[For IACUC Use Only]
Initial Approval Date: [redacted]

Current Approval Date: [redacted]
Version 2 Approval Date
Version 3 Approval Date
Version 4 Approval Date

☐ 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:** The Investigation of Novel Imaging Approaches to the Circle of Willis

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 Neurosurgery Department 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:**

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.

The recent development of high resolution, ultra-thin cameras (most notably a device called the Scanning Fibre Endoscope) has allowed the potential to access the blood vessels of the brain in ways that were previously not possible with current fibre-optic devices. The SFE device is already in clinical trials in Seattle (Washington, USA) for bile duct, pancreatic duct, and fallopian tube imaging, and has been used in vivo for intravascular imaging. Preliminary data has shown that this camera (1.5mm) can be used to view the inside of peripheral blood vessels in a large animal model (pig). We wish to perform a pilot study to demonstrate that this camera can also be used to view the inside of blood vessels of the brain. The dog is specifically chosen as the arrangement of blood vessels to the brain closely resembles the human condition. Having direct vision of endovascular procedures would introduce a novel field of intravascular intervention, which currently relies on indirect views of the procedure obtained via X-Rays and injection of contrast material to produce 'luminograms' rather than viewing directly the target area of the procedure, in addition to requiring ionizing radiation. The technical accuracy and safety of performing procedures such as aneurysm obliteration, stent deployment and retrieving clots during stroke thrombectomy could be improved. An important unmet need in the interventional treatment of stroke is the ability to visualize the occluding lesion directly – currently the lesion position is inferred from planar x-ray angiography images and the placement and deployment of retrieval devices is based on estimated sizes and times rather than actual images of the clot from inside the vessel. By using this endoscope we will be able to directly visualize the clot from inside the vessel, as well as guide the placement and incorporation of retrieval stents into the lesion. We anticipate this will lead to a higher percentage of clots which are removed intact, decreasing the morbidity associated with partial removal and the necessity for repeated stent application.

Preliminary unpublished studies have shown the ability to analyze the temporal endothelial coverage of stents in vivo in peripheral vasculature of porcine blood vessels. There is currently no standardized way of direct high resolution in vivo analysis of the temporal course of stent integration into the vasculature, without animal sacrifice or use of surrogate imaging techniques such as ultrasound or non-invasive imaging. This ability to directly analyze stent endothelial coverage of both the temporal as well as biological process (using fluorescence of biomarkers) would be a major advance in understanding this process, particularly for neurovascular stents, particularly novel devices such as flow diverting stents.

Furthermore, we wish to attempt to access the outside of the blood vessels via a keyhole approach to the base of the brain where these blood vessels are situated. The purpose behind imaging both the inside of blood vessels and the outside is firstly for diagnostic purposes, but more importantly to deliver therapy with the assistance of micro-instruments to disorders of the blood vessels of the brain such as aneurysms. This is currently not possible without the use of fluoroscopic X-rays (from the inside of blood vessels) or an open surgical procedure with brain retraction (from the outside of blood vessels).

Other intended applications would be to correlate the findings of MRI scanning of brain blood vessel walls or (vessel wall imaging) with the abnormalities that can be seen under direct vision with an endoscope. MRI of brain blood vessel walls is an emerging field of brain imaging that would benefit significantly from validation of findings. Lesions of blood vessel wall (such as a tear or dissection) would be simulated in the dog model under direct vision and then with corresponding MRI vessel wall imaging to obtain both structural/ anatomical and temporal course of these changes with serial imaging.

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.

We intend to perform a series of pilot experiments initially to assess the feasibility of imaging the intracranial vasculature in a dog model.

1. Endovascular angioscopic imaging

These experiments would involve a canine model under general anesthesia in which we would access the carotid/ vertebral circulation via femoral artery cannulation using sheaths and catheters used in clinical practice to perform endovascular neurosurgery. The camera would then be fed through the lumen of a guide catheter positioned within the cerebral circulation to visualize the blood vessel lining surface, which is enabled by a combination of flow arrest through balloon inflation and or saline irrigation around the camera via the catheter. Once visual optimization has been achieved, subsequent studies would focus on performing procedures within the blood vessels under direct vision (which are currently performed clinically using fluoroscopy with contrast or X-Rays).

We would also introduce micro-catheters and micro-wires via the guide catheter alongside the camera to perform coiling and or inserted stents under direct vision. The dog would then be euthanized.

2. Novel approaches to the basal cisterns

For the second arm of the study, the animal will be placed under general anesthesia. Using aseptic conditions, access to the basal cisterns will be gained at the base of the brain via either a lumbar or cervical cisternal puncture. We would then introduce the camera into the subarachnoid space via a cannula, to obtain a view of the basal cisterns. We would hope to perform multiport access with one channel for camera access and other ports serving as working channels to introduce micro-instruments.

We anticipate that a maximum of ten animals would be needed to complete both pilot experiments with the hope of using the five animals for each of the experimental arms. Our aim would be to perform these as a non-survival surgery to reduce pain and suffering.

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.**

Two different groups of 5 dogs each will be used for two different types of surgeries: 1. Endovascular angioscopic imaging and 2. Novel minimally invasive approaches to basal cisterns.

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

Because this is a pilot study assessing 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.

1. Endovascular angioscopic imaging will be performed via direct cannulation to achieve visualization of the cerebral circulation within the blood vessels themselves. Coiling and stent application with be performed using the visualization provided by the camera, rather than current methods used in surgery. For current methods, please see appendix 5.
2. Access to the basal cisterns will be obtained via two different insertion points, one for the camera, and one for the introduction of micro-instruments. Once access is achieved, coiling and stent application may also be performed within the vessels.

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.

The canine model is chosen due to the similarity of the anatomical configuration of the cerebral vasculature (circle of willis) which his homologous to the human anatomical arrangement.

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".

[redacted] will be assisting in training [redacted] with the differences in using a canine model from his previous porcine experience. If necessary, Veterinarian staff will be contacted. Canine vascular anatomy will also be studied based on literature that was provided from the Veterinarian staff.

VMU staff, particularly [redacted] in the monitoring and anesthesia of the canine model. In addition, [redacted] with the euthanizing of the animals.

[redacted]

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 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 Identify persons responsible for monitoring Category D procedures in Appendix 1.	Frequency of monitoring after the procedure and how long animals will be monitored	Analgesic, sedative, or anesthetic used, plus dose, route, and duration
Endovascular Angioscopy	N/A (non-survival)	Pre Surgery: 7mg/kg Telazol IM; 4mg/kg Carprofen; Maintenance throughout: 2-3% isoflurane;
Minimally invasive approach to basal cisterns	N/A (non-survival)	Pre Surgery: 7mg/kg Telazol IM; 4mg/kg Carprofen; Maintenance throughout: 2-3% 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 cannot 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.

Initial [redacted]
Version 2
Version 3
Version 4 [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.

☐ 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 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:

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)*

*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."

   ☐ 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 following grading scale will be used:

Body condition
- 0-normal
- 1-Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.
- 2-Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvis bones becoming prominent. Obvious waist.
  3-Ribs, lumbar vertebrae, and pelvis bones easily visible, and some visible evidence of other bony prominences. No palpable fat. Muscle loss evident.

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
- 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.

   100mg/kg Beuthanasia-D delivered intravenously

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.

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.

					Mandates:				
					Lack of unnecessary duplication (Item W5.) ⇩				
					Refinement to minimize pain or distress (Item W4.) ⇩				
					Reduction in number of animals used (Item W3.) ⇩				
					Replacement of animals (Item W2.) ⇩				
					Key words and/or search strategy used ⇩				
				Potentially painful or distressing procedures addressed ⇩					
			Years covered by the search ⇩						
		Date of search ⇩							
Database	⇩								
PubMed	July 2016	1956-2016	Cerebral Angioscopy	Cerebral Angioscopy; canine model; scanning fibre endoscope	x	x	x	x	
ALTBIB	July 2016	All up to 2016	Cerebral Angioscopy	Cerebral Angioscopy; canine model; scanning fibre endoscope	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.

Porcine models have previously been used. It has been found that canine models are better used to represent the vessels of the human brain and therefore are desired.

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 sizes were 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.

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

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

The camera being used has never been studied in a canine model, only porcine. Since the canine model has a much closer vessel structure to humans, it will give more relevant information than in the porcine model. Since this has not been done before, it is necessary to determine the feasibility of such a procedure.

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, Beuthanasia-D VMU 70-B-123

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.

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)	Signature	Date
[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]		
Name of IACUC Chair	Signature	Date
[redacted]		[redacted]

Comments:

Effective date: [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	

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 10/13)

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 ⇩

Hazard list: 1= Mutagen, 2=Carcinogen, 3=Teratogen, 4=Exempt select agent, 5=Other- Describe:

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 ⇩

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.

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 ⇩
Omnipaque	Water	129-650mg/kg; 3.4-26ml	Catheter Flush	As needed	To assist with the use of fluoroscopy. Omnipaque is a contrast dye that enhances the visualization of vessels. It will be administered as a flush to guide the path of the micro camera.

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 ⇩
Omnipaque	Isoflurane	2-3%	Inhalation	Continuous during surgery

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?**

☒ 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.

Principal Investigator(s)	Signature(s)	Date

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
Chair, Subcommittee for Research Safety	Signature	Date
Chair, Biosafety Subcommittee	Signature	Date
Radiation Safety Officer	Signature	Date
IACUC Chair	Signature	Date

ACORP Appendix 5
SURGERY
(REV 10/13)

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

	Surgery	Terminal	Survival	
#	Brief Description		Minor	Major
1	Endovascular Angioscopic Imaging	☒	☐	☐
2	Minimally invasive approach to the basal cisterns	☒	☐	☐
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	Endovascular Angioscopic Imaging: The entire procedure will be performed within the animal operating room using sterile devices and aseptic technique. Dogs will be placed under general anesthesia using Telazol (7mg/kg) to initiate and isoflurane (2-3%) to maintain. Surgical site will be prepared using Betadine scrub and spray. The femoral artery will be catheterized using the same technique as done clinically using the Seldinger technique under ultrasound guidance. For most procedures both femoral arteries will be accessed to provide for dual access, with one groin used as the access point for the camera and the second for the introduction of devices to be introduced alongside the camera in the target vessel. Using clinically available guide catheters distal the internal carotid/ vertebrobasilar system will be cannulated under fluoroscopic guidance. The same meticulous technique of avoiding air bubbles and systemically

	heparinizing the animal will be used throughout, with all catheters attached to continuous heparinized flush. The endoscope will be advanced through the guide catheter and using a combination of proximal balloon inflation (if required) and saline flush the endothelium will be visualized. We anticipate that a maximum of two animals will be required to optimize the process of visualization in the cerebral circulation. We will attempt to cannulate both the anterior and posterior cerebral circulation. Fluoroscopy will be used concurrently to allow for precise navigation within the vasculature, including the use of Omnipaque contrast to better visualize the vascular system. We anticipate that the first series of experiments will be devoted to optimization of visualization of the endothelial surface. Once this process has been optimized and standardized we will proceed with to introduce sterile micro catheters and micro wires via another guide catheter system in the common carotid artery, and to visualize the navigation and deployment of endovascular coils and stent deployment. Following the procedure, the groin puncture site will be sealed after all devices are removed. The animal will then be euthanized.
2	Minimally invasive approach to the basal cisterns: The entire procedure will be performed within the animal operating room using sterile devices and aseptic technique. Dogs will be placed under general anesthesia using Telazol (7mg/kg) to initiate and isoflurane (2-3%) to maintain. Surgical site will be prepared using Betadine scrub and spray. Under fluoroscopic guidance, a Touhy needle (16Guage) will be used to access the intradural space using a standard aseptic technique. This will be performed initially to access the lumbar thecal sac. Following confirmation of intradural placement (obtaining CSF) we will attempt to introduce the camera through the upturned bevel of the Touhy needle and under direct vision and concurrent fluoroscopic guidance attempt to reach the basal cisterns posterior to the brainstem where the cerebral vasculature is located. We will also attempt, to perform this procedure via a cervical cisternal puncture. This will be performed at the C1/C2 interspace using aseptic technique and fluoroscopic guidance to insert a Touhy needle into the cervical cistern. Fluoroscopic guidance will be enhanced with the use of Omnipaque contrast dye. Once confirmation of CSF flow is obtained via the Touhy needle, the camera will be introduced to allow for direct visualization of basal cistern structures. Following the procedure, the Touhy needle will be removed and the puncture site sutured to prevent CSF leakage and the animal will be euthanized. It is anticipated that modifications to the procedure will be required, particularly a form of saline flush attached to the Touhy needle to allow for CSF replacement. Further modifications of the introduced sheath/ needle are also anticipated to allow for both irrigation and stability.
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]	☒	☐
2		☒	☐
		☐	☐
		☐	☐

6. **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) (from item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	☒ Overnight	☐	☐	☐
2	☒ Overnight	☐	☐	☐
	☐	☐	☐	☐
	☐	☐	☐	☐

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)
1,2	Tiletamine/zolazepam Telazol	7mg/kg	IM	Once	Immediate
1,2	Enrofloxacin Baytril	5mg/kg	IM	Once	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 #(s) (from item 1)	Surgical Site	Preparation Prior to Surgery
1	Femoral Artery Access Point	Remove hair with clippers, clean area with betadine, cover surrounding areas with sterile drapes.
2	Lumbar or Cervical Area of Back	Remove hair with clippers, clean area with betadine, cover surrounding areas with sterile drapes.
3		
4		

7. **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.**

Surgery #(s) (from item 1)	Agent	Paralytic Y/N	Dose (mg/kg) & volume (ml)	Route Administered	Frequency of dosing
1,2	Saline	N	10-15ml/kg/hour	Through inserted catheters in the femoral artery (surgery 1), or the Touhy needle/spinal catheter (surgery 2)	Will be delivered as needed throughout the surgery, but not exceeding the hourly recommended dose
1,2	Isoflurane	N	2-3%	Inhalation	Continuous during surgery
1,2	Carprofen	N	4mg/kg	SC	Once
1,2	Omnipaque	N	129-650mg/kg; 3.4-26ml	Through the inserted catheter containing the camera, but not through the spinal canal for surgery 2	As needed to enhance the visualization for fluoroscopy

1,2	Furosemide	N	2-4mg/kg	IV	Once, only if needed as recommended by the veterinarian

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)
1,2	Aqua tears	Heart rate monitor, pulse oximeter, eye reflex, pain reflex between toes and rate of respiration will be physically monitored
1,2	Temperature controlled heating pads	Heart rate monitor, pulse oximeter, eye reflex, pain reflex between toes and rate of respiration will be physically monitored
1,2	Sterile draping	Heart rate monitor, pulse oximeter, eye reflex, pain reflex between toes and rate of respiration will be physically monitored

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 #(s) (from item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐
		☐	☐	☐	☐	☐	☐	☐	☐

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

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

Surgery #(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)

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.**

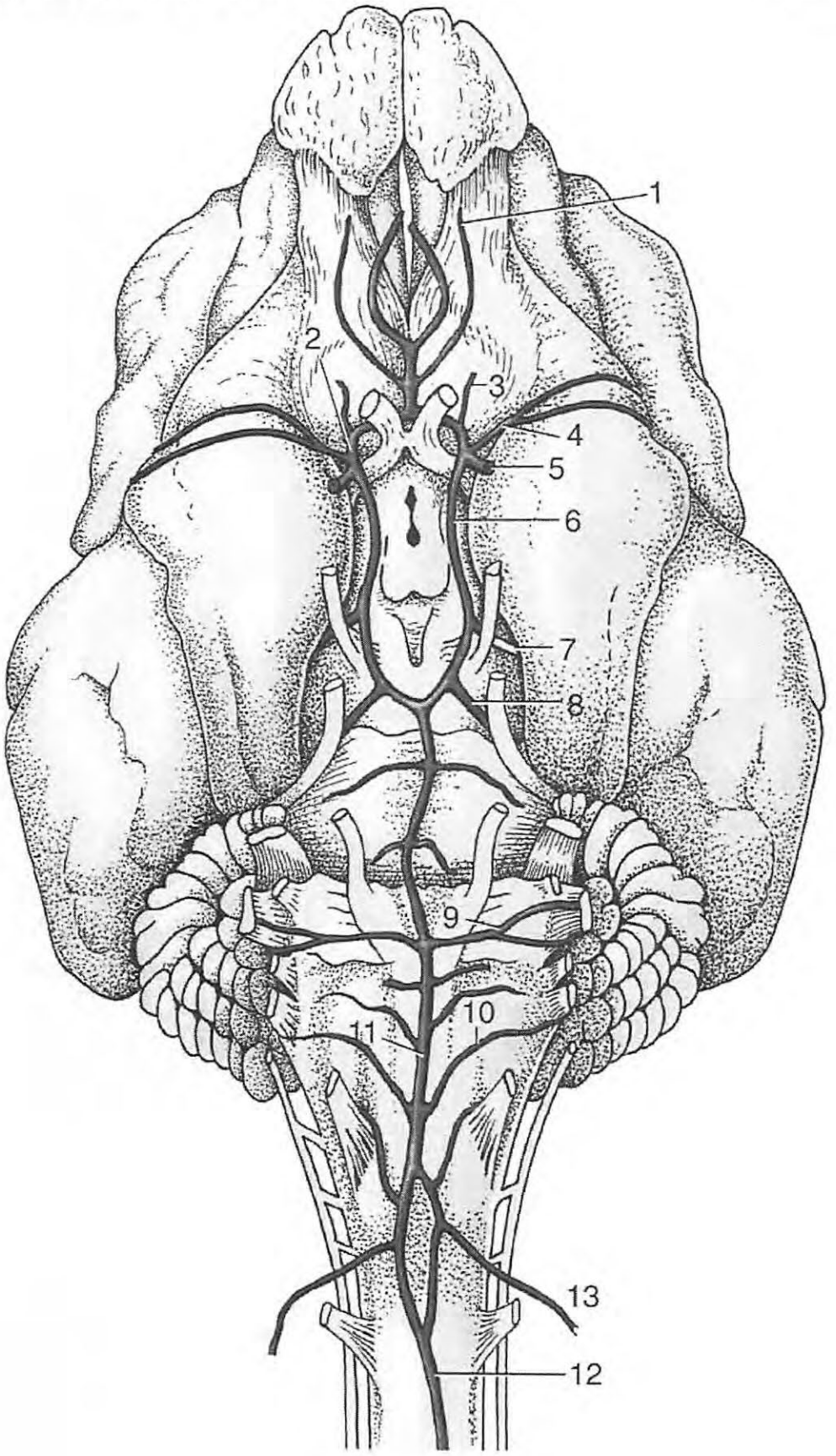
9. **Certification.** The PI must sign the certification statement.

I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- 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)	Signature(s)	Date
[redacted]		


1
2
3
4
5
6
7
8
9
10
11
13
12

Revision Signatures
Version 4 signatures

1. Certification by Principal Investigator(s).

To the best of my knowledge, I certify that the information provided in this Animal Component of Research Protocol (ACORP) Amendment is complete and accurate.

I further certify that:

- **No personnel will perform any animal procedures until they have been approved by the IACUC. When new or additional personnel become involved in these studies, I will submit their qualifications, training, and experience to the IACUC and seek IACUC approval before they are involved in animal studies;**
- **I will ensure that all personnel are enrolled in an Institutional Occupational Health and Safety Program prior to their contact with animals, or have declined in writing to participate, if allowed by local policy;**

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

2. Approval Signatures. To the best of their abilities, the undersigned verify that the care and use of the animals described in this ACORP amendment has been evaluated 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*, VA Policy and local IACUC Policy, and find the use of animals described in this ACORP to be appropriate.

Institutional Veterinarian	Signature	Date
[redacted]	[redacted]	[redacted]
[redacted]	Signature	
[redacted]		

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Milwaukee, WI - 695	Medical College of Wisconsin-Dept. of Neurosurgery funds	The Investigation of Novel Imaging Approaches of the Circle of Willis

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 study involving the use of a scanning fibre endoscope to directly view the lumen of the brain's blood vessels; this technology is expected to significantly improve the accuracy of diagnosis and treatment of vascular brain disorders such as blood clots and aneurysms. [redacted] Several aspects of protocol should be clarified. 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.1	The investigator outlined two experiments - endovascular angioscopic imaging and a new approach to the basal cisterns; however, the language used is quite technical (e.g. endovascular, subarachnoid space, basal cisterns, etc.) Understanding of the proposed study would be improved if this item was revised using non-technical (lay) language.
Item C.2	Item C.2.a – This section should address the rationale for the proposed procedures and methods and also explain the sequence of the experiments. Information found in Appendix 5 outlines the sequence of the two experiments (see below) and should be added to item C.2: <u>*Endovascular Hagioscopic Imaging:*</u> *"We anticipate that the first series of experiments will be devoted to optimization of visualization of the endothelial surface. Once this process has been optimized and standardized we will proceed with to introduce sterile micro catheters and micro wires via another guide catheter system in the common carotid artery, and to visualize the navigation and deployment of endovascular coils and stent deployment."*

(cont.)

	Minimally invasive approach to the basal cisterns: *"This will be performed initially to access the lumbar thecal sac. We will also attempt, to perform this procedure via a cervical cisternal puncture."* Please revise Item C.2.a. Item C.2.b – Although this study is being conducted as a pilot, the investigator should explain the rationale for using 10 dogs as opposed to six dogs. Please address. Item C.2.c -The procedural descriptions lack sufficient detail. The specific concerns are: • Endovascular angioscopic imaging procedure - The investigator does not explain that the femoral artery is punctured with a hollow needle containing a guide wire, the needle is removed and a sheath is inserted over the guide wire and threaded from the femoral artery up through the vasculature tree (e.g. femoral artery, aorta, heart, common carotid artery, internal carotid artery) to reach the Circle of Willis. All devices and procedures performed should be addressed. • Access to the basal cisterns – Where are the two insertion points and how are the basal cisterns accessed? Please also address coiling and stent application.
Item D	As the investigator pointed out, in dogs and humans, the Circle of Willis is supplied by the internal carotid and basilar arteries. Nonetheless, it would be worthwhile to specifically address why *in vitro* models and other animal models are unacceptable.
Item H	The investigator should be aware that the structure of the Circle of Willis may differ among breeds of dogs, with a complete lack of the rostral communicating cerebral artery being the most common anomaly (see: https://vetsci.wordpress.com/2010/02/09/arterial-blood-supply-to-the-brain/).
Item T	Do the dogs undergo an acclimation period prior to use in non-survival experiments?
Item U	If dogs are euthanized by an overdose of euthanasia solution, how is death confirmed?
Item W	Please elaborate on why porcine models are no longer used.
Appendices 3 and 5	The information in Appendix 5 – item 3 tends to be a description of what will be done as opposed to how the experimental manipulations will be performed. Specific concerns include: (1) Endovascular Angioscopic Imaging: • Please explain the Seldinger technique. • Please explain proximal balloon inflation and saline flush used to advance the endoscope. • The dog is heparinized; please list heparin in Appendix 3. • Please explain the deployment of endovascular coils and stents. (2) Minimally invasive approach to the basal cisterns • Please specify where (anatomically) the Touhy needle is inserted to access the lumbar thecal space and the path taken to reach the basal cisterns. • The investigator expects to make modifications to (1) the form of saline flush used to allow replacement of cerebral spinal fluid and (2) modifications of the introduced sheath/needle to allow for both irrigation and stability). Modifications to the protocol must be fully described in a protocol amendment and approved by the IACUC before the procedures are

(cont.)

	implemented. Appendix 5 - item 6: Enrofloxacin Baytril is listed as a pre-operative medication, are the experiments expected to exceed six hours in duration? Appendix 5 - item 7: Carprofen and furosemide are listed as being administered, please add to Appendix 3 and explain their purpose. Appendix 5 – item8.f (2): The response to this item should be deleted.

(cont.)

Literature search Milwaukee [redacted]

1) How is this research relevant to Veterans health?

This study will develop improved methods for seeing exactly where a stroke is occurring in the brain, and for removing the blood clot causing the stroke.

Stroke is the fourth leading cause of death in the United States, accounting for more than 1 out of every 18 deaths. Approximately 6,000 VA admissions are for Veterans with acute ischemic stroke, with new strokes costing an estimated $111 million for acute inpatient care, $75 million for post-acute inpatient care, and $88 million for follow-up care in the first six months post-stroke (see https://www.hsrd.research.va.gov/news/feature/stroke.cfm *accessed 3/11/18).* Improving stroke treatment will benefit thousands of Veterans each year and the improved patient outcomes may result in potential cost savings.

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

Name of the database	Date of search	Period of years covered by the search	Key words and/or search strategy used	How many papers were found?
PubMed	3/11/18	All available years	scanning fiber endoscope, cerebral angioscopy	0

A PubMed search for this work brought up no papers at all. This is new imaging technology that has only been used in peripheral blood vessels thus far, and this is the first group to expand it to cerebral blood vessels.

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 <u>Animal Use Alternatives</u> as the main topic	3/11/18	All available years	scanning fiber endoscope, cerebral angioscopy	0

An ALTBIB search for “alternatives to using animals” for this work 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	scanning fiber endoscope, cerebral angioscopy	0

An ALTBIB search for all citations brought up no papers. There are no other animal models for this work. The scanning fiber microscope has been used in the pig for peripheral blood vessels, however the porcine brain has a plexus of very small vessels, (rete mirabile), at the base of the brain that makes it unsuitable for these endoscopy studies [Burbridge 2004].

By comparison, the blood vessel structure of the canine brain is much closer to that of the human brain [Kapoor 2003]. The information from doing this study in dogs will be more relevant for designing clinical studies in humans than a study in pigs would be.

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

The dogs will be under general anesthesia throughout the procedure. At the end, while they ae still anesthetized, the dogs will be euthanized by a standard method used in veterinary clinics. The animals should experience no pain or distress.