[For IACUC Use Only]

Initial Approval Date: [redacted] *Current Approval Date:* [redacted]

☐ Maj. Surv. Surgery ☐ Departures ☐ Cat. E studies ☐ Haz. Agents ☒ USDA reg.

Clement J. Zablocki VA Medical Center

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)

(Rev 10/13)

Directions: Use a separate form for each species. DO NOT include individual appendices if they are not relevant to the protocol being described. To check (☒) boxes, position cursor to the left of the check box, double click and then select "Checked". Define all abbreviations the first time they are used. To add a row to a table, click inside one of the existing table cells, then select Table, Insert, Rows from the main menu of the program.

A. ACORP Status.

1. **Name of Principal Investigator:** [redacted]

2. **Proposal Title:** Determining organ perfusion during vascular compromise

3. **Animal Species covered by this ACORP (only one):** Canine

4. **Funding Source. Indicate the source(s) of funds that will be used to perform these animal procedures once approved by the VA Institutional Animal Care and Use Committee (IACUC), known as the Subcommittee for Animal Studies at this facility:**

   ☐ Department of Veterans Affairs
   ☐ U.S. Public Health Service (e.g. NIH)
   ☐ Private or Charitable Foundation: **Identify:**
   ☒ University Intramural Funds: **Identify University and Funding Component:** Medical College of Wisconsin; Department of Anesthesiology Departmental Funds
   ☐ Private Company: **Identify:**
   ☐ Other: **Identify:**

5. **Indicate the status of this ACORP below.**

   ☒ This is a new ACORP for a new project.
   ☐ This is a revised ACORP that reflects changes or additional, new studies.
   ☐ This ACORP is submitted as a three-year (3-year) renewal. **Provide a separate progress report describing work accomplished during the last approval period.** Include the number of animals used, the objectives that were met and how the work proposed in this renewal extends the previous studies.
   ☐ Other: **Specify:**

6. **Indicate the type of animal use.**

   ☒ Research
   ☐ Teaching or training
   ☐ Testing
   ☐ Breeding and colony management only (no experimental procedures)
   ☐ Holding protocol (as specified by local requirements)
   ☐ Other: **Specify:**

[redacted]

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.

During liver and kidney transplantation surgeons must connect the vessels of the new organ with the patient's vessels to supply the organs with oxygen and nutrients. Various factors (surgical technique, hemodynamic instability, blood pressure and medication effects) may compromise the flow of blood through the newly anastomosed vessels. The current practice for liver transplants is a scheduled ultrasound 2-3 times a day and frequent blood tests to determine whether the blood vessel is still open. The ultrasound may miss a vascular compromise by hours till the graft has suffered permanent damage. The blood tests are not specific for vascular occlusion and can be elevated if acute rejection of the organ takes place.
Near Infrared Spectroscopy (NIRS) is a technology that measures the oxygen content of organ tissue and thus indicates oxygen delivery to and oxygen consumption in the measured tissue. It is frequently used in clinical practice in adult and pediatric patients where it is placed on the forehead to evaluate the cerebral circulation and oxygen delivery or on the abdomen and flank to measure perfusion of the abdominal organs or the kidneys. NIRS is a very reliable monitor but its use is limited because it uses infrared light that can only penetrate 3-4cm of tissue, i.e., when applied to the skin it may not reach the tissue of interest. We plan to evaluate a novel approach to measuring organ perfusion by placing a modified NIRS probe directly on the surface of the liver, kidneys and small bowel and then to change the blood flow to these organs mechanically and pharmacologically to determine how sensitive NIRS measurements are to detect organ ischemia compared to other established methods like blood sampling and Doppler ultrasound measurements.

C. **Experimental Design.**

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

These experiments will be performed on live dogs that will be deeply anesthetized for the entire duration of the experiment. The animals are monitored and treated with medication for anesthesia, blood pressure control, fluid status like human patients. Surgery is performed to open the abdomen and access the blood vessels leading to the individual organs. We will place NIRS probes on the skin overlying these organs and also modified NIRS probes (encased in silicone) directly on the organs. We will then reduce the blood flow to the vessels mechanically and with intravenous medication and observe the changes in NIRS values as well as in liver function tests and lactic acid values that are drawn from the arterial line. We will modify the geometry of the NIRS probe to determine the tissue penetration that yields clinically most valuable results.

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.**

This study is designed to determine whether internal NIRS measurements can be a valid tool to assess organ oxygen delivery. We will use clinically used transdermal patches and reconfigure them with silicone casing to rest on the organs of interest. We will determine the sensitivity of internal NIRS measurements for decreases in organ perfusion from different causes, i.e., mechanical or pharmacological flow

[redacted]

restriction. A total of 10 dogs will be used to establish a surgical experimental model and to determine the optimal configuration for an internal NIRS probe.

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

Because this is a pilot study establishing feasibility we do not anticipate the use of more than 10 animals.

c. **Describe each procedure to be performed on any animal on this protocol.**
Any procedures that involve "departures" from the standards in the *Guide for the Care and Use of Laboratory Animals* require justification.

a) Animals will be fasted for 12 hours before surgery. Animals will receive intramuscular premedication before leaving the VMU.
b) Inhalational induction: The animal is placed on a table and the head is gently placed in a facemask. The animal spontaneously breathes 5% isoflurane in 100% oxygen until asleep. A sufficient anesthetic level is ascertained by pinching the ear and the anesthetic is immediately deepened if the animal shows any movement.
c) Intubation and ventilation: In case of no reaction to the painful stimulus, the animal is turned supine and intubated with a 7.0 cuffed endotracheal tube. The tube is then connected to an anesthesia machine (Ohmeda CD) and the animal is ventilated with isoflurane in air/oxygen mixture with FiO2 0.6. CO2 is maintained at mild hypercapnia (~45 mmHg). General anesthesia with isoflurane is continued until the end of the experiment. Endtidal anesthetic concentration is measured continuously with a POET IQ (Criticare) and maintained at 1-2 Vol%. A heat-moisture exchanger is added between endotracheal tube and ventilator circuit to reduce temperature loss.
d) Line placement: Both groins are injected with lidocaine SQ. A cut-down is performed on both sides and lines are inserted in one femoral artery and both femoral veins. The arterial line is used for continuous blood pressure monitoring. The line is flushed with heparinized Lactated Ringer's. One venous line is used for continuous administration of fluids (Lactated Ringer's solution) and drug infusion (phenylephrine, phentolamine, norepinephrine, epinephrine, milrinone). The second IV allows intermittent fluid boluses, application of bolus drugs and blood draws.
e) Surgical procedure: For the surgical procedure the animal is placed on a warming blanket and the temperature is maintained between 36.5 and 37.5 °C for the entire experiment. The abdominal skin is infiltrated with lidocaine and a midline incision or star incision will be used to allow access to the abdominal viscera (liver, kidneys, small bowel) and their vasculature.. Vessels will be dissected free and controlled with vessel loops.
f) Experimental measurements, vascular occlusion and drug infusion: Transcutaneous NIRS probes will be placed on the shaved skin overlying the organ of interest, i.e., where the NIRS value is typically obtained clinically. Modified (soft, silicone-encased) NIRS probes will be placed directly on various parts of the organ. Blood flow to the organ will then be restricted by reducing systemic arterial, and for the liver also portal arterial, blood flow mechanically or through vasoactive drugs, and the changes in organ oxygenation will be measured with NIRS. Additional monitors of tissue oxygenation will be used to validate the NIRS measurements. Simultaneously, oxygen saturation, lactic acid and other metabolic indicators are determined from blood samples drawn from the respective organ draining veins.
g) Multiple protocols are performed for liver, gut and kidney during every experiment.
h) Euthanasia: At the end of the experiment KCl 2mEq/ml, 3 ml/kg is injected intravenously and cardiovascular collapse is verified by the arterial tracing and complete loss of expiratory CO2. Only then ventilation and anesthesia are discontinued.

D. **Describe the characteristics of the selected species, strain, stock, mutant, or breed that justify its use in the proposed study.**
Consider such characteristics as body size, species, strain, breed, availability, data from previous studies, and unique anatomic or physiologic features.

We hope to translate the results from these studies directly to our pediatric transplant patients where compromised organ perfusion either from blood clot or vasoactive medication plays a critical role in

[redacted]

organ survival and patient welfare. The canine model was chosen because the anatomy and organ size closely resemble pediatric patients.
The animals are donated by a commercial contract research laboratory as part of their commitment to reduce the number of animals used in research. Experiments will only be performed when donated animals are available.

Personnel

E. **Complete Appendix 1, providing the names of all research staff expected to work with the animals in this study.**

F. **If any of the personnel listed in Appendix 1 do not have experience with the exact procedures described in this ACORP, indicate how they will be trained, who will train them, and provide the training experiences or qualifications of the person(s) doing the training.** If not applicable, enter "N/A".

G. **Occupational Safety and Health.**

1. **In Appendix 1, if personnel listed are enrolled in the VA Occupational Health and Safety Program for those with laboratory animal contact, check "In VA Occupational Health Program". If personnel have declined to participate or are enrolled in another equivalent program, check "Declined Occupational Health enrollment".**
The PI is responsible for confirming the current status of those involved in the project.

2. **Are there any non-routine measures such as special vaccines or additional health screening techniques that would potentially benefit research, husbandry, or veterinary staff participating in or supporting this project?**
Routine measures included in the Occupational Health and Safety Program (vaccination for tetanus, rabies, and hepatitis B, and TB screening) need not be mentioned here.

☒ No. Proceed to item H.
☐ Yes. **Describe them.** Then proceed to item H.

Animal Use Information

H. **Complete the table below.** Then proceed to item I.

Description	Gender	Age/Size	Source (Vendor, Collaborator)	Health Status*
Canine	Male or Female	Adult/ 8-12+kg	[redacted]	Conventional

*Provide information about the expected status of the animals:
-For rodents and rabbits, indicate specific-pathogen-free (SPF), gnotobiotic (germ-free or defined flora), conventional, feral, or other description.
-For dogs, cats, pigs, and other "large animals", indicate specific-pathogen-free (SPF), conditioned, conventional, feral, or other description.
-Also indicate here if animals will be surgically altered by the vendor (e.g., ovariectomized rats).

I. Complete the tables below, assigning all requested animals to a USDA category of pain/distress.
If several different procedures are planned, the animal should be placed in a category based on the most painful/distressful procedure. If the period of funding extends beyond three years, you may include entries for years 4 and 5. However, IACUC approval does not extend beyond 3 years.

USDA Category B: List by year the number of animals that will be bred or purchased for

[redacted]

breeding, but not used for experiments.
This includes breeders, young that cannot be used because of improper genotype or gender, and any other animals that will not have any research procedures performed on them or participate in research studies. If numbers cannot be determined exactly, estimate as closely as possible. (Note: If tail snips are necessary for genotyping, this category is not appropriate.)

Description	Year 1	Year 2	Year 3	Year 4	Year 5

USDA Category C: List, by year the number of animals that will undergo procedures that involve no or only very brief pain or distress, with no need for or use of pain relieving drugs.
Examples include observational studies, most intravenous and parenteral injections of non-irritating agents, most blood collections from peripheral vessels, and the collection of cells and/or tissues from animals after euthanasia has been performed.

Description	Year 1	Year 2	Year 3	Year 4	Year 5

USDA Category D: List by year the number of animals that will undergo procedures involving potential pain or distress that is relieved by appropriate anesthetics, sedatives, or analgesics.
Examples include major and minor surgery performed under anesthesia (survival or non-survival), tissue or organ collections prior to euthanasia, painful procedures performed under anesthesia (such as retro-orbital blood collection in rodents), prolonged restraint accompanied by tranquilizers or sedatives, and experiments involving infectious or other hazardous materials in animals that have provisions for immediate euthanasia if they become sick to effectively prevent pain and/or suffering. If an endpoint is used that involves significant pain or distress, consideration should be given to putting animals into Category E.

Description	Year 1	Year 2	Year 3	Year 4	Year 5
Canine	10				

USDA Category E: List, by year, the number of animals that will undergo procedures in which pain or stress is NOT relieved with the use of anesthetics, analgesics, tranquilizers, or by euthanasia.
Examples include studies in which animals are allowed to die without intervention (e.g. LD_{50}, mortality as an end-point), studies that allow endpoints that are painful or stressful, addictive drug withdrawals without treatment, pain research, and noxious stimulation.

Description	Year 1	Year 2	Year 3	Year 4	Year 5

TOTALS: Bring all totals for each year down.

Description	Year 1	Year 2	Year 3	Year 4	Year 5
	10				

J. **Description of USDA Category D. Are any USDA Category D studies planned?**

☐ No. Proceed to item K.
☒ Yes. **Complete the table below.** Then proceed to item K.

List and describe all category D procedures.
For any surgical procedures you will describe in Appendix 5, enter only a brief description in the "Procedure" column, then enter "See Appendix 5 for details."

Procedure	Frequency of	Analgesic, sedative,

[redacted]

Identify persons responsible for monitoring Category D procedures in Appendix 1.	monitoring after the procedure and how long animals will be monitored	or anesthetic used, plus dose, route, and duration
General anesthesia, ventilation, laparotomy and measurement of organ perfusion with near infrared spectroscopy probes and other validated measures of organ perfusion on the internal organs. Reduction of organ perfusion and assessment of the NIRS measurements/ tissue oxygenation during the state of relative and total ischemia.	N/A (non-survival)	Premedication with Telazol (6-10 mg/kg, IM). Induction with isoflurane 5%. Maintenance throughout: 1-2% isoflurane;

K. **Description of USDA Category E procedures. Are any USDA Category E studies planned?**

☒ No. Proceed to item L.

☐ Yes. **Describe each category E procedure, and justify completely why pain or distress relief cannot be provided for each procedure.** Then proceed to item L.

If animals will be allowed to experience natural death as a result of experimental procedures (e.g. infectious disease or oncology studies), or an endpoint is used that allows the animals to experience significant pain or distress, you must justify why an alternate endpoint (such as weight loss, clinical signs, tumor size, etc.) prior to death or pain or distress can not be used.

L. **Laboratory Animal Veterinary Support.**

1. **Give the name of the laboratory animal veterinarians responsible for providing adequate care to the animals that will be used and their institutional affiliation:**

[redacted]

2. **Indicate the name of the laboratory animal veterinarian(s) consulted during the planning of procedures involving animals.**

VA Policy requires that a laboratory animal veterinarian be consulted during the planning stages of any procedure involving laboratory animals, before IACUC review. As an alternative to an actual meeting, the veterinarian may perform a pre-review of the ACORP and provide comments to the PI so that the ACORP may be revised prior to IACUC review. (If you want the IACUC Administrator is to arrange for veterinary pre-review, skip to item M.)

☐ [redacted]
☐
☒
☐ Other: **Identify:**

3. **Give the date of the veterinary consultation:**

Date can be provided by the IACUC Administrator based on the VMC review of information provided herein.

Pre-submission
Initial Review [redacted]

M. **Husbandry.**

1. **Caging needs. Indicate the type of caging that you will need.**

This will help the animal care staff with caging needs.

[redacted]

- ☐ Gnotobiotic (germ-free and defined flora) isolators
- ☐ Biohazard or other special hazard containment caging
- ☐ Sterile rodent microisolator caging, with filtered cage top
- ☐ Non-sterile rodent microisolator caging, with filtered cage top
- ☐ Standard rodent shoebox caging with no filter top
- ☒ Standard non-rodent caging, appropriate for species
- ☐ Other: **Describe:**

2. **Will social animals be housed singly?**

- ☐ Not applicable; the species involved is not a social animal. Proceed to item M3.
- ☒ No. Proceed to item M.3.
- ☐ Yes. **Provide a justification for housing social animals singly.** Then proceed to item M3.

3. **Will rodents be housed on suspended wire mesh floors or other flooring in which the animals do not rest on bedding?**

- ☒ Not applicable; this ACORP does not describe rodent use. Proceed to item M4.
- ☐ No. All rodents will be housed in shoebox or other caging in which the animals rest directly on bedding. Proceed to item M4.
- ☐ Yes. **Justify the need for wire mesh flooring.** Then proceed to item M4.

4. **Enrichment. Will "standard" exercise and environmental enrichment be provided to the animals on this protocol?**

- ☒ Yes. Proceed to item M5.
- ☐ No. **Describe any special supplements or restrictions that will be required.** Then proceed to item M5.

5. **Does this protocol involve the use of genetically engineered or modified (e.g. transgenic, knock-out or knock-in) animals?**

- ☒ No. Proceed to item M6.
- ☐ Yes. **Describe any characteristic clinical signs or abnormal behavior related to their genotype.** Then proceed to item M6.

6. **Will any cannulae, acrylic implants, venous catheters, or other similar medical devices be implanted into an animal such that the device extends chronically through the skin?**

- ☒ No. Proceed to item N.
- ☐ Yes. **Explain what implantation and wound management measures will be taken to minimize the chances of chronic infections around the device(s) where they penetrate the skin**. Then proceed to item N.

N. **Housing Sites**. **Give the location(s), inside or outside the animal facility, where animals will be housed permanently or temporarily.**
Institutions housing animals must have full AAALAC accreditation.

[redacted]

VA Veterinary Medical Unit- Room to be determined by husbandry staff.

VA location outside the VMU: Specify location/room:

Non-VA animal facility- Room to be determined by husbandry staff.

Non-VA location outside the animal facility: Specify location/room:

[redacted]

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

☒ No. Proceed to item P.
☐ Yes. **Complete and attach Appendix 2, "Antibody Production."** Then proceed to item P.

P. **Test Substances. Will test substances be administered to animals?**

☐ No. Proceed to item Q.
☒ Yes. **Complete and attach Appendix 3, "Test Substances."** Then proceed to item Q.

For the purposes of this question, test substances are defined as materials administered to animals. This includes, but is not limited to, radioisotopes, toxins, antigen, pharmacological agents, infectious agents, carcinogens or mutagens, biomaterials, prosthetic devices, and cells, tissues, or body fluids. The following substances do not need to be entered in Appendix 3 unless they are hazardous: drugs used for routine husbandry or veterinary care, routine pre- intra- or post-operative drugs described in the Surgery Appendix [Appendix 5], antigens, adjuvants, hybridomas described in the Antibody Production Appendix [Appendix 2], and euthanasia agents entered in item U, Euthanasia.

Q. **Location of procedures. Complete the table below, indicating where all non-surgical procedures and manipulations will be performed.**

The IACUC must be aware of all procedures performed outside of the animal facility. Give the location of any laboratory or other areas outside of the animal facility in which animals will be manipulated in any way. Be sure to include the sites of procedures such as radiography, fluoroscopy, computed axial tomography (CT), or magnetic resonance imaging (MRI) that may be performed outside the animal facility.

Non-surgical Procedure	Building and Room Number	Method of discreet transport, if required through non-research areas (enter N/A if not applicable)*
[redacted]	[redacted]	N/A

*Transportation must be in accordance with the *Guide*, USDA regulations, and PHS policy in climate-controlled vehicles and sanitizable transport cages when appropriate. Such transport must be discreet such that hospital staff and patients are not aware of the transport, and are not exposed to allergens and/or body fluids from the transported animal(s).

R. **Body Fluid, Tissue, and Device Collection.**

1. **Will any body fluids, tissues, or devices be collected from animals AFTER euthanasia?**

   ☒ No. Proceed to item R2.
   ☐ Yes. **List the fluids, tissues, and/or devices.** Then proceed to item R2.

2. **Will any body fluids, tissues, or implanted devices or materials be collected from animals BEFORE euthanasia?**

   ☐ No. Proceed to item S.
   ☒ Yes. Proceed to item R3.

3. **Is collection in live animals limited to blood collection associated with antibody production?**

   ☐ Yes. Proceed to item S.
   Because blood collection associated with antibody collection is already described in Appendix 2, "Antibody Production", DO NOT complete Appendix 4, "Antemortem Specimen Collection."

[redacted]

☒ No. **Complete and attach Appendix 4, "Antemortem Specimen Collection."** Then proceed to item S.
If the body fluid, tissues, or devices are collected as a surgical procedure, please be sure to also describe these collections as part of the surgical protocol in Appendix 5, "Surgery".

S. **Surgery. Will survival or non-survival surgery be performed?**

☐ No. Proceed to item T.
☒ Yes. **Complete and attach Appendix 5, "Surgery."** Then proceed to item T.

T. **Endpoint Criteria. What specific endpoint criteria will be used for determining when sick animals, either on and off study, will be euthanatized or otherwise removed from a study?**
Examples of appropriate criteria that should be considered include a weight loss limit as a percentage of initial or expected body weight, allowable durations of anorexia, allowable tumor size or total tumor burden expressed as a percentage of body weight, the presence of health problems refractory to medical intervention, and severe psychological disturbances. Other criteria appropriate for the species under consideration should also be considered.

The experiments will be acute non-survival experiments with the animals euthanized at the end of the experimental procedure. The animals will be delivered just in time to minimize caging periods. The PI will defer to animal facility staff on treatment of sick animals.

The following grading scale will be used:

Body weight changes (compared to arrival values)
- 0-normal
- 1-<10 percent weight loss
- 2-10-15 percent weight loss
- 3- >20 percent weight loss

Body condition
- 0-normal
- 1-slight segmentation of vertebral column and dorsal pelvic bones slightly palpable
- 2-segmentation of vertebral column evident and dorsal pelvic bones are readily palpable
- 3-animal is emaciated; skeletal structure extremely prominent with little or no flesh cover and vertebrae distinctly segmented

Respiratory (compared to other animals)
- 0-normal
- 1-minor increase in rate or effort
- 2-moderate increase in rate and effort
- 3-dyspnea (major increase in respiratory effort/rate and/or open-mouth breathing

Behavioral responses to external stimuli
- 0-normal
- 1-minor depression (mildly decreased alertness, responsiveness to handlers, and/or interest in play)
- 2-moderately decreased activity (further decreased alertness, depressed responsiveness to handlers, and/or no interest in activity/play)
- 3-comatose or minimal (forced) response to tactile stimulation

Gastrointestinal
- 0-normal
- 1-Vomiting up to 3 times in one day
- 2-vomiting up to 4-5 times/day and/or small amount of blood noted in vomit

[redacted]

- 3-vomiting 6 or more times/day and/or large amount of blood noted in vomit

A VMC will be consulted for any scores of 1 or more to determine if medical intervention is warranted. If an animal scores a 3 in any category, or earlier if determined necessary by the VMC, it will be euthanized.

U. **Euthanasia. Will animals be euthanatized as part of the planned studies?**

☐ No. **Describe the final disposition of the animals here.** Then proceed to item U4.
☒ Yes. **Complete items U1 - U4** Then proceed to item V.

1. **Describe the exact method of euthanasia for each animal used.** Include the agents used, dose (as applicable), and route of administration.

KCl 6 mEq/kg delivered intravenously. Animals are anesthetized with isoflurane at that time.

2. **Are all euthanasia methods acceptable according to the latest report of the AVMA Panel on Euthanasia?**

☒ Yes. Proceed to item U3.
☐ No. **Justify any method that is not considered "acceptable" by the latest report of the AVMA Panel on Euthanasia.** Then proceed to item U3.

3. **In Appendix 1, list the personnel who will perform euthanasia and indicate their training and experience with the method of euthanasia and the species involved.**

1. **If the animal care staff find an animal dead, how should the carcass be handled (e.g. refrigerated or frozen), and should a member of your staff be contacted immediately?**

Label carcass and place in cooler. Notification of the PI and/or a staff member should be done immediately.

V. **Other Procedures.**

1. **Are any special husbandry procedures planned that are NOT described in the VMU standard operating procedures (SOP) manual?**
Examples include provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

☒ No. Proceed to item V2.
☐ Yes. **Complete and attach Appendix 6, "Special Husbandry and Other Procedures."** Then proceed to item V2.

2. **Are any other procedures planned that are NOT described in detail elsewhere in this ACORP or an attached SOP?**
Examples include prolonged restraint, use of noxious stimuli, forced exercise, behavioral conditioning, irradiation and imaging studies.

☒ No. Proceed to item W.
☐ Yes. **Complete and attach Appendix 6, "Special Husbandry and Other Procedures."** Then proceed to item W.

W. **Consideration of Alternatives and the Prevention of Unnecessary Duplication. Does this protocol involve any potentially painful or distressing procedures?**

☐ No. Proceed to Item X.
☒ Yes. **Complete items 1 - 6 below.** Then proceed to Item X.

[redacted]

1. **Complete the table below for each database search you conduct to answer items W2 - W5.**

Investigators must consider less painful or less stressful alternatives to procedures, and provide assurance that proposed research does not unnecessarily duplicate previous work. You should perform one or more database searches to meet these mandates unless compelling justifications can be made without doing so. You must provide complete information in the first four columns of the table to comply with USDA Policy. Keep copies of computer database search results in your files to demonstrate your compliance with the requirement if regulatory authorities or the IACUC should choose to audit your project.

Database ⇩	Date of search ⇩	Years covered by the search ⇩	Potentially painful or distressing procedures addressed ⇩	Key words and/or search strategy used ⇩	Replacement of animals (Item W2.) ⇩	Reduction in number of animals used (Item W3.) ⇩	Refinement to minimize pain or distress (Item W4.) ⇩	Lack of unnecessary duplication (Item W5.) ⇩
Mandates:								
PubMed	December 2016	till December 2016	Laparotomy	Liver surface Near Infrared Spectroscopy	x	x	x	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 refers to methods that use animals lower on the phylogenetic scale or that avoid the use of animals.

Because of the direct clinical relevance of these studies, animal studies investigating organ blood flow and mechanical and pharmacological effects on such are usually performed in larger mammals, i.e., dogs and pigs. The volume of blood draws necessary to validate the new, investigational monitor requires an animal size > 5kg. Our laboratory has extensive experience with in vivo dog experiments, which should minimize attrition. In addition, these dogs will preferably be obtained as part of a donation program from a commercial research laboratory, i.e., use of these animals will not add to the number of research animals used in the country. If meaningful results are obtained with less animals, the number of experiments will be reduced.

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.**

This refers to design or technological methods for obtaining comparable information using fewer animals.

The sample size is kept to the smallest possible number to allow for a relevant sample size.

4. **Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.**

This refers to modification of procedures to enhance animal well-being and minimize pain and distress.

[redacted]

Each animal will be completely anesthetized during the procedure and euthanized immediately following, which means they will not consciously experience any pain or distress.

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

There is one single published study where near infrared spectroscopy was used on the internal surface of the liver in a porcine model. This study was limited to discrete obstruction of liver vessels and used internal measurements only to contrast with transcutaneous measurements rather than to systematically investigate the feasibility and validity of internal NIRS measurements to determine blood flow under a variety of conditions.

X. **Other Regulatory Considerations**.

1. **Will drugs classified as controlled substances be used in animals?**

☐ No. Proceed to item X2.
☒ Yes. **Complete item X1a and X1b** Then proceed to item X2.

a. **List the controlled substances that will be used in animals for this project here, and include the building and room number where they will be stored.**
Telazol 6-10 mg/kg IM for premedication, stored in VMU

b. **Will the use of all controlled substances comply with VA Pharmacy policies?**
To comply with VA Pharmacy policies, all controlled substances used on VA property must be ordered through and received by the local VA pharmacy prior to issue for research use. Controlled substances must be stored in a double-locked cabinet and be accessible only to authorized personnel. Controlled substances procured through the VA Pharmacy cannot be removed from VA property.

☒ Yes. Proceed to item X2.
☐ No. **Explain.** Then proceed to item X2.

2. **Will any non-pharmaceutical grade chemicals or substances be used in any animal-related procedures?**
This includes all test substances, drugs administered pre-, intra- or post-operatively or administered for routine husbandry or veterinary care.

☒ No. Proceed to item X3.
☐ Yes. **List each non-pharmaceutical grade chemical or substance, explain why the use of the formulation is necessary and describe how it will be ensured that the material is suitable for use.** Then proceed to item X3.

3. **Will any human patient procedural areas be used for these animal studies?**

☒ No. Proceed to item X4.
☐ Yes. **Complete and attach Appendix 7, "Request to Use Patient Procedural Area."** Then proceed to item X4.

4. **Will an explosive anesthetic or other explosive agent be used in any portion of these animal studies?**

☒ No. Proceed to item Y.
☐ Yes. **Complete and attach Appendix 8, "Request to Use Explosive Agent."** Then proceed to item Y.

[redacted]

Y. **Appendices. Please indicate which of the following Appendices are completed and attached.** Do not attach blank appendices, which are not applicable to this ACORP.

- ☒ Appendix 1, *Personnel* (Required.)
- ☐ Appendix 2, *Antibody Production* (ref item O.)
- ☒ Appendix 3, *Test Substances* (ref item P.)
- ☒ Appendix 4, *Antemortem Specimen Collection* (ref item R.)
- ☒ Appendix 5, *Surgery* (ref item S.)
- ☐ Appendix 6, *Special Husbandry and Procedures* (ref item V.)
- ☐ Appendix 7, *Request to Use Patient Care Procedural Areas for Animal Studies* (ref item X3.)
- ☐ Appendix 8, *Request to Use Explosive Agent in the Animal Facility or in Animals* (ref item X4.)

Z. **Certifications.**

1. **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)		Date
[redacted]	[redacted]	[redacted]

[redacted]

2. **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 as indicated below (check one):
  - ☐ No minority opinions were submitted by any IACUC participant for inclusion.
  - ☐ Minority opinions submitted by IACUC participants are attached.

Name of Attending Veterinarian	Signature	Date
[redacted]		[redacted]
Name of IACUC Chair	Signature	
[redacted]		

[redacted]

ACORP Appendix 1

PERSONNEL

(Rev 10/13)

1. **Complete the table below listing the names of all research staff expected to work with the animals in the study, indicating each assigned duty with an "X" in the appropriate box.**

Name	In VA Occupational Health program	Declined Occupational Health enrollment	Monitors Category D procedures	Performs euthanasia	Performs surgery	Assists with surgery	Manages anesthesia	Responsible for immediate post-operative monitoring	Responsible for extended post-operative monitoring	Maintains surgical records	Responsible for performing/monitoring **other procedures** (Describe.)
[redacted]	X		X	X	X	X	X			X	
		X	X	X	X	X	X			X	
	X		X		X	X	X			X	

2. **For each person listed above, describe their education, training, and experience with experimental animals in general AND describe their experience performing the exact procedures corresponding to each assigned duty above in the species described <u>in this ACORP</u>.**

A listing of academic degrees alone is <u>not</u> an adequate response. Be sure to include a description of qualifications to perform euthanasia as applicable.

[redacted]

ACORP Appendix 3
TEST SUBSTANCES
(Rev 09/13)

1. **Toxic Agents. Will toxic chemicals, known or suspected mutagens, carcinogens, teratogens, or other similar agents be used in animals?**
Chemicals, including investigational drugs, for which the hazard level is unknown, must be considered hazardous for the purposes of this item. List and describe under "Other".

☐ No. Proceed to item 2.
☒ Yes. **Complete the table below.** Then proceed to item 2.

Table of toxic agents.

Material	Hazard(s) (from list) ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩
Isoflurane	5a	None	1-3 vol%	ETT	Entire case	Induction and Maintenance anesthetic
LR	None	None	1-20 ml/h	IV	continuous	Carrier fluid, line flush
Telazol	5b	Sterile Water	6-10 mg/kg	IM	once	Premedication
Lidocaine	5c	LR	5 mg/kg	SQ	once	Local anesthesia
Epinephrine	5d	LR	0.1-2 mcg/kg/min	IV	10-30 min	Study drug
Norepinephrine	5d	LR	0.1-2 mcg/kg/min	IV	10-30 min	Study drug
Phenylephrine	5d	LR	1-10 mcg/kg/min	IV	10-30 min	Study drug
Vasopressin	5d	LR	0.5-5 mcg/kg/min	IV	10-30 min	Study drug
Phentolamine	5e	LR	0.5-5 mcg/kg/min	IV	10-30 min	Study drug
Milrinone	5e	LR	0.5-2 mcg/kg/min	IV	10-30 min	Study drug
Nicardipine	5e	LR	0.5-5 mcg/kg/min	IV	10-30 min	Study drug
Dexmedetomidine	5f	LR	1-5 mcg/kg/h	IV	10-30 min	Study drug
KCl	5g	None	2 mEq/ml, 3 ml/kg	IV	once	Euthanasia

Hazard list: 1= Mutagen, 2=Carcinogen, 3=Teratogen, 4=Exempt select agent, 5=Other- Describe:
5a: sedation, only if inhaled in small, sealed compartment (mask, ETT), adequate ventilation of laboratory ensured
5b: sedation and apnea when applied IV, none with skin contact
5c: arrhythmia, seizure when applied IV, non with skin contact

[redacted]

5d: blood pressure increase, arrhythmia, none with skin contact
5e: blood pressure decrease, arrhythmia, none with skin contact
5f: sedation, blood pressure and heart rate changes, none with skin contact
5g: cardiovascular collapse when applied IV, none with skin contact

2. **Infectious Agents. Will bacteria (including rickettsia), viruses, fungi, protozoa, prions, or other infectious agents be used in animals?**

☒ No. Proceed to item 3.
☐ Yes. **Complete items 2a and 2b.** Then proceed to item 3.

a. **Table of infectious agents.**

Agent	Hazard level (from list) ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩

Hazard level list: 1= BSL1, 2=BSL2, 3=ABSL1, 4=ABSL2, 5=Exempt select agent

b. **Has an antibiogram, anti-viral drug sensitivity screen, or other appropriate drug sensitivity panel been determined for the agent(s) listed to assist physicians in selecting proper therapy if an inadvertent human infection occurs?**

3. **Biological Materials. Will serum, cell lines, tissue, nucleic acid or other biological materials be administered to animals?**

☒ No. Proceed to item 4.
☐ Yes. **Complete items 3a and 3b.** Then proceed to item 4.

a. **Table of biological materials.**

Material	Source (vendor, colleague) ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩

[redacted]

b. **How will these materials be screened to make sure they do not harbor infectious agents that could infect other laboratory animals or people?**

4. **Radioactive Agents. Will radioactive compounds or agents be administered to animals?**

☒ No. Proceed to item 5.
☐ Yes. **Complete items 4a and 4b.** Then proceed to item 5.

a. **Table of radioactive agents.**

Agent and isotope	Activity (mCi or μCi) ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩

b. **Which investigator has been given authorization by the Radiation Safety Committee to utilize the isotope(s) indicated above?**

5. **Nucleic Acids. Will agents containing recombinant or synthetic nucleic acids be administered to animals?**

☒ No. Proceed to item 6.
☐ Yes. **Complete the table below.** Then proceed to item 6.

Table of nucleic acid agents.

Agent	Subject to NIH Guidelines? Y/N ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩

6. **Other Agents. Will any other test substances that are not listed in items 1 - 5 be administered to animals?**

☒ No. Proceed to item 7.
☐ Yes. **Complete the table below.** Then proceed to item 7.

[redacted]

Table of other agents.

Agent ⇩	Diluent or vehicle ⇩	Dose and volume administered ⇩	Route of administration ⇩	Frequency or duration of administration ⇩	Reason for administration and expected effects ⇩

7. **Will the animals be anesthetized or sedated when any of the test substances listed in items 1 - 6 above are administered?**

☐ No. Proceed to item 8.
☒ Yes. **Complete the table below.** Then proceed to item 8.

Test Substance	Anesthetic, tranquilizer or analgesic agent ⇩	Dose (mg/kg) and volume (ml) ⇩	Route of administration ⇩	Frequency of administration ⇩
LR	isoflurane	1-5%	inhalation	continuous
Lidocaine	isoflurane	1-5%	inhalation	continuous
Epinephrine	isoflurane	1-5%	inhalation	continuous
Norepinephrine	isoflurane	1-5%	inhalation	continuous
Phenylephrine	isoflurane	1-5%	inhalation	continuous
Vasopressin	isoflurane	1-5%	inhalation	continuous
Phentolamine	isoflurane	1-5%	inhalation	continuous
Milrinone	isoflurane	1-5%	inhalation	continuous
Nicardipine	isoflurane	1-5%	inhalation	continuous
Dexmedetomidine	isoflurane	1-5%	inhalation	continuous
KCl	isoflurane	1-5%	inhalation	continuous

8. **Pain or Distress. Will animals potentially experience pain and/or distress as a result of the administration of agents listed above in items 1 - 6 that will not be treated with anesthetic, tranquilizer or analgesic agents?**

☒ No. Proceed to item 9.
☐ Yes. **Describe the nature of the pain and/or distress that animals might experience and describe measures that will be taken to alleviate any pain and/or distress here**. Then proceed to item 9.

9. **Protection of Animal Facility Staff. Is animal facility staff at risk of exposure to any of the agents listed above in items 1 - 6?**

[redacted]

☒ No. Proceed to Item 10.
☐ Yes. **Complete items 9a and 9b.** Then proceed to item 10.

a. **Complete the table for each hazardous agent that represents a potential risk to animal facility staff.**

Toxic or hazardous agent(s) from items 1-5 above.	Safety, biosafety, or radiation safety committee that has approved the use of this hazardous agent	Indicate whether VA or affiliate committee	List all animal facility staff who will come in contact with animals given these agents or with contaminated bedding, cages, or other items.

b. **Detail how the individuals listed in the table above (item 7a) 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.**

10. **PI Signature.** By my signature, I certify that:

a. Before any animal experiments involving the agents listed in item 9a are performed, SOPs designed to protect all animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and the IACUC; and

b. All staff that might be exposed to these agents will be informed of possible risks and will be properly trained to follow the SOPs to minimize the risk of exposure. As is appropriate, concurrence signatures from biosafety or radiation safety personnel are also required as shown.

[redacted]	Date [redacted]

[redacted]

10. **Approval Signatures.** By our signatures, we certify that:

a. Before any animal experiments involving the agents listed in item 9a are performed, SOPs designed to protect all animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and the IACUC; and

b. All staff that might be exposed to these agents will be informed of possible risks and will be properly trained to follow the SOPs to minimize the risk of exposure. As is appropriate, concurrence signatures from biosafety or radiation safety personnel are also required as shown.

Institutional Veterinarian	Signature	Date
[redacted]		[redacted]
Chair, Subcommittee for Research Safety	**Signature**	
[redacted]		
Chair, Biosafety Subcommittee	**Signature**	**Date**
NA	NA	NA
Radiation Safety Officer	**Signature**	**Date**
NA	NA	NA
IACUC Chair	**Signature**	**Date**
[redacted]		[redacted]

[redacted]

ACORP Appendix 4

ANTEMORTEM SPECIMEN COLLECTION

(Rev 10/13)

NOTE: Appendix 4 is not required for collections described in Appendix 2, "Antibody Production".

1. **Summary. Complete the table below for each specimen to be collected from a live animal on this protocol.**

We will limit the blood draws to less than 14ml/kg for an assumed dog blood volume of 92 ml/kg.

Specimen collected ⇩	Site and method of collection ⇩	Anesthesia required? Y/N ⇩	Amount collected each time ⇩	Volume replacement? Y/N/NA ⇩	Total number of collections per animal ⇩	Time interval between collections ⇩
Venous Blood Gas	Direct abdominal venous puncture and/ or central venous line	Y	0.7ml	Y	7-10	~30-60min
Lactic Acid	Direct abdominal venous puncture and/ or central venous line	Y	1ml	Y	1-3	~30-60min
LFT	Direct abdominal venous puncture and/ or central venous line	Y	1ml	Y	1-3	~30-60min
hemoglobin	Direct abdominal venous puncture and/ or central venous line	Y	1ml	Y	1-3	~30-60min

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

a. **For each specimen described in item 1, above, as being collected WITHOUT anesthesia, complete the following table:**

Specimen collected ⇩	Reason measures to prevent pain are not required* ⇩	Description of physical restrains that may be used ⇩

* E.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain.

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

[redacted]

Specimen collected ⇩	Anesthetic, tranquilizer or analgesic agent ⇩	Dose (mg/kg) and volume (ml) ⇩	Route of administration ⇩	Frequency of administration ⇩
Blood samples	Isoflurane	1-3%	inhalation	Continuous

3. **Volume Replacement for Fluid Collections. For each fluid specimen described in item 1, above, for which NO volume replacement will be provided, provide justification.**

[redacted]

ACORP Appendix 5

SURGERY

(REV 10/13)

1. **Surgery Classification. Complete the table below for each surgery included in this protocol.**

#	Surgery Brief Description	Terminal	Survival	
			Minor	Major
1	General anesthesia, ventilation, laparotomy and measurement of organ perfusion with near infrared spectroscopy probes on the internal organs. Reduction of organ perfusion and assessment of the NIRS measurements/ tissue oxygenation during the state of relative and total ischemia.	☒	☐	☐
2		☐	☐	☐
3		☐	☐	☐
4		☐	☐	☐

2. **Multiple Survival Surgery. Are any of the above surgeries (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) being performed in addition to any other such surgery (on this or another protocol) on the same individual animal?**

☐ Yes. **Complete items 2a and 2b.** Then proceed to item 3.
☒ No. Proceed to item 3.

a. **Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:**

b. **Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):**

3. **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 6, 7, and 8, below.

Surgery #(s) (from item 1)	Detailed Description
1	i) Animals will be fasted for 12 hours before surgery. Animals will receive intramuscular premedication before leaving the VMU. j) Inhalational induction: The animal is placed on a table and the head is gently placed in a facemask. The animal spontaneously breathes 5% isoflurane in 100% oxygen until asleep. A sufficient anesthetic level is ascertained by pinching the ear and the anesthetic is immediately deepened if the animal shows any movement. k) Intubation and ventilation: In case of no reaction to the painful stimulus, the animal is turned supine and intubated with a 7.0 cuffed endotracheal tube. The tube is then connected to an anesthesia machine (Ohmeda CD) and the animal is ventilated with isoflurane in air/oxygen mixture with FiO2 0.6. CO2 is maintained at mild hypercapnia (~45 mmHg). General anesthesia with isoflurane is continued until the end of the experiment. Endtidal anesthetic concentration is measured continuously with a POET IQ (Criticare) and maintained at 1-2 Vol%. A heat-moisture exchanger is added between endotracheal tube and ventilator circuit to reduce temperature loss. l) Line placement: Both groins are injected with lidocaine SQ. A cut-down is performed on both sides and lines are inserted in one femoral artery and both femoral veins. The arterial line is used for continuous blood pressure monitoring. The line is flushed with heparinized Lactated

	Ringer's. One venous line is used for continuous administration of fluids (Lactated Ringer's solution) and drug infusion (phenylephrine, phentolamine, norepinephrine, epinephrine, milrinone). The second IV allows intermittent fluid boluses, application of bolus drugs and blood draws. m) Surgical procedure: For the surgical procedure the animal is placed on a warming blanket and the temperature is maintained between 36.5 and 37.5 °C for the entire experiment. The abdominal skin is infiltrated with lidocaine and a midline incision or star incision will be used to allow access to the abdominal viscera (liver, kidneys, small bowel) and their vasculature.. Vessels will be dissected free and controlled with vessel loops. n) Experimental measurements, vascular occlusion and drug infusion: Transcutaneous NIRS probes will be placed on the shaved skin overlying the organ of interest, i.e., where the NIRS value is typically obtained clinically. Modified (soft, silicone-encased) NIRS probes will be placed directly on various parts of the organ. Blood flow to the organ will then be restricted by reducing systemic arterial, and for the liver also portal arterial, blood flow mechanically or through vasoactive drugs, and the changes in organ oxygenation will be measured with NIRS. Additional monitors of tissue oxygenation will be used to validate the NIRS measurements. Simultaneously, oxygen saturation, lactic acid and other metabolic indicators are determined from blood samples drawn from the respective organ draining veins. o) Multiple protocols are performed for liver, gut and kidney during every experiment. p) Euthanasia: At the end of the experiment KCl 2mEq/ml, 3 ml/kg is injected intravenously and cardiovascular collapse is verified by the arterial tracing and complete loss of expiratory CO2. Only then ventilation and anesthesia are discontinued.
2	
3	
4	

4. **Personnel. In Appendix 1, indicate personnel involved in any of the surgeries on this protocol.** Surgical training and qualifications of those listed should be described in Appendix 1.

5. **Location of surgery. Review the local guidelines below and complete the table below for each location where surgery on this protocol will be performed.**

Local Surgical Guidelines:

- Non-Rodent Mammalian Survival Surgery: Aseptic surgery conducted only in facilities intended for that purpose (i.e., VMU Surgery Suite).
- Rodent Survival Surgery: Separate room dedicated for aseptic procedures or conventional laboratory setting using aseptic technique.
- Terminal Surgery: Clean instruments and surrounding area, surgical site clipped, surgeon wearing gloves.

Surgery #(s) (from item 1)	Location/Room No.	Meets local guidelines?	
		Yes	No (Justify)
1	[redacted]	☒	☐
		☐	☐
		☐	☐
		☐	☐

6. **Pre-operative protocol.**

[redacted]

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) (from item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	☒ Overnight	☐	☐	☒premedication, see below
2	☐	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

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.

Surgery #(s) (from item 1)	Agent	Dose (mg/kg) & volume (ml)	Route Administered	Frequency (e.g., times/day)	Pre-op period of treatment (immediate, or # of days)
1	Telazol	6-10 mg/kg	IM	once	Immediately before transport from VMU to 70-A-110-A

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 #(s) (from item 1)	Surgical Site	Preparation Prior to Surgery
1	Femoral Artery and Venous Access Point	Remove hair with clippers, clean area with betadine.
1	Abdominal wall	Remove hair with clippers, clean area with betadine.

7. **Intra-operative management.**

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

Surgery #(s) (from item 1)	Agent	Paralytic Y/N	Dose (mg/kg) & volume (ml)	Route Administered	Frequency of dosing

[redacted]

1	Lactated Ringers	N	20ml/kg, 160-300ml	IV	Continuous during surgery
1	Isoflurane	N	1-5%	Inhalation	Continuous during surgery
1	Lidocaine	N	Up to 3 mg/kg	SQ	Before skin incision

b. **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.**

c. **Intra-operative physical support and monitoring. For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.) and describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.**

Surgery #(s) (from item 1)	Physical support	Monitoring method(s)
1	Depth of anesthesia, pain	Continuous heart rate and blood pressure monitoring with PowerLab; continuous respiratory gas composition including volatile anesthetic concentration with POET IQ (Criticare)
1	Hemodynamic stability	Continuous heart rate and blood pressure monitoring with PowerLab; intravenous fluid replacement and vasoactive medication PRN
1	Normothermia	The animal will be placed on a servo-controlled heating blanket to maintain temperature between 36.5-37.5 °C; a heat-moisture exchanger will be used to reduce heat loss; bowel will be covered with moist, warmed sponges to avoid heat loss and dehydration

8. **Survival surgery. Are any of the surgical procedures indicated in Item 1 survival surgeries?**

☒ No. Proceed to Item 9.
☐ Yes. **For each survival surgical procedure indicated in Item 1 and described in Item 3, complete Items 8a – 8g.** Then proceed to item 9.

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

Surgery	Survival Period	Measures for Maintaining Sterility

#(s) (from item 1)		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐

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

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

Surgery #(s) (from item 1)	Immediate Post-operative Support

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

Surgery #(s) (from item 1)	Agent (Enter "None" if none is provided*)	Dose (mg/kg) & Volume (ml)	Route Administered	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)

***For each surgery for which NO post-operative analgesic will be provided, give justification:**

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 #(s) (from item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route Administered	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)

[redacted]

e. **Post-operative monitoring.**

(1) **Complete the table below for immediate and extended post-operative monitoring.**

Surgery #(s) (from item 1)	Immediate Post-operative Period		Extended Post-operative Period	
	Frequency of Monitoring	Duration at this Frequency	Frequency of Monitoring	Duration at this Frequency

(2) **In Appendix 1, list personnel responsible for post-operative monitoring.** After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

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. List the criteria for euthanasia related specifically to post-operative complications:**

Surgery #(s) (from item 1)	Complication	Treatment	Euthanasia Criteria

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

g. **Maintenance of post-surgical medical records.**

(1) **Indicate where the post-surgical records will kept.**

(2) **In Appendix 1, identify at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records.**

9. **Certification.** The PI must sign the certification statement.

I certify that:

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

[redacted]

- 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 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)	[redacted]	Date
[redacted]		[redacted]

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Milwaukee, WI-695	Medical College of Wisconsin – Dept. of Anesthesiology funds	Determining organ perfusion during vascular compromise

ACTION NEEDED BY IACUC
The IACUC must review the 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) and the revised ACORP(s) must be forwarded to the CVMO for archiving.

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

REVIEWER FEEDBACK

ACORP Item number(s)	Comments/Concerns
ACORP (dog)	This ACORP uses dogs in a non-survival surgery to determine if Near Infrared Spectroscopy (NIRS) can be used to accurately measure organ perfusion by using an internal probe. If successful, the internal NIRS probe would provide earlier and more sensitive measurements than currently used ultrasound examinations or blood tests. The investigator is an experienced anesthesiologist and is supported by a well-qualified research team. Some aspects of the protocol should be clarified. An appendix to this review provides additional information for the IACUC's consideration. The specific numbered comments provided below must be reviewed by the IACUC, to determine what responses are needed. These actions must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP and the revised ACORP provided to the CVMO for archiving.
Item C.2	Ten dogs are expected to be used in this pilot study but the investigator has not clearly explained why ten dogs are needed. Elsewhere in the protocol, the investigator indicates blood flow will be controlled mechanically or by using intravenous drugs (eight listed) and protocols for the liver, gut, and kidney will be performed during every experiment; please explain how this information factors into the need for 10 dogs.
Item C.2 and Appendix 3	The investigator has provided an overview of the proposed study in item C.2. In Appendix 3, epinephrine, norepinephrine, phenylephrine, vasopressin, milrinone, nicardipine, and dexmedetomidine are listed as study drugs. Understanding of the experiments would be improved, if the investigator explained why each drug was chosen. Please reconcile.
Item C.2, U, and Appendix 5	According to the information in item U, dogs are euthanized while under anesthesia with intravenously administered KCl. In item C.2 and Appendix 5, the investigator notes that death will be verified by arterial tracings, which confirm cardiovascular collapse and complete loss of expiratory CO_2, please add to item U.

(cont.)

Items D and W	The investigator has made several valid points about why dogs were chosen for this pilot study. Nonetheless, it would be worthwhile to address why *in vitro* models cannot be used and why pigs were not the preferred animal model.
Item T	The investigator indicates the dogs will be delivered shortly before they are needed for experiments. The criteria listed in the scoring system suggest the animals will be held for at least a few days in advance of the study to allow for acclimation. Is this correct?
Appendix 4	The abbreviation LFT is used; does LFT stand for liver function tests? If so what proteins and enzymes are assessed to gauge the health of the liver?
Appendix 5	Item 3 - Are vessel loops used as a mechanical means of altering blood flow? Item 8.f (2) – The response to this item should be deleted.

Appendix - Additional Suggestions for Improvement

Comment 1: <u>Part B.</u> This section would benefit from a brief discussion of the relevance to Veterans' health. Try something like this at the very beginning of this section:
This project will help improve the success rate for kidney and liver transplants. The main reason people need kidney transplants is they have diabetes that has seriously damaged their kidneys ("kidney failure"). The Department of Veterans Affairs reports that almost 25% of Veterans have diabetes, compared to just 8% of the general population (https://www.va.gov/health/NewsFeatures/20111115a.asp accessed on 1/31/18), which makes methods to improve kidney transplant success especially important for the Veteran population.

Comment 2: Part D: The species justification looks sound, however section W5 later on in the ACORP states that the only other relevant study was in a porcine model. Since pigs are available in a range of sizes (including Yucatan miniature swine), it would strengthen this section if an explanation were included for why pigs cannot be used for this project.

Comment 3: <u>Part J:</u> There is a problem with the layout of this table. Please correct the table layout so it has a separate column listing who will be responsible for monitoring the category D procedures.

Comment 4, <u>section W1 table</u> (literature search).
This literature search would be strengthened by running specific searches on the ALTBIB (alternatives to animal testing) website at https://toxnet.nlm.nih.gov/altbib.html .

1) **Include a search on ALTBIB specifically for papers with "Animal Use Alternatives" as the main topic to see if there are non-animal alternatives that could work for studying near infrared spectroscopy on liver. (This search covers the replacement of animals in the fourth column from the right of the table and addresses section W2).**
2) **Include a search on ALTBIB for "PubMed using ALTBIB animal alternatives search strategy citations from 2000 to present" using the keywords laparotomy and dog. This**

(cont.)

addresses whether there might be further refinements (better anesthetics, etc.) and ways to use fewer animals.

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
PubMed	2/15/18	Till 2/2018	N/A	liver surface near infrared spectroscopy	( )	( )	( )	(X)
ALTBIB Citations with Animal Use Alternatives as the main topic	2/15/18	Till 2018	N/A	Liver, near infrared spectroscopy	(X)	( )	( )	( )
PubMed using ALTBIB animal alternatives search strategy	2/15/18	2000-2018	Laparotomy	laparotomy, dog	( )	(X)	(X)	( )

Comment 5, section W2: (Replacement)
This section would be strengthened by including an explanation as to why this research cannot be done with computer models or *in vitro* methods (it may be obvious to any biomedical scientist, but this still has to be included). You could put something like this at the beginning:
"We ran a search specifically looking for animal use alternatives for this kind of research, and there were no in vitro, computer, or other non-animal models available."

Comment 6, section W4 (refinement): This section could be strengthened by adding a sentence:
"Our search for refinements did not bring up any improvements over the anesthetic regimen we are already using."

Comment 7, section W5 (lack of unnecessary duplication):
The answer provided in this ACORP would be strengthened by providing some more detail. Try something like this, which is based on a new literature search run on 2/15/18:
Our PubMed literature search for "liver surface near infrared spectroscopy" yielded 37 papers, none of which duplicate this study. Of these papers, 11 were using nanoparticles and two used dyes – our method does not need either. Ten papers looked at tumors, two at endotoxemia, and one each at water content, fat content, biliary excretion, doppler, and $tcpO_2$/pO2, none of which are relevant to

(cont.)

this project. There were three papers doing basic work showing the promise of this technology which this work will build upon. Of the remaining papers, there was one each on woodchucks and mule ducks, neither of which are good models for the pediatric patients that are our focus. The remaining two papers were on pigs, which this work will also build upon. (As noted above in section D, we are not able to use pigs for this study).

(cont.)

Literature search Milwaukee [redacted]

1) How is this research relevant to Veterans health?

This project will help improve the success rate for kidney and liver transplants. The main reason people need kidney transplants is they have diabetes that has seriously damaged their kidneys ("kidney failure"). The Department of Veterans Affairs reports that almost 25% of Veterans have diabetes, compared to just 8% of the general population (https://www.va.gov/health/NewsFeatures/20111115a.asp *accessed on 3/11/18*). which makes methods to improve kidney transplant success especially important for the Veteran population.

The dogs used for this study will all be donated from a commercial contract research laboratory as part of their commitment to reduce the number of animals used in research. Studies will only take place when donated dogs are available.

2) Is this work unnecessarily duplicating work already documented in the literature?

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
PubMed	3/11/18	All available years	liver surface near infrared spectroscopy	37

Our PubMed literature search for "liver surface near infrared spectroscopy" yielded 37 papers, none of which duplicate this study. Of these papers, 11 were using nanoparticles and two used dyes – our method does not need either. Ten papers looked at tumors, two at endotoxemia, and one each at water content, fat content, biliary excretion, doppler, and $tcpO_2$/pO2, none of which are relevant to this project. There were three papers doing basic work showing the promise of this technology which this work will build upon. Of the remaining papers, there was one each on woodchucks and mule ducks, neither of which are good models for the human patients that are our focus. The remaining two papers were on pigs, which this work will also build upon. As noted below, dogs are used for this study because the anatomy and organ size resemble pediatric patients, which are a focus of this group. Pigs would not be suitable due to their anatomy.

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

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

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

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

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
ALTBIB animal alternatives search strategy - all citations	3/11/18	2000-present	liver surface near infrared spectroscopy	16

An ALTBIB search for all citations brought up 16 papers. If these, 15 relied on nanoparticles or dyes, while the method used in this study requires neither. The one paper that was using a method like the one in this study was only using the near infrared spectroscopy on tissues removed from euthanized mice [Cai, 2007], while the current study is developing a method to monitor blood flow in tissues in the living body without needing dyes or nanoparticles with their attendant side effects. The canine model is used because the anatomy and organ size resemble pediatric patients, which are a focus of this group.

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

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	How many papers were found?
ALTBIB animal alternatives search strategy - all citations	3/11/18	All available years	Laparotomy	laparotomy, dog	9

Each animal will be completely anesthetized during the procedure and euthanized immediately afterwards, so they will not experience any pain or distress. The search for refinements did not bring up any improvements over the anesthetic regimen and surgical methods they are already using.