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], MD

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► N/A

   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 I accordingly
      ►N/A

      (3) Describe any study results that have prompted changes to the protocol, and briefly summarize 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.
►

Atrial fibrillation (AF) is disorganized beating of the top chambers of the heart (atria), and it is the most common rhythm problem of the heart. AF commonly occurs after heart surgery; postoperative atrial fibrillation (POAF) occurs in 15% to 65% of the 750,000 patients who undergo heart surgery annually. POAF is strongly associated with increased risk of stroke, longer hospital stays, increased medical care costs (greater $10,000 per patient), and hospital mortality (death) as well long-term mortality. During cardiac surgery, the lining around the heart (pericardium) must be opened to access the heart; opening the pericardium draws large numbers of white blood cells into the lubricating fluid (pericardial fluid) between the heart and the pericardium. These white blood cells produce substances called cytokines that cause inflammation (swelling, redness, and electrical disturbances), which we believe are directly related to the development of postoperative atrial fibrillation (POAF). Although, many preventative treatments have been developed for POAF, a study from our institution has shown that over the last twenty years none of these treatments have reduced the incidence or severity of POAF. The aim of this study is to investigate the fluid around the heart after surgery using pericardial fluid and blood collected from human patients (Aim 1), and to determine which type of white blood cells and their products damage the heart and cause POAF using our canine model (Aim 2). In this study, dogs will be anesthetized and the upper chambers of the heart (atria) and blood will be collected. Dogs will be euthanized without recovery from anesthesia. The benefits of identifying new treatments to prevent POAF are improved survival and outcome for patients undergoing heart surgery.

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.

►Based on our preliminary studies, we think POAF occurs in two phases. The first phase of POAF occurs

when neutrophils and monocytes enter the pericardial fluid immediately after the pericardium is opened; these cells secrete cytokines that cause inflammation, which interferes with electrical conduction (electrical signals that cause the heart to beat) and increases the likelihood of atrial fibrillation. The second phase begins approximately 18 hours after heart surgery and peaks at 48 hours when large numbers of white blood cells are present in the blood and pericardial fluid; high levels of inflammation are found that correlate with the higher rates of POAF. By analyzing the pericardial fluid and blood of patients before and after heart surgery, we have identified several inflammatory cytokines produced by neutrophils and monocytes that are likely indicators of POAF. It is likely that the abundant inflammatory neutrophils and monocytes adhere to the myocardium (heart muscle) and change the normal rhythm and conduction of heart muscle cells through direct contact or through secretion of highly reactive molecules (hydrogen peroxide) and/or cytokines. In Aim 1, we will determine the extent to which of these inflammatory molecules and cytokines are associated with the development of POAF. Using our canine atrial tissue model, we can test the electrophysiologic function (electrical properties of heart tissue) in vitro (performed in a test chamber) when the atrial tissue is exposed to activated neutrophils, monocytes, and cytokines. Dogs will be anesthetized and undergo a non-survival surgery were blood will be collected and whole heart removed. Atrial tissue will be collected and placed in a sterile controlled glass chamber for the testing procedure described above.

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.

► We previously have showed that POAF is associated with increased morbidity, prolonged length of stay after cardiac surgery, and is associated with increased long−term mortality. POAF 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 proinflammatory and pro−oxidant species. 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. An enormous body of literature examining POAF has been summarized by Steinberg, which illustrates a major impediment to finding good treatments is a lack 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. All dogs will have sternotomies performed under general anesthesia and their hearts excised (the dogs are euthanized without recovery from anesthesia) for study in a perfused tissue set up. 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 (9 dogs per group X 4 groups = 32 dogs)

- Group 1) neutrophils activated by exposure to myocardial lysate
- Group 2) monocytes activated by exposure to the myocardial lysate.
- Group 3) H2O2 to simulate oxidative stress

- Group 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 (20 ml/min) and super fused (continuous flow of medium 20 ml/min) 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 (vascular cell adhesion protein1 – mediates adhesion of neutrophils, monocytes, and other white blood cells to vascular endothelium) is used, is 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

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► Each adult dog of either gender 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.

The primary outcome is inducibility of AF. The inducibility is defined by the duration of AF in seconds. In control tissue the duration is ~40 seconds based on previous studies in our laboratory. AF<60 seconds is considered non-sustained and is not clinically significant. To confirm a clinically significant effect it needs to be sustained, which is at least 60 seconds. We used a target mean duration of 90 seconds, or a 50 second increase over the control data. Using G*Power 3.1.9.2 we determine the sample size using a power of 0.8, alpha=0.5, and a two tailed paired t-test:

t tests - Means: Difference between two dependent means (matched pairs)

Analysis:	A priori: Compute required sample size	
Input:	Tail(s)	= Two
	Effect size dz	= 1.4285714
	α err prob	= 0.05
	Power (1-β err prob)	= 0.8
Output:	Noncentrality parameter δ	= 3.7796447
	Critical t	= 2.4469119
	Df	= 6
	Total sample size	= 7
	Actual power	= 0.8799389

Reducing the group to only 6 dogs lowers the power to less than 80%. Since we are supposed to have a statistical power of at least 80%, we will need 7 dogs.
We are also requesting one additional animal due to anatomic variability in dogs in which <10% have a left sided sinus node artery and the RA cannot be isolated and perfused.

Summarizing: The studies will then be divided into 4 groups:
Group #1 − perfused neutrophils =8
Group #2 − perfused monocytes = 8
Group #3 − oxidative stress =8
Group #4 − cytokines = 8

Total=32

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 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.)

► **Terminal Surgery:**
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 covered with sterile drapes. 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.

Note: The agents listed in Appendix 3 section 2 are only used during the acute surgical procedure to isolate the atria. Most of the drugs will not be used in most of the animals, in that the surgery is fairly straight forward and short; most conditions that would require their use will not occur. As an example, if the potassium levels are low we would supplement potassium.

Canine isolated atrial tissue model:

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. 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.

The inflammatory process in the patient is both systemic and within the pericardial space. The isolated atrial tissue model 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 propose 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 right coronary artery in the isolated atria.

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

► At least at this time, dogs are the only species in which this study can be successfully conducted. Atrial fibrillation can be readily induced in dogs and the canine model of atrial fibrillation is well-characterized. Sheep have been used for some types of cardiac studies, but they have a number of anatomical differences compared to humans. Sheep have an os cordis, a bone that blocks the usual route of the His bundle, an important part of the electrical conduction system of the heart; this structure is not found in pigs, dogs, and humans. Furthermore, the cells found in the His bundle and bundle

branches of sheep differ from those of humans. Likewise, pigs are not a suitable model for this work because the electrophysiology of the pig heart is substantially different from that of the human and canine heart. "Specifically, the endocardium and epicardium are activated simultaneously in the swine heart but not in the human or canine heart [Lelovas 2014]…" "Additionally, dogs have a His-Purkinje system located in endocardium, very similar to the human heart, that pigs and other larger animals do not have [Newton 2004]."

Smaller animal models are also unacceptable. Rabbits have substantial differences in the action potential configuration of the atrium and ventricle in comparison to humans. Recent studies have shown that the distribution of potassium-ion-channel variants in the mouse heart and in the human heart are different. Rodents have ion channels in the atria that are different from those in the ventricles; humans do not have this arrangement.

Consequently, the dog is the only acceptable model; it is necessary to have a well described in vitro model of inducible AF with large enough atria to closely mimic human clinical studies. Our lab has over 30 years of experience and data utilizing this dog atrium 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. The canine model is highly representative of the human conditions and its use is essential to the development of safe and effective cardiovascular drugs.

Sources:

https://www.guwsmedical.info/heart-failure/comparative-cardiac-anatomy.html
https://www.sciencedaily.com/releases/2011/08/110803174755.htm
https://physoc.onlinelibrary.wiley.com/doi/abs/10.1113/jphysiol.1988.sp017325
https://link.springer.com/chapter/10.1007/978-1-4419-6658-2_14

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], MD
Animal research experience ► I completed my undergraduate training at [redacted] and earned a Bachelor of Science with Honors in Zoology. I then went on to medical school at the University of [redacted] and graduated in [redacted]. I did my surgical training at [redacted] Hospital and [redacted] taking two years off from surgical training to do research in the laboratory of Drs. [redacted]. and [redacted]. After I completed four years of General Surgery clinical training, I did three years of training in the Early Specialization Program in Cardiothoracic Surgery. As part of that experience, I spent three months in [redacted] as a traveling fellow. Since then I completed three years as an Assistant Professor at the University of [redacted]. Currently I am

an Assistant Professor of Surgery in the Division of Cardiothoracic Surgery at [redacted] and [redacted] Hospital specializing in adult cardiac surgery, including minimally invasive valve and a small incision access for valve replacement. My research interests include ongoing efforts in the investigation of atrial fibrillation and the effects of postoperative fibrillation. Other clinical interests include valve, coronary bypass grafting, and aortic surgery in the adult population.

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	2 years

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

Name► [redacted], PhD
Animal research experience ► 35 years of research in cardiac and thoracic surgery performing sternotomies, thoracotomies, vascular access, cannulation, and perioperative and post operative care in large animals including dogs, sheep, rabbits, pigs, baboons

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	35 years

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

Name► [redacted], DVM, DACLAM

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	DVM, Director of surgical services Dept. Comparative Medicine, [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], MD	Sept 2016	Canine	USDA regulations, large animal ([redacted] IACUC training)

[redacted], PhD	Sept 2016	Canine	USDA regulations, large animal ([redacted] IACUC training)

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] MD	( )	(x) [redacted] EH&S	( )	Yes
[redacted] PhD	( )	(x) [redacted] EH&S	( )	Yes
	( )	(x) [redacted] EH&S	( )	
	( )	(x) [redacted] EH&S	( )	

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], LLC	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 (non-survival)	**0**	**0**	**16**	**16**		**32**

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 (non-survival)	**0**	**0**	**16**	**16**		**32**

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)
Non-survival sternotomy	Continuous monitoring of vital signs and other parameters until euthanized under anesthesia	[redacted] PhD	Propofol induction and isoflurane maintenance; depth of anesthesia assessed by elevation of HR and blood pressure, and response to palpebral eye and toe pinch reflex.

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], DVM
   Institutional affiliation► [redacted] School of Medicine
   email contact► [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted► Dr. [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ►11-7-2016
   VA Veterinary Pre-review performed by [redacted] DVM and VMC on 11-15-16.

M. **Husbandry.** As a reference <u>for the animal husbandry staff</u>, 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

[1] If one dog of an original pair remains, the animal will receive additional environmental enrichment and positive human interaction and play time.

*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] School of Medicine	(x) CFA	( )**	[redacted] Bldg	[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.

Special Features

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
Non-survival sternotomy	**(x)**	**( )**	[redacted] **Building**	**( )**	**(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)**	**( )**
heart	**( )**	**( )**	**(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.)

► Dogs will be examined upon arrival to ensure they are in good health. If health concerns are noted, animals will be euthanized or removed from the protocol based on the recommendations of the attending veterinarian. This is an acute surgery and at the end of the surgery, the dog is euthanized and the heart is removed for tissue analysis.

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

► (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 that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable

( )	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►		( )	( )	( )
( )	Anesthetic overdose Agent► Dose► Route of administration►		( )	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
(x)	Exsanguination under anesthesia Agent► propofol, isoflurane, potassium chloride Dose► 5-7mg/kg, 1-5%, 40-60mEq 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] 2 years experience in the research lab performing surgery and euthanasia using exsanguination.
[redacted] – 35 years in research 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 who may be contacted)
   Name►[redacted], MD
   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. **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 6
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. **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.
   ► non-survival 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)
ALTBIB	4/22/19	2000-present	Sternotomy	Sternotomy, canine	(x)	(x)	(x)	( )
ALTBIB	4/22/19	2000-present	femoral cut down	Femoral cut down, canine	(x)	(x)	(x)	( )
ALTBIB Citations with Animal Use Alternatives as the main topic	4/22/19	All available years	Search for alternatives to using animals	Postoperative atrial fibrillation	(x)	( )	( )	( )
PubMed	4/22/19	All available years	N/A	POAF and (activated neutrophils OR activated monocytes)	( )	( )	( )	(x)
PubMed	4/22/19	All available years	N/A	POAF and inflammatory cytokines	( )	( )	( )	(x)
PubMed	4/22/19	All available years	N/A	POAF and oxidative molecules	( )	( )	( )	(x)

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 will document the electrophysiology of cardiac tissue and its response after being exposed to inflammatory mediators and how they affect its ability to sustain normal/abnormal cardiac rhythms. Our search for alternatives to using animals for this work yielded no papers at all – there are no computer models or other in vitro models available for this research. Cardiac 3-D printing is a promising technology but it has not sufficiently advanced to the point that 3-D printed atria could accurately address the questions we hope to answer in this study (see: https://www.sciencedirect.com/science/article/pii/S1936878X16310142).

   Tissue and cell culture do not have the anatomic detail, or the cellular interconnections and normal gap junction distributions seen in human and canine atria. Cell size and shape are also not the same in tissue and cell culture models as that seen in the canine and human atria. These are essential substrates for the study AF since these affects both conduction and conduction velocity in the tissue.

   Computer simulations are also currently inadequate for studying the effects of inflammation on the atrial tissue. Data generated from the proposed studies will be used to incorporate these details in future models.

   The in vitro canine atria model has been well developed and AF is easily induced using established methods. Testing the inducibility of AF requires a minimal mass of tissue from each animal; canines are the only species that can provide enough tissue and have heart anatomy and physiology that closely matches that of the human heart. As discussed in the species justification above (section D), the canine heart is the far more comparable to the human heart than other available species. Mice, rabbits, pigs, and sheep all have anatomical or physiological differences from humans that make them unsuitable for this research.

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.
► We have calculated the minimal number of animals needed for this study – please see section C2b for details. 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 non-survival surgery, which is performed under general anesthesia and euthanasia is completed while still under anesthesia. No further refinements to the protocol are possible at this time.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
► Our PubMed searches for lack of unnecessary duplication brought up only six papers. Two were meta-analyses of patient studies, two were studies of correlations between various factors and the

occurrence of POAF, one was a clinical trial of statins that showed a non-significant effect, and one was on the effect of low-level vagus nerve stimulation on POAF and inflammation. None of these studies were directly studying the effects of various inflammatory components on heart tissue, which this study will do.

X. **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 Phar-macy	Non-VA
	( )	( )*		( )	( )	( )	(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. **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	Non-survival Sternotomy and harvest of cardiac tissue		9-16-16
M.1	Husbandry		2011
M.2	Enrichment		2011
U.4.a	Animal care staff		9-16-16
U.4.b	Animal care staff		07-2016
V			

Reference to congruency Memo re: Deficiencies 10-19-2017

C.2.c. sternotomy and harvest of cardiac tissue has no SOP. Surgical procedures are written as needed by the research staff.
M.1 husbandry: Guide for the care and use of laboratory animals, eighth edition. Husbandry. (2011) Nat'l Academies press, Washington DC, pp.65-75.
M.2 enrichment: March 2015, Policies procedures and guidelines: Exercise and environmental enrichment for dogs, retrieved from: https://research.[redacted].edu/exercise-environmental-enrichment-dogs-policy/
February 2016, Policies procedures and guidelines: Social Environment and Enrichment, retrieved from: https://research[redacted].edu/social-housing-environmental-enrichment-policy/
U.4.a animal care staff: there is no SOP for disposing of a carcass. As stated above in U.4.a ► Animal undergoes a necropsy to determine cause of death and then is bagged and stored in the freezer until incineration.
U.4.b animal care staff: July, 2016, Policies procedures and guidelines: Reporting Unusual or Unexpected Adverse Events. Retrieved from: https://research.[redacted].edu/reporting-unusual-unexpected-adverse-events-guideline/

Z. **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]	[redacted]	4/26/19

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]**, VMC**		
Name of IACUC Chair	Signature	Date
[redacted]**, M.D.**		

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]	4/26/19
Name of Institutional Veterinarian	Signature	Date
[redacted]**, VMC**		
Name of IACUC Chair	Signature	Date
[redacted]**, M.D.**		

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]	4/26/19

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 <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> 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 – cessation of heartbeat)	Hospira	( )	( )BSL_	( )	( )	( )	x	( )
Saline	Baxter	( )	( )BSL_	( )	( )	( )	x	( )
Calcium chloride	Hospira	( )	( )BSL_	( )	( )	( )	x	( )
Lactated ringers	Baxter	( )	( )BSL_	( )	( )	( )	x	( )
Insulin	Eli Lilly	( )	( )BSL_	( )	( )	( )	x	( )
Sodium bicarbonate	Hospira	( )	( )BSL_	( )	( )	( )	x	( )
Dextrose	Hospira	( )	( )BSL_	( )	( )	( )	x	( )
Epinephrine	International med sys	( )	( )BSL_	( )	( )	( )	x	( )
Phenylephrine	West-ward pharm	( )	( )BSL_	( )	( )	( )	x	( )

Atropine	American regent	( )	( )BSL_	( )	( )	( )	x	( )
Lidocaine	Hospira	( )	( )BSL_	( )	( )	( )	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	vehicle	IV	As needed	Fluid maintenance	App. 5, 2.) surgery 1	Y
LRS	10-20ml/kg, 500ml	vehicle	IV	As needed	Fluid maintenance	App. 5, 2.) surgery 1	Y
Propofol	5-7mg/kg, 20ml	vehicle	IV	once	anesthesia	App. 5, 2.) surgery 1	N
Isoflurane	1-5%, 100ml	vehicle	IH	During surgery	anesthesia	App. 5, 2.) surgery 1	Y
KCl	2-10MEq in saline	vehicle	IV	As needed	Hypokalemia; Cessation of cardiac activity	App. 5, 2.) surgery 1	Y
CaCl	3−20mg/kg, 10ml	vehicle	IV	As needed	hypocalcemia	App. 5, 2.) surgery 1	Y
Insulin	0.25−0.5 U/kg, 5 ml	vehicle	IV	As needed	hyperkalemia (insulin drives glucose into cells, which results in an intracellular shift of potassium). Hyperkalemia is unlikely	App. 5, 2.) surgery 1	Y
Sodium Bicarbonate	Calculated Meq According To Base Excess	vehicle	IV	As needed	acidosis	App. 5, 2.) surgery 1	Y

Dextrose	0.5 – 1.0 G/kg, 50 ml	vehicle	IV	As needed	hypoglycemia	App. 5, 2.) surgery 1	Y
Epinephrine	0.01−0.02mg/ kg	vehicle	IV	As needed	Cardiac support, arrest	App. 5, 2.) surgery 1	Y
Phenylephrine	1−3ug/kg/min or 100ug Bolus	vehicle	IV	As needed	hypotension	App. 5, 2.) surgery 1	Y
Atropine	0.05mg/kg	vehicle	IV	As needed	bradycardia	App. 5, 2.) surgery 1	Y
Lidocaine	30−70 ug/kg/hr IV, 2−4mg/kg Bolus		IV	As needed	Arrhythmias	App. 5, 2.) surgery 1	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):

Isoflurane 1-5%, IH During surgery anesthesia
NaCl 10-20ml/kg, 500ml, IV As needed, Fluid maintenance
Lactated Ringers Solution (LRS) 10-20ml/kg, 500ml, IV As needed, Fluid maintenance
KCL 2-10MEq, IV As needed, hypokalemia
CaCl 3−20mg/kg, 10ml, IV As needed, hypocalcemia
Insulin 0.25−0.5 U/kg, 5 ml, IV As needed, hyperkalemia
Sodium Bicarb Calculated Meq according To Base Excess, IV As needed, acidosis
Dextrose 0.5 – 1.0 G/kg, 50 ml, IV As needed, hypoglycemia
Epinephrine 0.01−0.02mg/kg, IV As needed, Cardiac support, cardiac arrest
Phenylephrine 1−3ug/kg/min or 100ug Bolus, IV As needed, hypotension
Atropine 0.05mg/kg, IV As needed, bradycardia
Lidocaine 30−70 ug/kg/hr Iv,2−4mg/kg Bolus IV As needed Arrhythmias

   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).

		b. Drug Sensitivity Panel Available? (Describe)	c. Select Agent?

Name and BSL Number of Infectious Agent	a. ABSL Number *		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**	**210 ml**	**no**	**1**	**Terminal collection**

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**	**Propofol induction with isoflurane maintenance**

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. Blood is also collected for isolation of neutrophils and monocytes for in vitro experiments. 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 to expose the heart. Briefly, the skin subcutaneous tissue over the sternum are incised with a scalpel to expose the sternum.. Using a bone saw, the sternum is

cut on midline, which exposes the heart. The heart is removed en bloc. 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.
The following drugs will be administered before and during surgery. Each dose, route of administration and reason is listed.

Propofol 5-7mg/kg, 20ml, IV, once anesthesia
Isoflurane 1-5%, IH During surgery anesthesia
NaCl 10-20ml/kg, 500ml, IV As needed, Fluid maintenance
LRS 10-20ml/kg, 500ml, IV As needed, Fluid maintenance
KCL 2-10MEq, IV As needed hypokalemia
CaCl 3−20mg/kg, 10ml, IV As needed, hypocalcemia
Insulin 0.25−0.5 U/kg, 5 ml, IV As needed, hyperkalemia (drives glucose into cells causing intracellular shift of potassium).
Sodium Bicarbonate Calculated Meq according To Base Excess, IV As needed, acidosis
Dextrose 0.5 – 1.0 G/kg, 50 ml, IV As needed, hypoglycemia
Epinephrine 0.01−0.02mg/kg, IV As needed, Cardiac support, cardiac arrest
Phenylephrine 1−3ug/kg/min or 100ug Bolus, IV As needed, hypotension
Atropine 0.05mg/kg, IV As needed, bradycardia
Lidocaine 30−70 ug/kg/hr Iv,2−4mg/kg Bolus IV As needed, arrhythmias

As stated above, the dog is “EUTHANIZED AS A RESULT OF EXSANGUINATION”. The heart is removed and the atria is prepared and studied utilizing an isolated perfused preparation.

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, and 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], MD	1	(x)	( )	( )	( )
[redacted], PhD	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]	[redacted]	1	(X)	( )*	( )*
			( )	(*)	( )*
			( )	( )*	( )*
			( )	( )*	( )*

*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 and draped. Surgeons will wear sterile gloves and surgical attire.

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 <u>during</u> 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 <u>during</u> surgery (e.g., warming, cushioning, etc.).
►circulated water warming blanket placed under the dog and warm air blanket placed over the dog.

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 <u>during</u> surgery.

►elevation of HR, blood pressure, palpebral eye reflex, toe pinch, spontaneous breaths, mucus membranes' color, and eye position are assessed continuously.

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*
1	Non-survival[1]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*

[1]Note: Aseptic technique is not required for non-survival surgery but is used to limit recruitment of neutrophils to the atrial tissue.

* 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 ►N/A

   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	**N/A**				
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)
N/A					

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

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
N/A			

(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)
N/A			

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 ► N/A

Surgery 2 ►

Surgery 3 ►

Surgery 4 ►

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

Surgery 1 ► N/A

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.)
►N/A

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	N/A		( )	( )
2			( )	( )
3			( )	( )
4			( )	( )

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

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
[redacted]	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
● *6/5/19*	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.
● 12/15/16	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).* 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: (1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *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.) *A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov : (1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC.

			*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).*

12/15/16
The ACORP for Dr. [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 Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted] or ([redacted]) [redacted].

6/5/19
The ACORP for Dr. [redacted] has received an overall score of 0, which means that it is cleared and represents no bar to funding the application. The investigator and the IACUC are commended for their collective efforts to ensure humane animal care and use.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted] or ([redacted]) [redacted].

REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
	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.
ACORP (mouse)	*On 6/4/19, a revised ACORP and IACUC approval letter were provided; this study uses dogs in a non-survival study to investigate if fluid in the chest and pericardial fluid after heart surgery causes Postoperative Atrial Fibrillation. In the revised version of the ACORP, a strong justification documenting that only the canine model can be used as opposed to other species, such as sheep, pigs, rabbits, and rodents was included. The research team worked extensively with Dr. [redacted] (the reviewer) and Dr. [redacted] to optimize the revised ACORP. As a result, no new concerns were identified. No*

	further correspondence with the CVMO is needed; the ACORP is cleared and represents no bar to funding the application.
Item T (1) *(0)*	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. *Resolved.*

Melby updated literature search

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)
ALTBIB	4/22/19	2000-present	Sternotomy	Sternotomy, canine	(x)	(x)	(x)	( )
ALTBIB	4/22/19	2000-present	femoral cut down	Femoral cut down, canine	(x)	(x)	(x)	( )
ALTBIB Citations with Animal Use Alternatives as the main topic	4/22/19	All available years	Search for alternatives to using animals	Postoperative atrial fibrillation	(x)	( )	( )	( )
PubMed	4/22/19	All available years	N/A	POAF and (activated neutrophils OR activated monocytes)	( )	( )	( )	(x)
PubMed	4/22/19	All available years	N/A	POAF and inflammatory cytokines	( )	( )	( )	(x)
PubMed	4/22/19	All available years	N/A	POAF and oxidative molecules	( )	( )	( )	(x)

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 will document the electrophysiology of cardiac tissue and its response after being exposed to inflammatory mediators and how they affect its ability to sustain normal/abnormal cardiac rhythms. Our search for alternatives to using animals for this work yielded no papers at all – there are no computer models or in vitro models available for this research.

The in vitro canine atria model has been well developed and AF is easily induced using established methods. **Testing the inducibility of AF requires a minimal mass of tissue from each animal, and canines are the lowest species that can feasibly be used for this study because they can both provide enough tissue and they have suitable heart tissue physiology.** As discussed in the species justification above (section D), the canine heart is much closer to the human heart than other available species. Mice, rabbits, pigs, and sheep all have anatomical or physiological differences from humans that make them far less suitable than canines for this research.

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.

   ► We have calculated the minimal number of animals needed for this study – please see section C2b for details. 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 non-survival surgery, which is performed under general anesthesia and euthanasia is completed while still under anesthesia. No further refinements to the protocol are possible at this time.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.

   ► Our PubMed searches for lack of unnecessary duplication brought up only six papers. Two were meta-analyses of patient studies, two were studies of correlations between various factors and the occurrence of POAF, one was a clinical trial of statins that showed a non-significant effect, and one was on the effect of low-level vagus nerve stimulation on POAF and inflammation. None of these studies were directly studying the effects of various inflammatory components on heart tissue, which this study will do.