anesthetic, sedative, or chemical tranquilizer, and detail the dose and volume, and route of administration to be used. These agents should also be listed in Item 1 of this appendix.

   b. For each material to be administered without anesthetizing, sedating, or chemically tranquilizing the animals, explain why these are not necessary, or cannot be provided, and describe any alternate methods of restraint that will be used.

4. **Toxic Agents.** Examples include toxic chemical and pharmacologic agents, many cancer therapeutic agents with cytotoxic properties, known or suspected mutagens, carcinogens, teratogens, and DNA-binding agents. List in this table each agent that is marked as a "toxic agent" in the table in Item 1, above, and enter "X" in the ( ) for each property that applies:

   a. Mark as "mutagen" each agent that is a suspected or known mutagen.

   b. Mark as "carcinogen" each agent that is a suspected or known carcinogen.

   c. Mark as "teratogen" each agent that is a suspected or known teratogen.

   d. For each agent, indicate its status as a "select agent" (www.selectagents.gov/Regulations.html):

      - It is not on the CDC-USDA list of "select agents" that might have uses in bioterrorism

      - It is a "select agent", but the quantities to be used on this protocol fall below the threshold minima specified by select agent legislation, so the use of this agent is not subject to the requirements of that legislation.

      - It is a "select agent" that requires registration/approval before work with it may begin. "Select agent" legislation requires registration with CDC and/or USDA, and VA requires that VACO approval for the use of this agent be secured, before studies with it begin. Ask your research office to contact the VACO Biosafety Officer for specific instructions about registering and securing approval. Enter the information requested below the table with

regard to the registration and approval. Copy the lines shown for each additional agent to be documented.

e. Mark as "Other" each agent that has toxic properties other than the ones listed (e.g., corrosive agents, poisons, etc.) and specify the properties

5. **Infectious Agents.** These include, for example, bacteria (including rickettsia), viruses, fungi, protozoa, and prions. Copy into the table the name and BSL number of each agent that is marked as an "infectious agent" in the table in Item 1, above, and provide the information requested,

   a. Specify the ABSL level of the minimum measures that will be applied in handling each agent. The practices, safety equipment, and facilities that correspond to each ABSL level are described in Biosafety in Microbiological and Biomedical Laboratories, 5th edition (December 2009), available at www.cdc.gov/biosafety/publications/bmbl5/.

      ABSL1 is the recommended minimum for BSL1 agents.
      ABSL2 is the recommended minimum for BSL2 agents.
      ABSL3 is the recommended minimum for BSL3 agents.
      ABSL4 is the recommended minimum for BSL4 agents.

      It the handling of any agent is to be according to an ABSL level less than the BSL level of the agent, enter the justification for this as requested below the table

   b. Indicate whether an antibiogram, anti-viral drug sensitivity screen, or other appropriate drug sensitivity panel is available for each of these agents, to assist physicians in selecting proper therapy if human infection occurs. If "Yes", describe briefly.

   c. For each agent, indicate its status as a "select agent" (www.selectagents.gov/Regulations.html):

      - It is not on the CDC-USDA list of "select agents" that might have uses in bioterrorism

      - It is a "select agent", but the quantities to be used on this protocol fall below the threshold minima specified by select agent legislation, so the use of this agent is not subject to the requirements of that legislation.

      - It is a "select agent" that requires registration/approval before work with it may begin. "Select agent" legislation requires registration with CDC and/or USDA, and VA requires that VACO approval for the use of this agent be secured, before studies with it begin. Ask your research office to contact the VACO Biosafety Officer for specific instructions about registering and securing approval. Enter the information requested below the table with regard to the registration and approval. Copy the lines shown for each additional agent to be documented.

6. **Biological Agents.** These include, for example, antigens, serum, cell lines, tissue, and nucleic acid. List in the table each agent that is marked as a "biological agent" in the table in Item 1, above, and describe how the material will be screened to make sure that it does not harbor other agents that could infect other laboratory animals or personnel.

7. **Radioactive Agents.** List in the table each agent that is marked as a "radioactive agent" in the table in Item 1, above, and specify the radioactive isotope involved. Identify the individual who has been given permission to utilize the isotope(s) indicated, and identify the committee that has approved the use (e.g., Radiation Safety Committee or other equivalent committee).

8. **Recombinant nucleic acid and recombinant infectious agents.** These include both isolated recombinant nucleic acid and recombinant infectious agents. List in the table each agent that is

marked as “contains recombinant nucleic acid” in the table in Item 1 (Recombinant infectious agents should also be marked as “infectious agents” in the table in Item 1, and addressed in Item 5, above.), and indicate which of the following conditions applies:

- This work is subject to, and will be conducted according to, the animal research guidelines included in the latest version of the publication, *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, and the Biosafety Committee and veterinarian will be consulted to ensure compliance.
- The recombinant constructs are exempt from the animal research guidelines included in the latest version of the publication, *NIH Guidelines*.

9. **Potential for Pain or Distress**. Include any of the agents listed in Item 1 that is expected to have effects that are potentially painful or distressing to the animals (include even if measures will be taken to prevent animals on this protocol from actually experiencing the pain or distress). Focus on the effects of the agents, and not on the potential pain or distress associated with the procedures involved in administering them, which are addressed elsewhere in the protocol. Describe the nature of the potential pain and/or distress expected, and describe the measures that will be taken to alleviate that potential pain and/or distress. These measures may include not only administration of pharmacological anesthetics, analgesics, tranquilizers, or sedatives, but also appropriate special husbandry procedures (describe in Appendix 6). Any agents that will be administered to alleviate potential pain and/or distress should also be listed in the table in Item 1 of this appendix.

10. **Protection of Animal Facility Staff from Hazardous Materials.** This table addresses specifically the protection of members of the staff of the Animal Facility from the hazardous agents to be used on this protocol. (Make sure that protection of the research personnel from the risks associated with each of these agents is addressed in Item G of the main body of the ACORP.)

    a. Complete the table.

       “Hazardous Agents” – include each agent listed in Item 4, 5, 6, 7, of this Appendix.

       “Approving Committee or Official” – identify the specific committee or official (e.g., Safety, Biosafety, or Radiation Safety) that has approved the use of the hazardous agent on this protocol.

       “Institution (VA or affiliate)” – indicate “VA” or give the name of the affiliate institution that is represented by the approving committee.

       “Animal Facility Staff Members at Risk” – identify by name each individual member of the animal facility staff who is at risk of exposure to the hazardous agent (e.g., via contact with treated animals, or with contaminated bedding).

    b. Include what information will be posted, and where, and summarize any specific training to be provided.

11. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 4 – ANTEMORTEM SPECIMEN COLLECTION (ACORP APP. 4 INSTRUCTIONS)

**VERSION 4**

These instructions provide detailed guidance on completing Appendix 4 of the ACORP, and are referenced to the numbers of the items in Appendix 4. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 4 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

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.
- Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the header for the Main Body of the ACORP to which it applies, to identify each page of this Appendix with that ACORP:

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1. **Summary.** Include in this Appendix each body fluid, tissue, or device that is listed in Item R of the main body of the ACORP and marked in the last column of Item R as "Other collection". Post-mortem collection, blood collection associated with antibody production (detailed in Appendix 2), and collections that are part of the surgical procedures detailed in Appendix 5, need not be included here.

   Remember that the procedure(s) for each collection listed here should be described in Item C of the main body of the ACORP (including how the instruments will be sterilized and the method of hemostasis to be used).

   Indicate "yes" under "Anesthesia" if any measures will be taken to prevent pain or distress during collection of the specimens (including both administration of pharmacological anesthetics, analgesics, or tranquilizers, and use of non-pharmacologic methods such as cooling).

   For the "Amount Collected", enter the following:

- For fluids, enter the volume (ml). For blood samples, also give the % of total blood volume represented by each sample (total blood volume may be estimated for rodents and rabbits as 6% of lean body mass). Assume that 1 ml of blood weighs 1 gram.
- For any tail snips that will not be documented as surgical procedures in Appendix 5, enter here the length of tail to be removed (mm).
- For other solid tissues, enter the mass (g) or volume (ml)..

For "Volume Replacement", enter "N/A" for samples of solid tissues. Enter "yes" or "no" for each fluid specimen collected.

2. **Use of Anesthetics, Tranquilizers, or Analgesics**.

   For collection of specimens without application of any measures to prevent pain or distress, provide details of why such measures are not appropriate for this protocol, and describe how the animals will be restrained, if necessary.

   For collection of specimens that involves application of measures to prevent pain or distress, describe the measures to be taken. Any agents that will be administered should be included in Appendix 3.

3. **Volume Replacement for Fluid Collections.**

   For collection of fluid samples WITHOUT replacement of fluid volume, explain why the volume will not be replaced (give the calculations that show that the volumes removed are so small that replacement is not necessary, provide the scientific reasons, etc.).

   For collection of fluid samples WITH replacement of the removed volumes, describe the replacements that will be provided (their composition, volume, and route of administration). Be sure to include the replacement fluids in Appendix 3.

4. **Monitoring the animals.** The animals must be monitored after each collection of specimens to ensure that they recover appropriately. Include the methods of monitoring to be used, and how long the animals will be monitored specifically for recovery from specimen collection. Describe the criteria that will be considered indicators of the need for intervention, and describe the corresponding interventions to be made (e.g., administration of analgesics, application of pressure, euthanasia).

# INSTRUCTIONS FOR COMPLETION OF THE **ACORP APPENDIX 5 – SURGERY (ACORP APP. 5 INSTRUCTIONS)**

**VERSION 4**

These instructions provide detailed guidance on completing Appendix 5 of the ACORP, and are referenced to the numbers of the items in Appendix 5. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 5 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

Regulatory documents mentioned in the instructions are abbreviated as follows:

*Guide* – Guide for the Care and Use of Laboratory Animals, 8th 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)
*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.
Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

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1. **Surgery Classification.** List as a single "surgery" all of the procedures to be performed in a single session, from induction of, to recovery from, anesthesia. If more than a single species is covered by this protocol, list the surgeries separately for each species, and identify which species will undergo each surgery.

   Terminal surgery is any surgery during which the animal is euthanatized without being allowed to recover from anesthesia.

Survival surgery is any surgery after which the animal will be allowed to regain consciousness. This requires that provisions be made for recovery from anesthesia, and for post-operative care.

Major survival surgery is defined in the *Guide* (p. 117-118) as a surgical procedure in which a major body cavity is penetrated and exposed, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection. Examples of major surgeries include thoracotomy, craniotomy, joint replacement, and limb amputation.

One of multiple surgeries is each survival surgery (including any minor surgical procedures that may induce substantial post-procedural pain or impairment, as well as any major surgeries) that will be performed as part of this protocol, in addition to any other such surgery (on this or another protocol) on the same individual animal. For each surgery that is one of multiple surgeries to be performed on a single animal, complete items 1.a and 1.b, below the table, to describe why it is necessary to perform the multiple surgeries on a single animal, the time interval(s) between successive surgeries, and the rationale for the time intervals indicated. The multiple surgeries may be repetitions of the same surgery, or more than one different surgery. NOTE: If an animal regulated by USDA is to undergo survival surgery on this protocol in addition to undergoing surgery on a separate unrelated research protocol, the IO may be required to submit a request to the USDA for pre-approval (*Guide*, p 30; *OLAW FAQs*, F.9; *USDA APHIS Animal Care Policies*, #14).

2. **Description of Surgeries.** Describe each surgery in enough detail for the IACUC reviewers to be able to evaluate what the effects on the animals will be. (Details about pre-operative preparation, anesthesia, and post-operative recovery will be requested in Items 5, 6, and 7, respectively, so it is sufficient to provide only summaries of these here.)

3. **Personnel.** Include any research personnel and any VMU animal care personnel who will participate in performing any of the surgeries listed in Item 1, above. Each of these individuals, and their qualifications for their roles in the surgeries should be included in Item E of the main body of the ACORP.

4. **Location of surgery.** Provide the building and room number(s) where each surgical procedure listed in Item 1, above, will be performed. In general, any survival surgical procedure should be conducted in a dedicated surgical facility or space (*Guide*, p. 116 and 118). Major survival surgery on nonrodents must be performed only in dedicated surgical facilities (*USDA APHIS Animal Care Policies*, #3). If local policy allows, survival surgery may be performed on rodents in dedicated surgical space, which may be in animal procedure rooms or laboratories (*Guide*, p. 144). For each surgery that will be conducted in space other than in a dedicated surgical facility, provide the justification below the table. NOTE: Use Appendix 9 to document each "departure" from a "should" standard in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.

5. **Pre-operative protocol.**

   a. **Pre-operative procedures.** For each surgery listed in Item 1, above, mark each procedure that is to be performed before induction of anesthesia, to prepare the animal(s) for surgery, and enter the information requested. Medications to be administered pre-operatively will be documented separately, in Item 5.b, below.

      Fast – Pre-operative fasting is standard for larger animals, but is rarely required in rodents or rabbits. If fasting is necessary for this protocol, enter the duration of the fasting period.

      Withhold water – Withholding water pre-operatively is required for some procedures in some species. If it is necessary for this protocol, enter the length of time that water will be withheld.

Intravenous catheter placement -- Indicate the site(s) where any intravenous catheter(s) will be placed before surgery begins, for vascular access during surgery. Do not include here any catheters to be implanted as part of the surgery.

Other – Describe any other preparatory procedures to be performed on the animals before induction of anesthesia.

b. **Pre-operative medications.** Include all sedatives, tranquilizers, and other agent(s) to be used for induction of anesthesia, as well as any antibiotics or other pre-treatments to be administered in preparation for surgery, regardless of whether they are administered immediately before, or over longer periods before, preparation of the surgical site on the animal. Each of these agents should also be included in Item 1 of Appendix 3.

c. **Pre-operative preparation of the surgical site.** Include details about hair removal (such as whether clippers or chemical hair removal products will be used, and how the clipped hair or chemicals will be cleaned away), skin disinfection, and the use of surgical drapes.

6. **Intra-operative management.** Provide details of how the animals will be maintained during surgery.

a. **Intra-operative medications.** Include all anesthetic agents, paralyzing agents, fluids, and other pharmaceuticals that will be administered to the animal during surgery. Also include any experimental pharmaceuticals that will be administered during surgery. Each of these should also be included in Item 1 of Appendix 3.

Type "X" in the "Paralytic" column for each agent that is a neuromuscular blocking agent. Federal regulations prohibit the use of these agents for surgery unless other appropriate anesthetic agents are used to induce a surgical plane of anesthesia. Paralytics do not provide any pain relief, but animals are unable to respond physically to pain because motor control is blocked. **Very Important:** If any paralytics are to be administered, explain below the table why their use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.

b. **Intra-operative physical support.** Provide details about the physical support that will be provided during surgery, including information about, for example, the type of heating pads to be used, and how appropriate positioning of the animal will be maintained.

c. **Intra-operative monitoring.** Include the variables that will be monitored (e.g., mucous membrane color, heart rate, blood pressure, motor responses), the criteria for adjusting the level of anesthesia or other support, and the additional measures that may be taken, as appropriate.

7. **Survival surgery considerations.**

a. Survival Period – Enter how long the animal(s) are expected to survive after surgery (number of hours, days, weeks, etc.). For animals that will undergo multiple repetitions of any one survival surgery, enter the length of time after the last repetition before euthanasia.

Measures for Maintaining Sterility – Check each column that applies. Provide a description of any "other" measures to be taken.

b. Immediate post-operative support -- List all measures that will be taken to support each animal immediately after surgery, until it recovers sufficiently from anesthesia to ambulate without danger to itself. This commonly includes the use of circulating warm water heating pads and blankets, administration of fluids, etc.

c. Post-operative analgesia. Unless specifically justified to the satisfaction of the IACUC, you are obligated to routinely provide post-operative pain relief for all vertebrate animals undergoing survival surgery (*USDA APHIS Animal Care Policies*, #3; VA policy makes this applicable to rodents as well). Identify the analgesic(s) to be administered, and describe the protocol(s) of administration. Each of these should also be included in Item 1 of Appendix 3.

   If post-operative analgesics will be <u>withheld</u> from the animals after any of the surgeries on this protocol, enter "none" in the "Agent" column for that surgery, and provide the justification for withholding analgesics, in the space below the table.

d. Other post-operative medications. Describe the administration of any other medications (including, but not limited to, fluids, antibiotics, anti-coagulants, and other pharmacological agents) as part of post-operative care. (Each of these should also be included in Item 1 of Appendix 3.) Include as "post-operative care" all care that is specifically related to recovery from surgery.

e. Post-operative monitoring. The experience of each of the individuals responsible for post-operative monitoring should be listed in Item E of the main body of the ACORP. (<u>The names and after-hours contact information for these individuals must be provided to the VMU staff for use in case of any emergencies</u>.)

   (1) Immediate post-operative monitoring – Describe how each animal will be monitored until it recovers sufficiently from anesthesia to ambulate without danger to itself.

   (2) Post-operative monitoring after the immediate post-operative period – Describe how each animal will continue to be monitored until it fully recovers from surgery. Include the personnel who will be responsible for the post-operative monitoring and care after-hours, and on weekends and holidays.

f. Post-operative consequences and complications.

   (1) Surgery may have intended consequences for the health of the animals, or may be associated with common post-operative complications. This protocol must address how each of these will be managed or treated.

   (2) Animals may not recover as expected from surgery, so the criteria for euthanasia to prevent suffering must be specified. The endpoint criteria given here should be specific to the post-operative condition of the animals. The general endpoint criteria given in Item T of the main ACORP apply in any case.

   (3) In case of any emergency medical situation, VMU personnel will attempt to contact research personnel on this protocol, according to the standard operating procedures of the VMU. If none of the research personnel can be reached, VMU personnel are required to provide treatment as directed by the responsible veterinarian, consistent with currently accepted standards of veterinary care and the approved ACORP, to alleviate unacceptable levels of pain or distress. Provide as much guidance as possible to allow the VMU personnel to avoid any drugs or classes of drugs that would invalidate the data that could be collected from the animal. It is understood that, if the condition of the animal is such that these agents cannot be avoided, the animal will have to be removed from the study and will be euthanatized instead.

g. Maintenance of post-surgical medical records. The PI is responsible for ensuring that accurate, daily, post-surgical written medical records are maintained and accessible to the IACUC and to all research personnel and veterinary staff involved in the care and use of the

animals on this protocol. The PI must therefore assign at least one individual (this may be the PI) to maintain the records, and must identify an accessible location where the records will be kept. Each individual involved in maintaining the records should be listed in Item E of the main body of the ACORP (Items E.1 and E.2 for research personnel, and Item E.3 for VMU staff).

8. **Certification.** The PI must sign the certification in Item Z.5 of the main body of the ACORP.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 6 – SPECIAL HUSBANDRY AND PROCEDURES (ACORP APP. 6 INSTRUCTIONS)

**VERSION 4**

These instructions provide detailed guidance on completing Appendix 6 of the ACORP, and are referenced to the numbers of the items in Appendix 6. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 6 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

Regulatory documents mentioned in the instructions are abbreviated as follows:

*Guide* – Guide for the Care and Use of Laboratory Animals, 8th ed., 2011
*US Government Principles* – US Government Principles for the Utilization and Care of Vertebrate Animals Used I Testing, Research, and Training

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.
- Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

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1. **Description of Procedures.** The details of each special procedure to be included in this protocol, regardless of whether it will be carried out by the animal care staff or research staff, must be documented in a formal SOP, elsewhere in this ACORP, or in this Appendix, as indicated in Item V of the main body of the ACORP.

   Examples of special husbandry include housing under temperature extremes, food or water restriction, dietary manipulations, special housing/caging, modified light cycle, special health monitoring, wound management measures to be taken to minimize the chances of chronic infections where implanted devices penetrate the skin, and unusual means of identification.

Special procedures may also include non-husbandry procedures involving features such as prolonged physical restraint, noxious stimuli, forced exercise, behavioral manipulations, total body or local irradiation, and radiography or other imaging.

The details of special procedures that are documented in the local SOP manual or elsewhere in this ACORP need not be copied again here, as long as the level of detail and the specific information provided in the SOP or elsewhere in this ACORP are at least as much as requested here.

Include in the description of each special procedure not only what will be done, but also the duration of each procedure, how frequently it will be repeated in any one animal, what potential effects on the animal are expected (including both the intended effects and potential side effects such as skin lesions related to the use of restraint devices), how side effects will be addressed, and why the special procedure is necessary.

Be sure to document in Appendix 9 any of these procedures that represent "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.

2. **Personnel.** Identify the individuals who will carry out the special procedures, and the individuals who will be responsible for monitoring the animals specifically with regard to their condition during the procedures and during any post-procedural recovery period. Individuals responsible for routine monitoring unrelated to the special procedures need not be included here. Note that the experience of each of the individuals listed in the table should be described in Item E of the main body of the ACORP.

3. **Potential Pain or Distress.** In considering the potential for pain and/or distress that the animals may experience as a result of the special procedure(s) described in Item 1, above, it is important to keep in mind that the *US Government Principles* (IV) specifically state that "investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals".

   If potential pain and/or distress is expected unless measures are taken to prevent or alleviate it, provide a description of the potential pain and/or distress (the table will expand as needed), and indicate whether measures will be taken to relieve the pain and/or distress.

   a. If measures will be taken to prevent or alleviate the expected potential pain and/or distress, complete the table to describe the administration of analgesic(s) and/or stress-relieving agents (Each of these should be included in Item 1 of Appendix 3), and describe any other measures to be taken to address the potential pain and/or distress (e.g., training with positive reinforcement to promote adaptation to restraint devices, padding to prevent skin lesions).

   b. If no analgesic(s) or stress-relieving agents will be administered or other measures taken to prevent or alleviate the expected potential pain and/or distress, explain why this is necessary.

4. **Monitoring.** Describe the methods to be used in monitoring the animals specifically for effects of the special procedures, both while they are undergoing each of the procedures and afterwards. Include the frequency of monitoring, the variables to be monitored, and a description of the written records to be maintained. Then define the criteria for removal of individual animals from participation in any of these procedures because of pain or distress (e.g., if an individual animal fails to adapt to a restraint device). The table will expand as needed.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 7 -- USE OF PATIENT CARE EQUIPMENT AND/OR AREAS
# FOR ANIMAL STUDIES
# (ACORP APP. 7 INSTRUCTIONS)

**VERSION 4**

These instructions provide detailed guidance on completing Appendix 7 of the ACORP, and are referenced to the numbers of the items in Appendix 7. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 7 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

General Instructions:

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To check an item, type "X" inside the (  ) provided.

Define each abbreviation the first time it is used.

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1. **Full Name(s) of Principal Investigator(s).** Give the full name(s) of the Principal Investigator(s), who are responsible for the use of animals on this protocol.

2. **Equipment to be Used.**

   a. Identify the equipment – Describe the human patient care equipment and provide whatever additional identifying information is necessary to make it clear which piece(s) of equipment will be used on this animal protocol.

   b. Procedures to be performed – Specify the procedures that are to be performed on the animals, using this human patient care equipment.

   c. Describe the specific protocol to be followed to prevent contamination of the human patient care equipment by animal feces, urine, saliva, blood, or other body fluids, and to clean/sanitize the equipment before its subsequent use for human patients. The procedures used should be at least as thorough as the procedures established by the clinical facility for cleaning and

sanitizing the equipment between human patients.

3. **Human Patient Care Procedural Areas to be Used.**

   a. Location(s) -- Identify the location(s) of the human patient care area(s) in which animals will be used, specifying the building(s) and room number(s).

   b. Animal species to be studied or treated – Enter the species covered by this ACORP, so that this Appendix can be reviewed by officials responsible for the human patient care areas, without referring to other portions of the ACORP.

   c. Number of individual animals to be studied or treated – Enter the total number of animals to be studied or treated in human patient care areas on this protocol.

   d. Date(s) – Enter specific dates, or indicate the days of the week and the period of weeks over which animals will be studied or treated in the human patient care area(s).

   e. Time(s) of day – Specify the time(s) of day at which the animals will be studied or treated in the human patient care area(s), and address how these relate to the times of day at which human patients receive care in these areas.

   f. Procedures to be performed in these areas – Specify the procedures that are to be performed on the animals, in these human patient care areas.

   g. Protection and cleaning of patient care room surfaces – Describe the specific protocol to be followed to prevent contamination of the human patient care room surfaces by animal feces, urine, saliva, blood, or other body fluids, and for any cleaning/sanitizing necessary before subsequent use of the room for human patients. The procedures used should be at least as thorough as the procedures established by the clinical facility for cleaning and sanitizing the room between human patients.

   h. Benefits to VA patients – Address the potential value of this research to VA patients, which justifies the use of areas dedicated to care of human patients for research on animal subjects.

   i. Necessity for use of human patient care areas – Explain why the animal facility and research laboratory areas cannot be used for this work on research animal subjects, and the animals must be studied or treated in the human patient care areas.

   j. Animal transport – Transportation of animals through human clinical care areas must be discrete and secure. Corridors and elevators used by human patients must be avoided. Include descriptions of the transport enclosures (cages, carriers, etc.) and how they will be secured to prevent escape, any vehicles to be used, the planned route(s) for the transport, and any other precautions to be taken to minimize the likelihood that patients, visitors, or other non-research personnel will become aware of the animals.

   k. Preventing human patients and patient care personnel from being affected by the presence of the animals. Provide detailed descriptions of the measures to be taken to prevent patients and patient care personnel from becoming aware of the animals because of noises or odors, and the cleaning and disinfection protocols to be followed to protect them from exposure to allergens and transmission of zoonotic pathogens from the animals.

4. **Signatures.** Provide the signatures required on the signature pages (Item Z.7) of the main body of this ACORP. Note that the signatures required for use of human patient care equipment in the VMU or animal research areas are different from the signatures required for treatment or study of research animals in human patient care areas.

# INSTRUCTIONS FOR COMPLETION OF THE ACORP APPENDIX 8 -- USE OF EXPLOSIVE AGENT(S) WITHIN THE VMU OR IN ANIMALS (ACORP APP. 8 INSTRUCTIONS)

**VERSION 4**

These instructions provide detailed guidance on completing Appendix 8 of the ACORP, and are referenced to the numbers of the items in Appendix 8. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 8 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

General Instructions:

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Official Date of Approval – 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

1. **Full name(s) of Principal Investigator(s).** Give the full name(s) of the Principal Investigator(s), who are responsible for the use of animals on this protocol.

2. **Explosive agents to be used.** Identify each explosive agent to be used on this protocol, either within the animal facility (regardless of whether the agents will be administered to animals) or administered to animals (regardless of where the administration to the animals will be performed).

   a. Identify the explosive agents – Give all names used in this ACORP to refer to each agent, then describe the MSDS on file for each agent, including the primary name of the agent shown on the MSDS, the CAS registry number for the agent, and the location of the MSDS on file.

   b. Locations where the explosive agents will be used – Identify the building(s) and room number(s) where each of the agents will be used, indicating whether each location is within the VMU or outside of the VMU.

c. Procedure(s) to be performed – The use of explosive agents in the animal research facility is prohibited unless the IACUC and the Subcommittee on Research Safety (SRS) grant local approval. That approval should only be granted under exceptional circumstances, when scientific reasons preclude the use of non-explosive alternatives. Describe the reasons that this use of explosive agents should be approved.

d. Precautions to be taken to prevent explosions – Before approving the ACORP, the IACUC must ensure that all reasonable precautions to prevent explosions are to be taken. These precautions generally include, but need not be limited to, the following:

(1) Use of the agents only within a properly operating, ventilated safety hood.
(2) Locating and powering outside the hood any electrical equipment to be used with such agents.
(3) Storage only in an explosion-proof refrigerator or freezer.
(4) Provisions to ensure that all potentially explosive fumes have dissipated from animal carcasses and other objects before they are placed into storage.
(5) No disposal of empty containers or other items containing traces of any explosive agent by incineration or in receptacles for waste that is ordinarily incinerated.

e. Period of use. Define the period of time over which the explosive agent(s) may be used, by specifying the earliest and latest dates on which they may be used:

f. Animals that will be administered explosive agents – Describe any animals that will be administered any of the explosive agents listed in Item 2.a, above, noting the species, the range of average weights of individual animals to be treated, and the approximate number of animal subjects that will be administered the explosive agent(s). Note that any explosive agents to be administered to animals on this protocol must also be documented in Appendix 3.

3. **Personnel.** Identify each individual who will handle any of the explosive agents as part of this protocol, and describe the individual's training and experience with regard to use of explosive agents. Note that any of these individuals who will be involved in administering any of the explosive agent(s) to animals must also be included in Item E of the main body of the ACORP.

4. **Signatures.** Provide the signatures required on the signature pages (Item Z.8) of the main body of this ACORP.

# INSTRUCTIONS FOR COMPLETION OF THE
# ACORP APPENDIX 9 -- DEPARTURES FROM "MUST" AND "SHOULD" STANDARDS IN THE *GUIDE*
# (ACORP APP. 9 INSTRUCTIONS)
**VERSION 4**

These instructions provide detailed guidance on completing Appendix 9 of the ACORP, and are referenced to the numbers of the items in Appendix 9. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 9 of the ACORP, available at http://www.research.va.gov/programs/animal_research/, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

Regulatory documents mentioned in the instructions are abbreviated as follows:
- *Guide* – Guide for the Care and Use of Laboratory Animals, 8th 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

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.
- Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the 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
- Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record to the protocol as a whole
- Official Date of Approval – 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

*PHS Policy* (IV.B.3) requires that IACUC-approved departures from PHS Policy, including the provisions of the *Guide*, be reported to the Institutional Official in the report of each semiannual evaluation of the institutional animal care and use program. This appendix is to document the departures that have been approved by the IACUC for this protocol. The completed appendix may be copied for inclusion in semiannual reports.

Consider each instance in this protocol that requires care or use of animals in a way that does not meet the general standards described in the *Guide*. These are all considered "deviations", and most commonly appear in Items C.2.c, M, T, U, and V, and Appendices 2, 4, 5, 6, and 7 of the ACORP (procedures, husbandry, endpoint criteria, euthanasia, antibody production, antemortem specimen collection, surgery, transportation). Apply the series of test questions below to determine whether any

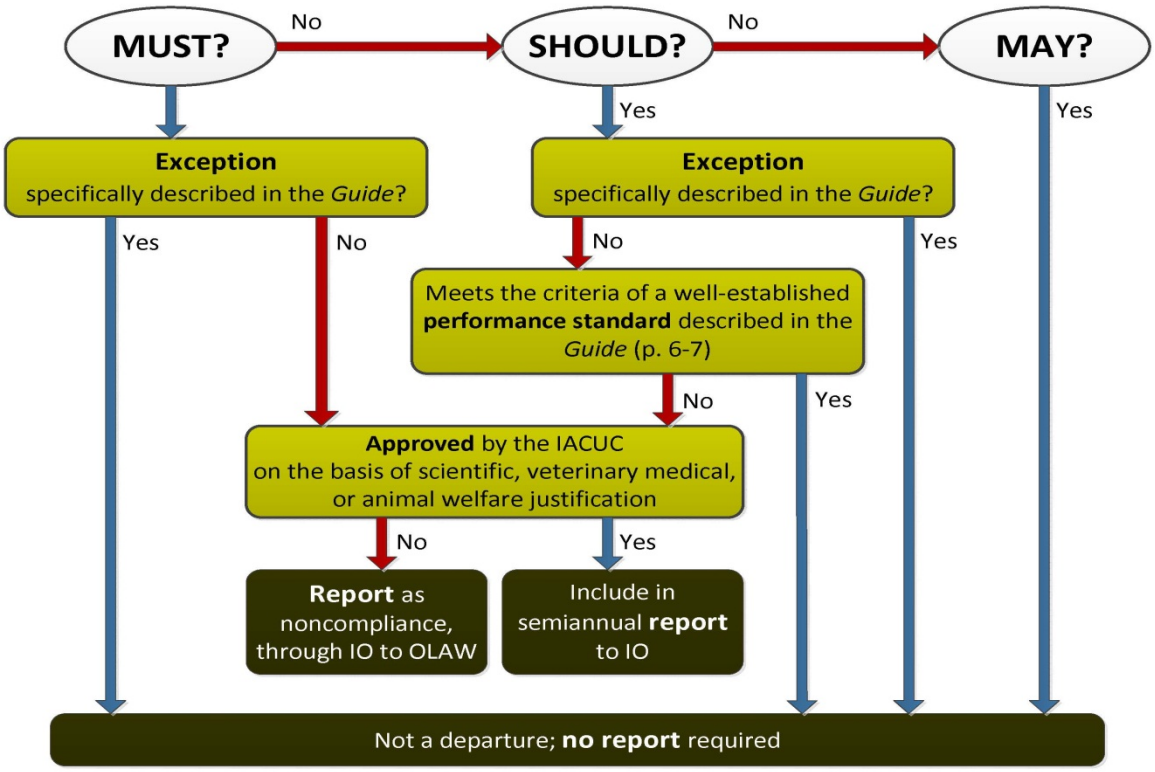
of the "deviations" are considered "departures" and therefore must be documented in Appendix 9 (see also *OLAW FAQs* C.7):

1. Does the *Guide* describe the general standard as a "May" standard? If so, this is NOT a "departure", and need not be documented in Appendix 9. Otherwise, for any "Should" or "Must" standard, proceed to the next question.

2. Does the *Guide* include an explicitly stated exception that allows for the "deviation"? If so, this is NOT a "departure", and need not be documented in Appendix 9. Otherwise, proceed to the next question.

3. Does the "deviation" meet a well-established performance standard for a "Should" standard, according to locally-defined and continuously monitored performance measures? If so, this is NOT a "departure", and need not be documented in Appendix 9. Otherwise, this IS a "departure", and may be approved by the IACUC only if justified on scientific, veterinary medical, or animal welfare grounds.

The test questions above are summarized in the following flow chart:




For each IACUC-approved departure included in this protocol, provide the following information:

Briefly summarize the standard that is described in the *Guide* and reference the page numbers on which it appears.

Describe the specific alternate standard(s) that will be met on this protocol, and how they will be monitored.

Provide the specific scientific, veterinary medical, or animal welfare considerations that justify this departure.