**Department of Veterans Affairs**

# Memorandum

Date: January 12, 2017

From: Institutional Animal Care and Use Committee (151/JC)

Subj: IACUC Letter of Approval re: A Just in Time (JIT) amendment to ACORP 1609-512 Contribution of Inflammation and Oxidative Stress in Pericardial Fluid to Postoperative Atrial Fibrillation after Cardiac Surgery

To: [redacted] 151B/JC)

Dear [redacted]

1.Your study was submitted to the IACUC for a Secondary JIT review with a Level 1 concern. Concern identified related to item "T" in your ACORP and you were requested to discuss the criteria that might necessitate exclusion of the animals from the study and/or require their euthanasia instead of providing a summary of the euthanasia method. In your response, you stated, *"Animals will be euthanized or removed from the protocol based on the recommendations of the attending veterinarian. Since this is an acute study, this would only occur if the animal vendor shipped an animal that was in poor health or the animal had significant change in health status during the quarantine period."*

2. The IACUC approved your JIT response on [redacted] and stated, "The euthanasia is adequately explained and justified in that the dogs will be subjected to non-survival surgery and will be exsanguinated under general anesthesia after harvesting their hearts."

3. The next annual review of ACORP 1609-512 is on [redacted] You will be notified 60-90 days in advance from your annual review date to submit ACORP 1609-512 for review.

4. If you have any questions, please contact the IACUC Administrator at [redacted] by e-mail [redacted]

[redacted]

Date [redacted]

[redacted]

Memorandum

Date [redacted]

To: Veterans Administration IACUC, St Louis VAMC

[redacted]

Subject: Revision of the ACORP Protocol 1609-512

1. I have made the changes to the protocol as requested by the committee. The revised document is attached.

# Secondary Just-In-Time ACORP Review

| [redacted] | STATION | CYCLE | APPLICATION TITLE |
|---|---|---|---|
| | St. Louis, MO-657 | MERIT/Spring 2016 | Contribution of Inflammation and Oxidative Stress in Pericardial Fluid to Postoperative Atrial Fibrillation After Cardiac Surgery |

| | SCORE | DESCRIPTION | ACTION NEEDED BY IACUC |
|---|---|---|---|
| ○ | 0 | No concerns noted. Any comments provided are for information only. | *None.* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application. |
| ● | 1 | Some concerns noted. | *The IACUC must review the level 1 concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).*<br>No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application. |
| ○ | 2 | Concerns are noted that must be addressed by the local IACUC and PI before funding can occur, but work described in the ACORP(s) may continue. | ***A response to each of the level 2 concerns noted below must be reviewed and cleared by the CVMO*** before funding can be released. Upload the following at https://vaww.gateway.research.va.gov:<br>(1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and<br>(2) (a) revised ACORP(s) approved by the IACUC.<br>*The IACUC must review each of the level 1 concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).* |
| ○ | 3 | Significant concerns are noted that must be addressed by the local IACUC and PI before funding can occur, and work described in the ACORP(s) listed below must cease immediately. | ***A response to each of the level 3 concerns listed below must be reviewed and cleared by the CVMO*** before work can resume and funding can be released. (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.)<br>*A response to each of the level 2 concerns noted below must be reviewed and cleared by the CVMO* before funding can be released.<br>For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov :<br>(1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and<br>(2) (a) revised ACORP(s) approved by the IACUC.<br>*The IACUC must review each of the level 1 concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated* |

(cont.)

| | | | |
|---|---|---|---|
| | | | *into the ACORP(s).* |

The ACORP fo[redacted]has received an overall score of 1, which means that it is cleared and represents no bar to funding the application, although some concerns were raised, as shown below.

Please note that a separate score is shown for each of the individual concerns (shown in parentheses under the Item number to which each of the individual concerns refers), to assist you in interpreting the review. An explanation of each of the levels of concern is shown above, in the chart on the previous page. The IACUC must review each of the **level 1** concerns listed and decide what response is needed. This action must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP, but no further correspondence with the CVMO is needed.

In case of questions about this review, please contact [redacted] Assistant Chief Veterinary Medical Officer [redacted]

## REVIEWER FEEDBACK

| ACORP Item number(s) (score) | Comments/Concerns |
|---|---|
| ACORP (dog) | This ACORP uses a canine isolated atrial tissue model to improve understanding of the underlying mechanisms of postoperative atrial fibrillation by investigating the pericardial space. Numerous literature citations were provided in support of the experimental plan. Only one concern was identified. |
| Item T (1) | The response is a summary of the euthanasia method as opposed to a discussion of criteria that might necessitate exclusion of the animals from the study and/or require their euthanasia. Please reconcile. |
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| | |

(cont.)

**ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)**
**Main Body**
**VERSION 4**

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

**A. ACORP Status.**

1. Full Name of Principal Investigator(s) [redacted]
2. VA Station Name (City) and 3-Digit Station Number► St. Louis -657
3. Protocol Title► Contribution of Inflammation and Oxidative Stress in Pericardial Fluid to Postoperative Atrial Fibrillation After Cardiac Surgery
4. Animal Species covered by this ACORP► Canine (Dogs)
5. Funding Source(s). Check each source that applies:

   ►( x ) Department of Veterans Affairs.
   ►( ) US Public Health Service (e.g. NIH).
   ►( ) Private or Charitable Foundation – Identify the Foundation:
   ►() University Intramural Funds – Identify the University and Funding Component:
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:

      (1) Title of project► N/A

      (2) If approved by the R&D Committee, give the date of approval► [redacted]

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

      (1) Identify the studies described in the previously approved ACORP that have already been completed
      ►N/A

      (2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in item 1 accordingly
      ►N/A

      (3) Describe any study results that have prompted changes to the protocol, and <u>briefly summarize</u> those changes, to guide the reviewers to the details documented in other items below.
      ►N/A

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(1) Title of other protocol ►N/A
(2) IACUC approval number of other protocol ►N/A
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►N/A

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

►( x ) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

## Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.
►

We previously showed that postoperative atrial fibrillation is associated with increased morbidity and prolonged length of stay after cardiac surgery. (1) Another recent study also showed that postoperative atrial fibrillation is an independent predictor of in hospital mortality (2) and the same study also identified an increased risk of stroke and increased length of stay (14 vs 10 days). This longer stay is directly related to the increased cost of care in these patients, upwards of $10,000 per patient. (2, 3, 4) Furthermore, POAF is associated with increased long–term mortality. (5) Postoperative atrial fibrillation has a very distinct time course. We recently showed that there are two phases of risk in the postoperative period in which the hazard for POAF is increased and that during the same postoperative period the pericardial space contains highly pro inflammatory and pro–oxidant species (6, 7).The first phase of risk occurs immediately after surgery and rapidly declines over 18 hours. The second phase begins afterward to peak at approximately 48 hours, and then diminishes over the next several days (6).
There is an enormous body of literature examining postoperative atrial fibrillation which has been summarized by Steinberg. (8) Most of these studies fall into two categories: (3) clinical studies documenting the incidence and risk factors, and (9) clinical studies evaluating the effects of various standard antiarrhythmic therapies.

This excellent summary illustrates a major impediment to finding good treatments for POAF: a paucity of basic information regarding the underlying mechanisms of POAF. Only by understanding these mechanisms can rational therapies be developed. We propose to examine the underlying mechanisms of postoperative atrial fibrillation in humans by novel investigation of a previously unexplored physiologic compartment (the pericardial space) and to determine basic mechanisms using a canine surgical model.

A canine isolated atrial tissue model extensively used in our lab will be utilized to investigate mechanisms of local action (i.e. pericardial space) of inflammatory cells, cytokines, and oxidative stress which contribute to changes in atrial repolarization, conduction velocity, spontaneous ectopy, and AF inducibility.

The overall experimental approach is to study alterations in atrial electrophysiology and AF inducibility in a canine isolated perfused right atrial preparation upon exposure to inflammatory agents belonging to four experimental groups 1) neutrophils activated by exposure to myocardial lysate 2) monocytes activated by exposure to the myocardial lysate. 3) H2O2 to simulate oxidative stress 4) candidate cytokines identified in Aim 1 of the grant (human studies examining the inflammatory components of pericardial fluid and blood). Groups 1 and 2 will utilize neutrophils and monocytes isolated from the blood of each dog. During the experimental protocol these cells will be perfused and super fused into an isolated right atrium, electro-physiologic data will be recorded 30, 60, 90, and 120 minutes into each two-hour period of perfusion.

For group 3, to simulate oxidative stress we will super fuse with 0.2mM and 2mM H2O2 for 30 min, followed by a 60 min washout of Krebs solution between each dose of H2O2. Electro-physiologic data will be taken during and after each infusion.

For group 4, candidate cytokine(s)/chemokine (s) as determined from human studies will be used to super fuse the atrial prep; we will use the mean peak concentration found in our study to determine physiologic levels in the pericardial fluid. For example, if sVCAM is used (a leading candidate based on our preliminary study) we will super fuse at the mean maximum concentration observed in patients with POAF. If multiple cytokine/chemokines are identified, we will use the agent with the highest correlation to POAF.

References:

1. Creswell LL, Schuessler RB, Rosenbloom M, Cox JL. Hazards of postoperative atrial arrhythmias. The Annals of thoracic surgery. 1993;56:539–549

2. Magee MJ, Herbert MA, Dewey TM, Edgerton JR, Ryan WH, Prince S, Mack MJ. Atrial fibrillation after coronary artery bypass grafting surgery: Development of a predictive risk algorithm. The Annals of thoracic surgery. 2007;83:1707–1712; discussion 1712

3. Echahidi N, Pibarot P, O'Hara G, Mathieu P. Mechanisms, prevention, and treatment of atrial fibrillation after cardiac surgery. Journal of the American College of Cardiology. 2008;51:793–801
4. Anselmi A, Possati G, Gaudino M. Postoperative inflammatory reaction and atrial fibrillation: Simple correlation or causation? The Annals of thoracic surgery. 2009;88:326–333
5. Bramer S, van Straten AH, Soliman Hamad MA, Berreklouw E, Martens EJ, Maessen JG. The impact of new–onset postoperative atrial fibrillation on mortality after coronary artery bypass grafting. The Annals of thoracic surgery. 2010;90:443–449
6. Melby SJ, George JF, Picone DJ, Wallace JP, Davies JE, George DJ, Kirklin JK. A time–related parametric risk factor analysis for postoperative atrial fibrillation after heart surgery. The Journal of thoracic and cardiovascular surgery. 2014
7. Kramer PA, Chacko BK, Ravi S, Johnson MS, Mitchell T, Barnes S, Arabshahi A, Dell'Italia LJ, George DJ, Steele C, George JF, Darley–Usmar VM, Melby SJ. Hemoglobin–associated oxidative stress in the pericardial compartment of postoperative cardiac surgery patients. Laboratory investigation; a journal of technical methods and pathology. 2014
8. Steinberg JS. Atrial fibrillation after cardiac surgery. Boston: Kluwer Academic; 2000.
9. Shen J, Lall S, Zheng V, Buckley P, Damiano RJ, Jr., Schuessler RB. The persistent problem of new–onset postoperative atrial fibrillation: A single–institution experience over two decades. The Journal of thoracic and cardiovascular surgery. 2011;141:559–570

**C. Experimental Design.**

1. **Lay Summary.** Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

▶

An isolated coronary perfused preparation of the canine right atria will be prepared as previously described in our laboratory. Normal mongrel dogs weighing between 20 and 30 kg, will be anesthetized, intubated and placed on a positive pressure respirator. Anesthesia will be maintained with inhaled isoflurane throughout the procedure. Animals will be surgically prepped and sterile precautions will be maintained throughout the procedure in order to minimize premature neutrophil activation and to produce a valid model of sterile cardiac injury. Arterial and venous catheters will be placed via femoral cut down for blood pressure monitoring and intravenous access during the operation. 10 ml of blood will be drawn for data of complete blood count. A median sternotomy

will be performed to access the atria for dissection.
When full exposure is completed, the aorta is cross clamped, cold cardioplegia is infused into the aortic root, and the heart is covered with normal sterile saline slush. Venous blood will be collected via a cannula to exsanguinate the animal into a sterile collection container to minimize exposure of neutrophils to the operative field. The whole heart is subsequently excised, the right atrium is dissected off of the heart, and any excess atrial and ventricular tissue is trimmed. A portion of left atrial appendage will be collected for later creation of a myocardial lysate. The isolated right atrium is then positioned in a sterile temperature controlled glass chamber for the electrophysiological study.

2. **Complete description of the proposed use of animals.** Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.
▶All dogs will have sternotomies performed under general anesthesia and their hearts excised (the dogs euthanized) for study in a perfused tissue set up. The studies will then be divided into 4 perfusion groups:
Group #1 – perfused neutrophils = 9
Group #2 – perfused monocytes = 9
Group #3 – oxidative stress = 9
Group #4 – cytokines = 9
Total=36

ARE YOU ONLY INCLUDING DOGS OF A CERTAIN AGE AND SEX?
Adult dogs (age> 6 months) of either gender will be used.

WHAT IS THE CONTROL GROUP? (NO INFLAMMATION/OXIDATIVE STRESS)
YOU MENTIONED SEVERAL CYTOKINES IN INTRODUCTION. WHICH WILL BE TESTED. ONE CYTOKINE, A MIXTURE?
Each dog will serve as its own control. Prior to exposure to of the agents listed above in groups 1-4, control data will be taken. With over 20 years of experience with this preparation we have shown that the electrophysiology stays stable for up to 6 hours.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.
▶
We have extensive experience using this model and historically a total of 8 complete studies is needed to have the data reach statistical significance. Eight animals in each group (for four groups) are needed to demonstrate a statistically significant difference in the electrophysiological parameters between groups. The statistical design is based on our previous studies, our preliminary data, and the literature and is designed to detect a 10% difference of outcome variables, an alpha=0.05, and a power =0.8.

Additionally, because we are working in and around the heart there is a significant chance of technical difficulties to occur and historically a loss of an animal is about 10%. Although fairly rare with our experience, we are asking for one additional animal for each scientific group to account for that potential loss.

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix B to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
▶

Dogs will be fasted overnight and allowed free access to water. An indwelling catheter will be placed in a peripheral vein and the dogs will be anesthetized with propofol, intubated and maintained to a surgical plane of anesthesia using isoflurane via mechanical ventilation. The chest and groin of the dog will be clipped and surgically scrubbed. Surgeons will surgically scrub, cap, mask, sterile gown, and apply sterile gloves. All instruments used are sterile. The dog is draped with sterile sheets. A catheter will be placed into the femoral artery and vein using a cut down approach. 10ml of blood will be withdrawn for complete blood count. Arterial blood gases (ABG)will be drawn for monitoring electrolytes and ventilation status.
Heart rate, respiration rate, arterial pressure, pulse oximetry, ETCO2 and body temperature will be monitored.
A sternotomy is performed and the heart exposed. The atrium is dissected and removed after cold perfusion of cardioplegia and cold saline. The dog is euthanized as a result of exsanguination. The atria is then prepared in the laboratory for isolated perfusion via the right coronary artery as per study outline

NEED TO INCORPORATE AGENTS DETAILED IN APPENDIX 3 TO STATE HOW THEY WILL BE USED. The agents listed in Appendix 3 section 2 are only used during the acute surgical procedure to isolate the atria. In the table the purpose, dose and route of administration are listed. Most of the drugs will not be used in most of the animals, in that the surgery is fairly straight forward and short and most conditions that would require their use will not occur. For instance, as an example, if the potassium levels are low we would supplement potassium. This is just our standard of animal care during surgery drug list used to maintain the animal during the procedure. Once the atria is removed the atria is perfused with Krebs solution and all these will be washed out during the stabilization period.

HOW WILL YOU CONTROL FOR DIFFERENTIAL EXPOSURE TO AGENTS (E.G. PRESSORS) IN APPENDIX 3 WHICH COULD INFLUENCE RISK OF AFIB IN THE ACUTE EX VIVIO PREP?
Once the heart is removed from the animal, the right atrium is perfused with Krebs Henselit and allowed to stabilize for a period of 30 minutes. We have found the effects of anesthesia and all the drugs we use are washed out in this time period.

WHAT IS THE TIME FROM SCALPEL TO GETTING THE ATRIA IN A BOTTLE?
The heart will be removed under sterile conditions, and from the incision of the skin until the atria is arrested and removed from the dog is less than 20 minutes.

HOW ARE INFLAMMATORY/OXIDATIVE AGENTS IN THE PERICARDIAL SPACE RELATED TO DIRECT INFUSION IN THE RCA AS THESE SPACES ARE SEPARATE.
The inflammatory process in the patient is both systemic and within the pericardial space. The animal protocol will attempt to determine if the changes that occur in the electrophysiology due to inflammation are a result of circulating inflammatory agents, inflammatory agents in the pericardial space, or a combined effect. Therefore we proposed in the grant to look at all three possible combinations. So to test the effect of the agents in the pericardial space we will bath the agents over the surface of the atria; to test the effect of the circulating agents, we will perfuse it through the RCA in the isolated atria.

D. **Species.** Justify the choice of species for this protocol.
▶

This model has been well developed in canine atria as a model to easily induce AF. It is necessary to have a well described model of inducible AF with large enough atria to closely mimic clinical studies.
Our lab has over 30 years of experience and data utilizing the dog as a model for atria tissue perfusion and electrophysiology. In order to utilize this data for control groups and comparison drug groups, we need to continue to use the dog as a tissue source. This in turn fulfills one of the prime directives of animal research to reduce the total number of animals used in research.

## Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. PI

Name [redacted]
Animal research experience ▶ [redacted]

[redacted]

Qualifications to perform specific procedures

| Specific procedure(s) that the PI will perform personally | Experience with each procedure in the species described in this ACORP |
|---|---|
| Sternotomy | [redacted] |
| | |

2. Other research personnel (copy the lines below for each individual)

Name [redacted]
Animal research experience ▶ [redacted] cic surgery performing operative and post operative

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| Sternotomy | [redacted] |
| | |

Name ▶ [redacted]

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| Sternotomy | [redacted] |

Name [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| Sternotomy | [redacted] |
| | |

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)

Name► [redacted]

Qualifications to perform specific support procedures in the animals on this protocol

| Specific support procedure(s) assigned to this individual | Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training) |
|---|---|
| veterinarian | [redacted] |
| | |

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course

| Name of Individual | Working with the VA IACUC | ORD web-based species specific course (Identify the species) | Any other training required locally (Identify the training) |
|---|---|---|---|
| [redacted] | | [redacted] | |
| | | | |
| | | | |
| | | | |

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"
► N/A

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

| Name | Enrollment in OHSP | | Declined optional services | Current on Interactions with OHSP? (yes/no) |
|---|---|---|---|---|
| | VA program | Equivalent Alternate Program – identify the program | | |
| [redacted] | ( ) | ( x) [redacted] | ( ) | |
| | ( ) | (x) | ( ) | |
| | ( ) | (x) | ( ) | |
| | ( ) | (x) | ( ) | |

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for,

personnel participating in or supporting this protocol?

► ( ) Yes. Describe them ►

► ( x ) No.

Animals Requested

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

| Description (include the species and any other special features not shown elsewhere in this table) | Gender | Age/Size on Receipt | Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony) | Health Status |
|---|---|---|---|---|
| canine | either | adult | [redacted] | Conditioned ( The dogs are healthy and free of diseases like rabies, distemper, heart worm, etc but the term SPF is not routinely used in this species. Conditioned means they have been determined healthy on veterinary exam and have received appropriate vaccinations and have been dewormed for elimination of gastrointestinal parasites). |
| | | | | |

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

| Procedures► |
|---|

| Species / Experimental Group / Procedures(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Category B TOTAL |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |

USDA Category C

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Category C TOTAL |
| | | | | | | |
| | | | | | | |

USDA Category D

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Category D TOTAL |
| Canine/sternotomy | 12 | 12 | 12 | | | 36 |
| | | | | | | |

USDA Category E

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Category E TOTAL |
| | | | | | | |
| | | | | | | |

TOTALS over all Categories

| Species / Experimental Group /Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | GRAND TOTAL |
|---|---|---|---|---|---|---|
| Canine/sternotomy | | | | | | 36 |
| | | | | | | |

J. **Management of USDA Category D procedures.** Indicate which statement below applies, and provide the information requested.

▶ ( ) This protocol does NOT include any Category D procedures.

▶ ( x ) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

| Procedure | Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery) | Person(s) responsible for the monitoring | Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered) |
|---|---|---|---|
| sternotomy | N/A | N/A | See Appendix 5 |
| | | | |
| | | | |

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

▶ ( x ) This protocol does NOT include any Category E procedures

▶ ( ) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.
▶

## Veterinary Care and Husbandry

L. **Veterinary Support.**

1. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

   Name[redacted]
   Institutional affiliation[redacted]
   email contact[redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted[redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI)[redacted]
   VA Veterinary Pre-review performed by[redacted]

M. **Husbandry.** As a reference for the animal husbandry staff, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

| a. Species | b. Type of housing* | c. Number of individuals per housing unit** | d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***) | e. Estimated maximum number of housing units needed at any one time |
|---|---|---|---|---|
| canine | Standard | 2 | yes | 2 |
| | | | | |

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
►

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
►

***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

| a. Species | b. Description of Enrichment* | c. Frequency |
|---|---|---|
| Canine | Standard, see below | |
| | | |

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here.

►All animals are housed in rooms with conspecifics and are pair or group housed whenever possible. When appropriate, cages are arranged to allow for visual, physical, olfactory and auditory communication. All dogs are housed in pens with 100% of the USDA required space and sufficient room for normal activity. If dogs are housed individually, the pens provide at least 200% of the USDA required space. Dogs are allowed access to exercise areas 1-2 times/ week in compatible groups where they receive positive human interaction and play activity is encouraged. Toys and other enrichment items are provided and rotated on a regular basis to prevent animal boredom.

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► (N/A ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized

routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.
▶

▶ (N/A) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.
▶

▶ (N/A) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
▶

▶ ( x ) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites.** Document in the tables below each location where animals on this protocol may be housed.

▶ ( N/A ) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU.

| Building | Room number | Inside of VMU? | |
|---|---|---|---|
| | | Yes | No |
| | | ( ) | ( ) |
| | | ( ) | ( ) |
| | | ( ) | ( ) |

▶ (x ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table.

| Name of Non-VA Facility | Is this facility accredited by AAALAC? | | Building | Room Number |
|---|---|---|---|---|
| | Yes – enter status* | No** | | |
| [redacted] | | | | |
| | ( ) | ( )** | | |
| | ( ) | ( )** | | |

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( N/A ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".

► (x ) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► ( x ) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► () This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

| Procedure | Surgical? | | Bldg/Room Number | Requires transport through non-research areas? | |
|---|---|---|---|---|---|
| | Yes | No | | Yes – describe method of discreet transport | No |
| sternotomy | ( x ) | ( ) | [redacted] | ( ) | ( x ) |
| | ( ) | ( ) | | ( ) | ( ) |
| | ( ) | ( ) | | ( ) | ( ) |
| | ( ) | ( ) | | ( ) | ( ) |

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

| Body Fluid, Tissue, or Device to be Collected | Collected AFTER Euthanasia | Collected BEFORE Euthanasia | | |
|---|---|---|---|---|
| | | Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production") | Collected as Part of a Surgical Procedure (Appendix 5, "Surgery") | Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection") |

| | | | | |
|---|---|---|---|---|
| blood | ( ) | ( ) | (x) | ( ) |
| | ( ) | ( ) | ( ) | ( ) |
| | ( ) | ( ) | ( ) | ( ) |

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► ( x) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► (N/A ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
► ~~The animal will be given potassium IC to stop the heart and the heart will be excised while animal is under surgical anesthesia allowed to exsanguinate, and therefore the animal euthanized as an endpoint.~~ Animals will be euthanized or removed from the protocol based on the recommendations of the attending veterinarian. Since this is an acute study, this would only occur if the animal vendor shipped an animal that was in poor health or the animal had significant change in health status during the quarantine period.

~~U.~~ **Termination or removal from the protocol.** Complete each of the following that applies:

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► ( N/A ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

► ( x ) Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

| Check each | Method of Euthanasia | Species | AVMA Classification |
|---|---|---|---|

| method that may be used on this protocol | | | Acceptable | Conditionally Acceptable | Unacceptable |
|---|---|---|---|---|---|
| ( ) | $CO_2$ from a compressed gas tank<br>Duration of exposure after apparent clinical death▶<br>Method for verifying death▶<br>Secondary physical method▶ | | ( ) | ( ) | ( ) |
| ( ) | Anesthetic overdose<br>Agent▶<br>Dose▶<br>Route of administration▶ | | ( ) | ( ) | ( ) |
| ( ) | Decapitation under anesthesia<br>Agent▶<br>Dose▶<br>Route of administration▶ | | ( ) | ( ) | ( ) |
| (x) | **Exsanguination under anesthesia**<br>**Agent▶ propofol, isoflurane, potassium chloride**<br>**Dose▶ 5-7mg/kg, 1-5%, 40-60mEq**<br>**Route of administration▶ IV, IH, IV** | canine | (x) | ( ) | ( ) |
| ( ) | Other (Describe) ▶ | | ( ) | ( ) | ( ) |
| ( ) | Other (Describe) ▶ | | ( ) | ( ) | ( ) |

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:
   ▶N/A

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:
   ▶N/A

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated. [redacted] in the research lab performing surgery and euthanasia using exsanguination. [redacted] performing surgery and euthanasia using exsanguination.

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
      ▶ Animal undergoes a necropsy to determine cause of death and then bagged and stored in the freezer until incineration.

   b. Describe how the PI's staff should be contacted.
      ▶ ( ) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual w[redacted])
      Name▶ [redacted]
      Contact Information▶

      ▶ ( x ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
      ▶ The veterinarian or care staff will inform the PI via email.

V.U. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

| Name of Procedure | Identify Where the Details of the Procedure are Documented | | |
|---|---|---|---|
| | SOP (title or ID number)* | Other Items in this ACORP – specify the Item letter(s) | Appendix B |
| N/A | | Items: | ( )** |
| | | Items: | ( )** |
| | | Items: | ( )** |
| | | Items: | ( )** |

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W.V. **Consideration of Alternatives and Prevention of Unnecessary Duplication. These are important to minimizing the harm/benefit to be derived from the work.**

1. **Document the database searches conducted.**
   **List each of the potentially painful or distressing procedures included in this protocol.**
   ► **sternotomy**

   **Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.**

| Name of the database | Date of search | Period of years covered by the search | Potentially painful or distressing procedures addressed | Key words and/or search strategy used | Indicate which mandate each search addressed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replacement of animals (Item W.2) | Reduction in numbers of animals used (Item W.3) | Refinement to minimize pain or distress (Item W.4) | Lack of unnecessary duplication (Item W.5) |
| PubMed | [redacted] | 16 | Sternotomy, femoral cut down | post -operative fibrillation, canine, animal welfare, animal research alternatives, sternotomy, femoral cut down, pericarditis, pericardial fluid, inflammation | ( x ) | (x ) | ( x) | ( x ) |
| Agricola | [redacted] | 16 | Sternotomy, femoral cut down | Post- operative fibrillation, canine, animal welfare, animal research alternatives, sternotomy, femoral cut down, pericarditis, | ( ) | ( ) | ( ) | ( ) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | pericardial fluid; inflammation | | | | |
| | | | | | ( ) | ( ) | ( ) | ( ) |
| | | | | | ( ) | ( ) | ( ) | ( ) |

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
   ▶ This study documents the electrophysiology of cardiac tissue and its response after being exposed to perfused materials and its ability to sustain normal/abnormal cardiac rhythms. There is no computer model to date to mimic the electrical components of live tissue.
   This model has been well developed in canine atria as a model to easily induce AF as determined by extensive research. Inducibility of AF requires a minimal mass and canines are the lowest species that can feasibly be used for this study because they fulfill the mass requirement.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
   ▶ The minimal number has been determined from 30 years of statistical data gathered in our laboratory to complete a study group that is designed to detect a 10% difference of outcome variables, an alpha=0.05, and a power =0.8.
   Our lab has over 30 years of experience and data utilizing the dog as a model for atria tissue perfusion and electrophysiology. In order to utilize this data for control groups and comparison drug groups, we need to continue to use the dog for tissue. This in turn fulfills one of the prime directives of animal research to reduce the total number of animals used in research.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
   ▶ This study only involves one surgery to be performed under general anesthesia and euthanasia is completed while still under anesthesia. No further refinements to the protocol are needed.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
   ▶ After an extensive search of the literature, this study does not duplicate any work already done.

## X.W. Other Regulatory Considerations.

1. Controlled drugs.

   a. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

| Controlled substances | Storage | | Personnel Authorized to Access | Location for Use | | Procurement | |
|---|---|---|---|---|---|---|---|
| | Double-locked | Not Double-locked | | VA Property | Not on VA Property | VA Pharmacy | Non-VA |
| propofol | ( x ) | ( )* | [redacted] | ( ) | ( x ) | ( ) | ( x ) |
| | ( ) | ( )* | | ( ) | ( ) | ( ) | ( ) |
| | ( ) | ( )* | | ( ) | ( ) | ( ) | ( ) |

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.
►N/A

b. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

► ( N/A ) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.

► ( N/A ) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

► ( N/A ) Other. Explain►

2. **Human patient care equipment or procedural areas.** Does this protocol involve use of any human patient care equipment or procedural areas?

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

► ( x) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents.** Does this protocol involve use of any explosive agent?

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

► ( x) No explosive agent(s) will be used as part of this protocol.

~~Y.~~ X. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

**Appendices.** Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► ( ) Appendix 1, "Additional Local Information"

- ▶ ( ) Appendix 2, "Antibody Production"
- ▶ ( x ) Appendix 3, "Biosafety"
- ▶ ( X ) Appendix 4, "Ante-mortem Specimen Collection"
- ▶ ( x ) Appendix 5, "Surgery"
- ▶ ( ) Appendix 6, "Special Husbandry and Procedures"
- ▶ ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
- ▶ ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
- ▶ ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"

**Standard Operating Procedures (SOPs).** List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

| Item | SOP | | Approval Date |
|---|---|---|---|
| | Title | ID | |
| C.2.c | Sternotomy and harvest of cardiac tissue | | 9-16-16 |
| M.1 | Husbandry | | 9-16-16 |
| M.2 | Enrichment | | 9-16-16 |
| U.4.a | Animal care staff | | 9-16-16 |
| U.4.b | Animal care staff | | 9-16-16 |
| V | | | |
| | | | |
| | | | |

Z.Y. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

   a. **Certification by Principal Investigator(s):**

   I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

   I understand that further IACUC approval must be secured before any of the following may be implemented:

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on

individual animals;

- Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
- Performing any additional procedures not already described in this ACORP;
- Use of any of these animals on other protocols, or by other investigators.

I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

| Name(s) of Principal Investigator(s) | Signature | Date |
|---|---|---|
| [redacted] | | |
| | | |

**b. Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

  ► ( ) No minority opinions were submitted by any IACUC participant for inclusion.

  ► ( ) Minority opinions submitted by IACUC participants are copied here ►

  ► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages► )

| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
|---|---|---|
| | | |

| [redacted] | | |
|---|---|---|
| Name of IACUC Chair | Signature | Date |
| [redacted] | | |

2. **Appendix 2. Antibody Production. No signatures required.**

3. **Appendix 3. Biosafety.**

   a. **Certification by PI(s) and IACUC Officials:**

   We certify that:

   - Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;
   - All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| [redacted] | [redacted] | [redacted] |
| | | |
| Name of Institutional Veterinarian | Signature | Date |
| [redacted] | | |
| Name of IACUC Chair | Signature | Date |
| [redacted] | | |

   b. **Certification by Biosafety Official.** I certify that:

   - Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant

nucleic acid";

- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

| Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee | Signature | Date |
|---|---|---|
| [redacted] | | |
| | | |

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

| Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee | Signature | Date |
|---|---|---|
| [redacted] | | |
| | | |

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.
5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;

- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:
    - Identification of each animal such that care for individual animals can be documented.
    - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
    - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
    - Daily records covering at least the period defined as "post-operative" by local policy.
    - The signature or initials of the person making each entry.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| [redacted] | | [redacted] |
| | | |

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

    a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| | | |
| | | |

b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of the Manager of the Human Patient Care Equipment | Signature | Date |
| | | |

c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
| | | |
| Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment | Signature | Date |
| | | |
| Name of ACOS for R&D | Signature | Date |
| | | |
| Name of Chief of Staff | Signature | Date |
| | | |
| Name of Director or CEO of the Facility (Hospital or Clinic) | Signature | Date |

| | | |
|---|---|---|
| | | |

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

   a. **Certification by the Principal Investigator(s).**

   I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

   I further certify that:

   - Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
   - All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
   - Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
   - Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| | | |
| | | |

   b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
| | | |
| Name of Safety/Biosafety Officer for the Facility | Signature | Date |

| | | |
|---|---|---|
| Name of ACOS for R&D | Signature | Date |
| | | |
| Name of VISN Regional Safety Officer | **Signature** | Date |
| | | |

9. **Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

ACORP APPENDIX 3

BIOSAFETY
VERSION 4

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of All Materials Administered to Animals on this Protocol.** Complete the table below for all materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

| **Material** (Identify the specific agent, device, strain, construct, isotope, etc.) | **Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors) | Nature of Material | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Toxic Agent (Item 4) | Infectious Agent (Item 5) – Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4) | Biological Agent (Item 6) | Radioactive Agent (Item 7) | Contains Recombinant Nucleic Acid (Item 8) | Routine Pre- or Post-Procedural Drug | Euthanasia agent |
| Propofol | hospira | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x) | ( ) |
| Isoflurane | Piramal critical | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x) | ( ) |
| potassium chloride(cardioplegia is potassium chloride and saline) | hospira | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x) | ( ) |
| Saline | baxter | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x) | ( ) |
| Calcium chloride | hospira | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x) | ( ) |
| Lactated ringers | baxter | | | | | | x | |
| Insulin | Eli lilly | | | | | | x | |
| Sodium bicarbonate | Hospira | | | | | | x | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dextrose | Hospira | | | | | | x | |
| Epinephrine | International med sys | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (x ) | ( ) |
| Phenylephrine | West-ward pharm | | | | | | x | |
| Atropine | American regent | | | | | | x | |
| Lidocaine | hospira | | | | | | x | |

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in item 1 above:

| Material* (Identify the specific agent, device, strain, construct, isotope, etc.) | Dose (e.g., mg/kg, CFU, PFU, number of cells, mCi) and Volume (ml) | Diluent* or Vehicle* | Route of admin | Frequency or duration of admin | Reason for Administration and Expected Effects | Location of Further Details in this ACORP (specify "Main Body" or "App #", and identify the item) | Administration Under Anesthesia, sedation, or tranquilization (Y/N) |
|---|---|---|---|---|---|---|---|
| Nacl | 10-20ml/kg, 500ml | | IV | As needed | Fluid maintenance | | Y |
| LRS | 10-20ml/kg, 500ml | | IV | As needed | Fluid maintenance | | Y |
| propofol | 5-7mg/kg, 20ml | | IV | once | anesthesia | | N |
| isoflurane | 1-5%, 100ml | | IH | During surgery | anesthesia | | Y |
| KCL | 2-10MEq in saline | | IV | As needed | hypokalemia | | Y |
| CaCl | 3-20mg/kg, 10ml | | IV | As needed | hypocalcemia | | Y |
| Insulin | 0.25-0.5 U/kg, 5 ml | | IV | As needed | hyperkalemia | | Y |
| Sodium Bicarb | Calculated Meq Accurding To Base Excess | | IV | As needed | acidosis | | Y |
| dextrose | 0.5 – 1.0 G/kg, 50 ml | | IV | As needed | hypoglycemia | | Y |
| Epinephrine | 0.01-0.02mg/kg | | IV | As needed | Cardiac support, arrest | | Y |
| phenylephrine | 1-3ug/kg/min Or 100ug Bolus | | IV | As needed | hypotension | | Y |
| Atropine | 0.05mg/kg | | IV | As needed | bradycardia | | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| lidocaine | 30–70 Ug/kg/hr iv, 2–4mg/kg Bolus | | IV | As needed | Arrhythmias | | Y |

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)
▶

3. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

   a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):
   ▶

   b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
   ▶

4. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

| Name of Toxic Agent | a. Mutagen | b. Carcinogen | c. Teratogen | d. Select Agent? | | | e. Other – specify toxic properties |
|---|---|---|---|---|---|---|---|
| | | | | Not a Select Agent | Select Agent Used in Sub-threshold Quantities | Select Agent that Requires Registration/Approval | |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )▶ |

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO ▶
Date of approval ▶

5. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name and BSL Number of Infectious Agent | a. ABSL Number * | b. Drug Sensitivity Panel Available? (Describe) | c. Select Agent? | | |
|---|---|---|---|---|---|
| | | | Not a Select Agent | Select Agent used in Sub-threshold quantities | Select Agent that Requires Registration/Approval |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ▶
Justification for applying ABSL measures that are less protective than those recommended ▶

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO ▶
Date of approval ▶

6. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name of Biological Agent | Screening for Infectious Agents |
|---|---|
| | |
| | |
| | |
| | |
| | |
| | |

7. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name of Radioactive Agent (specify the isotope) | Authorized Individual | Approving Committee or Official |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |

8. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

| Name of Agent that Contains Recombinant Nucleic Acid | Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules* | Exempt |
|---|---|---|
| | ( ) | ( ) |
| | ( ) | ( ) |
| | ( ) | ( ) |
| | ( ) | ( ) |
| | ( ) | ( ) |

| | | |
|---|---|---|
| | ( ) | ( ) |

9. **Potential for Pain or Distress.** Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

| Name of Agent | Nature of Potential Pain/Distress | Measures to Alleviate Pain/Distress |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |

10. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

a. **Complete the table below.**

| Name of Hazardous Agent | Approving Committee or Official | Institution (VA or affiliate) | Names of Animal Facility Staff Members at Risk |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.
▶

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

**ACORP Appendix 4**
**ANTEMORTEM SPECIMEN COLLECTION**
**VERSION 4**

See ACORP App. 4 Instructions, for more detailed explanations of the information requested.

1. **Summary.** Complete the table below for each specimen to be collected from a live animal on this protocol (see ACORP App. 4 Instructions, for details).

| Specimen Collected | Site and Method of Collection | Anesthesia (Yes/No) | Amount Collected Each Time | Volume Replacement (Yes/No/NA) | Total Number of Collections per Animal | Time Intervals Between Successive Collections |
|---|---|---|---|---|---|---|
| Blood | Femoral artery, syringe | yes | 200 ml | no | 1 | |
| | | | | | | |
| | | | | | | |

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

   a. For each specimen described in Item 1, above, as being collected WITHOUT anesthesia, complete items 2.a(1) and 2.a(2), below:

      (1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain).
      ▶N/A

      (2) Completely describe any method of physical restraint that may be used.
      ▶N/A

   b. For each specimen described in Item 1, above, as being collected WITH anesthesia, complete the following table:

| Anesthetic, tranquilizer, or analgesic agent | Dose (mg/kg) and volume (ml) | Route of administration | Frequency of administration |
|---|---|---|---|
| Propofol, isoflurane | 5-7mg/kg, 1-5% | IV, IH | During anesthesia |
| | | | |
| | | | |

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

   a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.
   ▶ Blood collected will be done during exsanguination and euthanasia.

b. For each fluid specimen described in Item 1, above, for which volume replacement WILL be provided, describe the replacement fluids that will be administered (including their composition, volume, and route of administration).
▶

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).
▶

**ACORP Appendix 5**
**SURGERY**
**VERSION 4**

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

| Surgery | | Terminal | Survival | | |
|---|---|---|---|---|---|
| # | Description (specify the species, if ACORP covers more than one) | | Minor | Major | One of Multiple* |
| 1 | Sternotomy | ( x) | ( ) | ( x) | ( )* |
| 2 | | ( ) | ( ) | ( ) | ( )* |
| 3 | | ( ) | ( ) | ( ) | ( )* |
| 4 | | ( ) | ( ) | ( ) | ( )* |

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) 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, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
► N/A

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
► N/A

2. **Description of Surgeries.** Describe each surgery listed in item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

Surgery 1 ►
Dogs will be fasted overnight and allowed free access to water. An indwelling catheter will be placed in a peripheral vein and the dogs will be anesthetized with propofol, intubated and maintained to a surgical plane of anesthesia using isoflurane via mechanical ventilation. The chest and groin of the dog will be clipped and surgically scrubbed. Surgeons will surgically scrub, cap, mask, sterile gown, and apply sterile gloves. All instruments used are sterile. The dog is draped with sterile sheets. A catheter will be placed into the femoral artery and vein using a cut down approach. 10ml of blood will be withdrawn for complete blood count. Arterial blood gases (ABG) will be drawn for monitoring electrolytes and ventilation status.
Heart rate, respiration rate, arterial pressure, pulse oximetry, ETCO2 and body

temperature will be monitored.
A sternotomy is performed and the heart exposed. The atrium is dissected and removed after cold perfusion of cardioplegia and cold saline. The dog is euthanized as a result of exsanguination. The atria is then prepared in the laboratory for isolated perfusion via the right coronary artery as per study outline.

Videos and/or pictures will potentially be recorded and or taken to document surgical technique for archiving or publication. The videos/pictures will be digitally derived and saved on a secure database within the secure departmental network. It will be protected with a password.

Surgery 2 ►

Surgery 3 ►

Surgery 4 ►

3. **Personnel.** Complete the table below for each individual who will be involved in any of the surgeries on this protocol.

| Name | Surgery #(s) (see Item 1) | Role in Surgery | | | |
|---|---|---|---|---|---|
| | | Surgeon | Assistant | Manage Anesthesia | Other (describe) |
| [redacted] | 1 | (x) | ( ) | ( ) | ( ) |
| | 1 | (x) | ( ) | ( ) | ( ) |
| | 1 | ( ) | ( ) | (x) | ( ) |
| | 1 | ( ) | ( ) | (x) | ( ) |
| | | ( ) | ( ) | ( ) | ( ) |

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

| Building | Room Number | Surgery #(s) (see Item 1) | Type of Space | | |
|---|---|---|---|---|---|
| | | | Dedicated Surgical Facility | Other Dedicated Surgical Space | Other Space not Dedicated to Surgery |
| [redacted] | | 1 | ( ) | ( )* | ( )* |
| | | | ( ) | ( *) | ( )* |

| | | | | | |
|---|---|---|---|---|---|
| | | | ( ) | ( )* | ( )* |
| | | | ( ) | ( )* | ( )* |

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
►

5. **Pre-operative protocol.**

a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

| Surgery #(s) (see Item 1) | Fast (Specify Duration) | Withhold Water (Specify Duration) | Place Intravenous Catheter(s) (Specify Site(s)) | Other – Describe |
|---|---|---|---|---|
| 1 | ( 12 hours) – | ( ) – | ( cephalic ) – | ( ) – |
| 2 | ( ) – | ( ) – | ( ) – | ( ) – |
| 3 | ( ) – | ( ) – | ( ) – | ( ) – |
| 4 | ( ) – | ( ) – | ( ) – | ( ) – |

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

| Agent | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) | Route of administration | Frequency of administration (e.g., times/day) | Pre-operative period of treatment (e.g., immediate, or # of days) |
|---|---|---|---|---|---|
| propofol | 1 | 5-7mg/kg | IV | 1 | Immediate |
| | | | | | |
| | | | | | |

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ►Chest – clipped of hair and surgically scrubbed, groin – clipped of hair and surgically scrubbed

Surgery 2 ►

Surgery 3 ►

Surgery 4 ►

6. **Intra-operative management.**

   a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal during surgery.

| Agent | Paralytic* | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) | Route of administration | Frequency of dosing |
|---|---|---|---|---|---|
| Isoflurane | ( )* | 1 | 1-5% | IH | During surgery |
| | ( )* | | | | |
| | ( )* | | | | |

   * For each agent shown above as a paralytic, explain why its 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.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).
   ▶circulated water warming blanket under and warm air blanket over

   c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.
   ▶elevation of HR, blood pressure, palpebral eye reflex, toe pinch, spontaneous breaths, mm's color, eye position

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a.–7.g.

   a. Complete the table below for each survival surgery listed in Item 1, above.

| Surgery # (see Item 1) | Survival Period | Measures for Maintaining Sterility | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sterile Instruments | Surgical Cap | Sterile Gloves | Surgical Scrub | Sterile Drapes | Sterile Gown | Face Mask | Other* |
| | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* |
| | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* |
| | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* |
| | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* |

   * Describe any "other" measures to be taken to maintain sterility during surgery.
   ▶

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

Surgery 1 ▶

Surgery 2 ▶

Surgery 3 ▶

Surgery 4 ▶

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

| Surgery # (see Item 1) | Agent* | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of Dosing (e.g., times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
▶

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

| Surgery # (see Item 1) | Medication | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of dosing (e.g. times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

e. Post-operative monitoring. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

(1) Immediate post-operative monitoring

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

(2) Post-operative monitoring after the immediate post-operative period

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

f. Post-operative consequences and complications.

**(1)** For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1 ▶

Surgery 2 ▶

Surgery 3 ▶

Surgery 4 ▶

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1 ▶

Surgery 2 ▶

Surgery 3 ▶

Surgery 4 ▶

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)
▶

g. **Maintenance of post-surgical medical records.** Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

| Surgery # (see Item 1) | Location of Records | Name(s) of Individual(s) Responsible for Maintaining Written Records | Research Personnel | Veterinary Staff |
|---|---|---|---|---|
| 1 | | | ( ) | ( ) |
| 2 | | | ( ) | ( ) |
| 3 | | | ( ) | ( ) |
| 4 | | | ( ) | ( ) |

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

**Literature search St Louis** [redacted]

## 1) How is this research relevant to Veterans health?

The most common complication following cardiac surgery is new-onset postoperative atrial fibrillation (POAF). The Veterans Affairs Randomized On/Off Bypass Trial found POAF patients stayed in the hospital an average of 3.9 days longer, costing an average of $13,993 more than no-POAF patients, and were also more than twice as likely to die within a year.

This study will focus understanding the roles of inflammation and oxidative stress in POAF, which in turn should lead to preventive treatments. Preventing POAF will greatly benefit both Veteran and non-Veteran cardiac surgery patients and reduce the overall cost of cardiac surgery care.

## 2) Is this work <u>unnecessarily</u> duplicating work already documented in the literature?

| Name of the database | Date of search | Period of years covered by the search | Key words and/or search strategy used | How many papers were found? |
|---|---|---|---|---|
| PubMed | 3/11/18 | All available years | POAF and (activated neutrophils OR activated monocytes) | 0 |
| PubMed | 3/11/18 | All available years | POAF and inflammatory cytokines | 5 |
| PubMed | 3/11/18 | All available years | POAF and oxidative molecules | 0 |

A PubMed search brought up only five papers, two of which were meta-analyses of patient studies, two of which were studies of correlations between various factors and the occurrence of POAF, and one was a clinical trial of statins that showed a non-significant effect. None of these studies were directly studying the effects of various inflammatory components on heart tissue, which this study will do.

**3) Could this work be done in computer models or in vitro (tissue culture)?**

| Name of the database | Date of search | Period of years covered by the search | Key words and/or search strategy used | How many papers were found? |
|---|---|---|---|---|
| ALTBIB Citations with Animal Use Alternatives as the main topic | 3/11/18 | All available years | POAF | 0 |

An ALTBIB search for "alternatives to using animals" for this study yielded no papers at all. No computer models or in vitro models were found for this work.

This study involves euthanizing animals, harvesting the heart tissues, and running the experiments in vitro. Unfortunately the technology does not yet exist to grow actual heart tissue in vitro, so it must be collected from living animals.

**4) Could it be done in non-mammals or in other mammals?**

| Name of the database | Date of search | Period of years covered by the search | Key words and/or search strategy used | How many papers were found? |
|---|---|---|---|---|
| ALTBIB animal alternatives search strategy - all citations | 3/11/18 | 2000-present | POAF | 0 |

An ALTBIB search for all citations for POAF yielded no papers at all.

A large animal has to be used for these studies so the atria are large enough to closely mimic clinical studies. Dogs are used for two reasons: 1) Atrial fibrillation can be readily induced in dogs and 2) The group has been using dogs for over 30 years. In order for the new data to be comparable with the previously collected data, they need to continue to use dog tissue. Switching to another species would to some degree be starting over, and require many more animals than this study will use.

5) **Are the methods used the best available (least painful or distressing to the dogs)?**

The animals will be anesthetized and euthanized, and the heart tissue harvested. They will not experience pain or distress.