Department of Veterans Affairs

Memorandum

Date: [redacted]

From: Associate Chief of Staff, Research and Development (151)

Subj: Notification of Project Modification Approval, PCC # 2017-090892
Project # 0013, *Glucose Sensing and Physiologic Insulin Delivery*
IACUC Approval: [redacted]
IRB Approval:
SRS Approval:

[redacted]

1. The above referenced submission has been approved by all required subcommittees and the R&D Committee. You may now implement the approved modifications. You will receive a separate notification from Research Administration when the approved documents, if any, are ready for pick-up/delivery. Please refer to the respective approval memos for details regarding each approval.

2. You are reminded that no further changes or modifications may be implemented on this study until you have requested and received full approval from all applicable committees. If you need assistance in submitting another modification, please refer to the applicable approval memo for specific contact information.

3. You are also reminded that all study personnel must remain current with all applicable training and compliance requirements. No person will be allowed to work on the project for any period during which they are not fully compliant, and non-compliance by the principal investigator may result in study termination.

4. Continuation of an approved research study requires review and approval by all applicable committees prior to the expiration date stated in each committee's approval memo. Expiration of any applicable committee approval will result in study termination, and necessitate a complete new submission.

5. Thank you for your cooperation in helping us adhere to the rules and regulations of the Department of Veterans Affairs for the conduct of research.

[redacted]

Department of Veterans Affairs

Memorandum

Date: [redacted]

From: Chair, Subcommittee for Research Safety (151)

Subj: PCC: 2017-090892 VA: 0013
Glucose Sensing and Physiologic Insulin Delivery
Approval of Modification

To: [redacted]

1. The above referenced request to modify an approved research study involving laboratory-based biohazards underwent a full review by the Subcommittee for Research Safety (SRS) and has been ***approved***. The modification does not change the biohazard status of the study. You are reminded that any future modification to this study must also be reviewed and approved by all relevant subcommittees prior to implementation.

MODIFICATION SUMMARY: This is a modification to correct items in the ACORP at the request of ORO.

2. This memo and the attached copies of the approved submission documents must be maintained in your study file. Please refer to your Protocol Safety Plan (PSP) for the study, as altered by this modification, and to your Laboratory Safety Plan (LSP), to review your responsibilities for minimizing the potential for harm or injury related to the biohazards involved in your research.

3. If you have questions regarding the SRS's decision or about your responsibilities, please contact the Program Analyst for Committees and Compliance, [redacted] or by email at [redacted]

4. Final approval by all relevant subcommittees is required before you may begin this change to your research. You will receive notices of such approvals separately, and will also receive an ACOS Approval Notification, verifying all required approvals are in place. All required committee approvals must remain current in order to continue the project work without interruption.

[redacted]

Department of Veterans Affairs

Memorandum

Date: [redacted]

From: Chair, GLA Institutional Animal Care and Use Committee (IACUC) (151)

Subj: Approval of Modification Submission (PCC #: 2017-090892)

To: [redacted]

Re: ***Glucose Sensing and Physiologic Insulin Delivery***
VA Project #: **0013**
Protocol #: **08075-02**
Approved Species: **Dog**
[redacted]
Annual Expiration: [redacted]
Triennial Expiratio[redacted]

1. The above referenced Modification submission was reviewed and approved by the IACUC at its meeting on [redacted]
2. The request was to add additional information to the ACORP.
3. Please be aware of the following:
   a. No modification to this study, including a change in personnel, may be implemented prior to IACUC approval. All personnel who work on this project must have a current VA or WOC appointment, a Scope of Practice, and must maintain compliance with all required training. To minimize delay in assigning new staff, please ensure that all requirements have been met before submitting a modification to add an employee to this project.
   b. The continued use of animal subjects beyond the above specified expiration dates will require the submission of documents for IACUC review at **least three weeks before the IACUC meeting that precedes the above specified expiration dates, which could be six or more weeks before the actual expiration date**. You will be informed about the documents and the required submission dates as they mature.
4. If you have questions about this submission, please contac[redacted]

[redacted]

VA Greater Los Angeles Healthcare System
RESEARCH SERVICE

APPLICATION TO MODIFY AN APPROVED RESEARCH PROJECT INVOLVING ANIMAL SUBJECTS

PROJECT TITLE: **Glucose Sensing and Physiologic Insulin Delivery**

PRINCIPAL INVESTIGATOR: [redacted]

PRIMARY CONTACT PERSON

TELEPHONE [redacted]	E-MAIL ADDRESS [redacted]

SECTION I – INVESTIGATOR'S ASSURANCE

I certify that the information in this application is complete and correct.

I understand that as Principal Investigator, I have ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of animal subjects, and strict adherence to any stipulations imposed by the Animal Research Committee.

The requested changes will not be implemented until full written R&D Committee approval has been received.

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

SECTION II – DESCRIPTION OF MODIFICATION/AMENDMENT

CHANGES REQUESTED:

a. Number and briefly describe and justify ***each*** change being requested.

During routine audits by ORO and AAALAC, several suggestions for improvement of the ACORP were provided. Modification of the following items is requested to implement these suggestions.

Item 1: Social housing of compatible dogs is now incorporated.

Item 2: Skin irritation as a result of adhesive tape removal is now listed as a potential medical complication, and treatment is described.

Item 3: Dogs are made diabetic either through surgical pancreatectomy or chemical induction before arriving at the VA. The latter method was not discussed in the previous version of the ACORP, and is now added in this version.

b. Complete the table below, separately listing every item on currently approved ACORP that is affected by the changes described above. Reference each change to the number of the item(s) above. *(Add rows to table, as needed.)*

REFERENCE ITEM (S)	ACORP ITEM	REPLACE EXISTING TEXT WITH THE FOLLOWING:
1	Main Body, Section M	Add "or two" to the table, and "Group housing of compatible dog pairs will be used."
2	Main Body, Section T	Add "Some dogs display mild-to-moderate localized skin irritation when adhesive tape (used to anchor gauze pads overlying glucose sensors) is removed, even when using adhesive solvent. Significant irritation will be treated with diphenhydramine (Benadryl) (125 mg qd po for three days) to provide comfort and protection from scratching."
2	App. 3, Sections 1 and 2	Add "Diphenhydramine" to both tables.
2	App. 6, Section 3	Add "Some dogs display mild-to-moderate localized skin irritation when adhesive tape (used to anchor gauze pads overlying glucose sensors) is removed, even when using adhesive solvent. Significant irritation will be treated with diphenhydramine (Benadryl) (125 mg qd po for three days) to provide comfort and protection from scratching."

3	**App. 6, Section 1**	**Add "or chemical induction".**

APPROVAL SIGNATURES

a. The undersigned have evaluated the care and use of the animals as described in this modification of an existing, approved protocol, in accordance with the provisions of the Animal Welfare Act, the PHS *Guide for the Care and Use of Laboratory Animals,* and VA Policy, and find the modifications of this ACORP to be appropriate.

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

b. The VA Research and Development Committee concurs with approval of the modification to this ACORP.

Name of R&D Committee Chair	Signature	Date
[redacted]		

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)

Main Body

VERSION 4 V2 6-17-2015

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

A. **ACORP Status.**

1. Full Name of Principal Investigator(s)► [redacted]
2. VA Station Name (City) and 3-Digit Station Number► **VA Greater Los Angeles 691**
3. Protocol Title► **Glucose Sensing and Physiologic Insulin Delivery**
4. Animal Species covered by this ACORP► **Dog**
5. Funding Source(s). Check each source that applies:

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

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review: ► **NO** ( ) **-- GO TO Item #7**
   Else, identify the project:
   (1) Title of project► **Glucose Sensing and Physiologic Insulin Delivery**
   (2) If approved by the R&D Committee, give the date of approval►

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:
   (1) Identify the studies described in the previously approved ACORP that have already been completed

   ► **This research has been successful in developing mathematical algorithms used to determine how much insulin must be delivered from a pump in order to maintain steady blood glucose levels. Much of the information used to define this algorithm has come from experiments that compare blood glucose levels to subcutaneous glucose levels, and the different rates of change between these two body compartments. In addition, changes to glucose sensor materials over the last three years have helped maximize sensitivity and make the sensors more resilient to changes in the subcutaneous environment (e.g., fibrosis). A number of glucose sensors tested in this study have gone on to clinical trials and are now available to diabetic patients.**

   (2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly

► **During the last three-year period, we used a total of 31 dogs to obtain these results, most of which were carried over from the previous three-year approval period, and all of the 24 current dogs will continue into the next three-year approval period. We plan to continue to test new glucose sensors as they are developed to verify good glucose-sensing properties and to define the correct mathematical algorithm necessary to provide the proper feedback to insulin pumps.**

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

► **There are no proposed changes to the protocol in this triennial submission.**

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).
(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):
►(**X**) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of veterans, the general population 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.

► **Approximately 3.7 million patients with both type 1 and type 2 diabetes are currently treated with insulin in the US, including a high percentage of veterans. In their advanced forms, the pancreas of patients with both type 1 and type 2 diabetes makes too little insulin, requiring insulin injections. When insulin is too low, blood levels of a kind of sugar called glucose become too high, which then damages blood vessels, nerves, eyes, kidneys, and other tissues. Low insulin also makes it impossible for cells that need glucose to absorb it, so they starve. It is a constant challenge for individuals with insulin-dependent diabetes to accurately measure and adjust their glucose levels by administering insulin. Traditionally, patients typically measure their blood glucose level by getting blood from needle sticks (also called "finger sticks") as often as possible per day in order to calculate and inject insulin according to their blood glucose values.**

This research is being performed to develop new, easier to use devices, or improve existing devices, that will automatically measure blood glucose levels and administer the right amount of

insulin continuously throughout the day with no effort on the part of the patient. Tight glucose control would significantly improve a patient's quality of life. It would also reduce the incidence of secondary, possibly life-threatening complications (e.g., eye diseases, kidney failure, skin disease, and nerve damage) and, therefore, would tremendously reduce health-care costs.

This work cannot be done using human pathology specimens because we need to see how these sensors work in a living body. This work cannot be done in a clinical trial because the FDA requires new devices to first be tested in animals to demonstrate safety and reliability before approving a clinical study that uses these devices.

The ultimate goal of this research is to develop accurate and long-lasting glucose sensors which patients can insert under their skin that will continuously monitor and relay glucose levels to a wearable insulin pump that automatically delivers insulin based on need.

C. **Experimental Design.**

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

   ► Our focus is on testing different kinds of glucose sensors to determine which ones work best, and to develop wireless communication systems that let the sensors communicate with the insulin pumps to determine how much insulin to give to the patient.

   Studies will be performed in dogs (normal and diabetic) during states of normoglycemia (normal blood-glucose levels), hyperglycemia (high blood-glucose levels), and hypoglycemia (low blood-glucose levels). These are called "clamp tests". Glucose levels will be altered by giving glucose, insulin, and/or glucagon. Standard oral glucose tolerance tests (similar to ones used in patients) and meals will be used to create normal glucose-level variations.

   Sensors will be placed under the skin to measure glucose levels, and the results will be compared to glucose levels measured in the blood. (If the sensor works perfectly, the glucose levels the sensor measures in the skin will match the glucose levels in the blood.) The withdrawal of blood samples from dogs is performed the same way as in humans.

   We will also work on developing good wireless communication between the sensor and the pump. In order to be sure this works properly, we must run tests with the sensor in a living body.

   The diabetic dogs will be treated with insulin every day just as if they were diabetic patients, either by insulin injections or with external insulin pumps (the same kind of pump that is used in human patients).

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.

► Both normal and diabetic dogs will continue to be used in this protocol at an approximate ratio of 2:1. The goals are to test new or existing glucose sensors and to develop the proper

algorithms to make them work with new or existing insulin pumps. Daily care of the dogs is described in detail in Appendix 6, while insertion of IV catheters, glucose sensors, and insulin delivery catheters is described in Appendix 5. In none of these tests will more than 10% of the total blood volume be taken within a two-week period to prevent the occurrence of anemia and hypovolemia. Basically, dogs will have glucose sensors inserted under the skin, and their blood glucose experimentally raised or lowered to determine the time it takes for the sensors to detect these changes.

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

► **There are two unknown factors that make it difficult to statistically determine the number of dogs to be used: 1) the number of sensor types that will be developed and tested during the three-year approval period, and 2) the number of times a sensor must be tested in order to arrive at an acceptable insulin-infusion algorithm. The more dogs that participate in these tests, the fewer the number of procedures performed on any one dog. Since some of the dogs currently enrolled may reach 10 years of age (the normal endpoint age for dog studies), the number of dogs requested includes those necessary to replace existing dogs. For space-related reasons, we will never enroll more than 34 dogs at any given time.**

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.) Describe each procedure in a few words or just one sentence, and then write "see appendix X for details". e.g. stereotaxic surgery – see appendix 5 for details, Morris water maze – see appendix 6 for details.

► <u>Hyperglycemic-Clamp Test (normal dogs only)</u>
This test will be performed to estimate insulin response and glucose sensor performance in normal dogs under experimental hyperglycemic conditions. The test will be conducted on conscious dogs equipped with two acute venous catheters (cephalic and/or saphenous)(one for blood sampling, the other for infusions) and up to six glucose sensors. Following an overnight fast, basal blood samples will be drawn once every 20-30 minutes for approximately 60-120 minutes. Blood samples will be taken with heparinized syringes and immediately analyzed or centrifuged and the supernatant (plasma) separated and frozen. Catheters for blood sampling will be flushed with heparinized saline (30 U/ml) to maintain patency, but heparin will not be injected into the dog. Once a steady basal blood-glucose level is obtained, blood-glucose levels will then be elevated to ~300 mg/dl with a primed, variable-rate infusion of 50% glucose through one of the IV catheters. Blood-glucose levels will be maintained at ~300 mg/dl for 60-120 minutes. After that period, the glucose infusion will be stopped and the blood sampling continued for another 60-120 minutes. At the end of the test, both IV catheters will be removed and the animal will be fed and returned to the kennel. Glucose sensors will remain in place for 4-7 days and a jacket will be placed on the dog to prevent dislodgement of the sensors. A total of 0.2-0.5 ml of blood will be collected in 35-140 samples.

<u>Closed-Loop Test (diabetic dogs only)</u>
This study is designed to test the stability and sensitivity of glucose regulation by infusion of insulin in a closed-loop system. The test will be conducted on conscious dogs equipped with two acute venous catheters (cephalic and/or saphenous)(one for blood sampling, the other for infusions), an insulin pump, and up to six glucose sensors. Following an overnight fast, basal

blood samples will be drawn every 10-15 minutes for approximately 60-120 minutes. Blood samples will be taken with heparinized syringes and immediately analyzed or centrifuged and the supernatant (plasma) separated and frozen. Catheters for blood sampling will be flushed with heparinized saline (30 U/ml) to maintain patency (heparin will not be injected into the dog). Once a steady basal blood-glucose level is obtained, the programmed basal insulin infusion rate will be stopped and the artificial beta cell (ABC) control on the pump will be switched on. After 1-3 hours, the dog will be fed and blood samples will be collected every 5-10 minutes for 6-9 hours. At the end of the test, both IV catheters and sensors will be removed, and the insulin pump will be switched back to the normal basal insulin infusion rate schedule. A total of 0.2-0.5 ml of blood will be collected in 35-140 samples.

These closed-loop tests will be performed in each dog to establish the optimal parameters of the glucose-sensing/insulin-delivery algorithm for each individual. Once the optimal parameters are established, additional tests will be performed varying the algorithm parameters ½ to 1½ of the optimal in order to establish a range in which the algorithm will function properly.

Bi-hormonal Closed-Loop Test (diabetic dogs only)
This study is designed to test the stability and sensitivity of glucose regulation by infusion of both insulin and glucagon in a closed-loop system. The test will be conducted on conscious dogs equipped with acute venous catheters (cephalic and/or saphenous)(one for blood sampling, the other for infusions), an insulin pump and a glucagon pump connected to separate subcutaneous infusion catheters, and up to six glucose sensors. Following an overnight fast, basal blood samples will be drawn every 10-15 minutes for approximately 60-120 minutes. Blood samples will be taken with heparinized syringes and immediately analyzed or centrifuged and the supernatant (plasma) separated and frozen. Catheters for blood sampling will be flushed with heparinized saline (30 U/ml) to maintain patency (heparin will not be injected into the dog). A steady basal blood-glucose level will be obtained using the regular proportional integrative derivative insulin feedback (PID-IFB) control algorithm on the insulin pump (or twice this rate). The glucagon pump will then be manually set to deliver glucagon at a rate of 0.05 U/hr. In some experiments, the glucagon pump will be set to automatically deliver a bolus of glucagon when hypoglycemia is predicted using a new glucagon-control algorithm. After 1-3 hours, the dog will be fed and blood samples will be collected every 5-10 minutes for 6-9 hours. At the end of the test, the IV catheters, sensors, and the glucagon pump and catheter will be removed, and the insulin pump will be switched back to the normal PID-IFB insulin infusion schedule. A total of 0.2-0.5 ml of blood will be collected in 35-140 samples.

These closed-loop tests will be performed to establish the optimal algorithm parameters for insulin/glucagon-delivery. Once these optimal parameters are established, additional tests will be performed varying the algorithm parameters in order to establish a range in which the algorithm will function properly.

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

▶ The diabetic dog is an appropriate and well-established model for testing insulin pharmacokinetics and dynamics, and has been used to study diabetes since 1880. The dog is very cooperative, its size allows frequent blood sampling, and its insulin sensitivity is comparable to humans. Several basic issues like insulin transport and glucose-distribution space have been investigated in dogs in the past so there is a good body of pre-existing information from which to draw.

A smaller animal cannot be substituted for this study because we need to test human-sized glucose sensors and insulin pumps. Other laboratory animals (rabbits, guinea pigs, rats, mice) are simply too small for these devices. Furthermore, we need to do frequent blood collections to measure blood glucose, and with smaller animals there is a possibility of anemia and hypovolemia from all the blood draws. The dogs are large enough and have a big enough blood volume that this is not a problem.

Other large animals have been used and found to not work nearly as well as dogs. Pigs literally rub the pump and sensor off against the wall, their skin is too tough for the sensor, and their subcutaneous fat interferes with the sensor. Sheep and other ruminants have not been well-characterized as a diabetic model, probably because of the profound differences in a ruminant's digestive system as compared to other mammals, including dogs and humans.

Personnel

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

1. PI
   Name▶ [redacted]
   Animal research experience [redacted]

   Qualifications to perform specific procedures

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

2. Other research personnel (copy the lines below for each individual)
   Name▶ [redacted]
   Animal research experience ▶ **More than 25 years of animal-research experience, including 20 years with dogs.**

   Qualifications to perform specific procedures

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

   Name▶ [redacted]
   Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

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

Name▶ [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

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

Name▶ [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

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

Name▶ [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

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

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)
Name► **To be determined by VMO.**

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

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
TBD by VMO	**TBD by VMO**

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

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

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

G. **Occupational Health and Safety.**

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

Name	Enrollment in Occupational Health and Safety Program		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
Krahl	**X**		**X**	
Loutseiko	**X**		**X**	
Montoya	**X**		**X**	
Pidkova	**X**		**X**	
Pranchenko	**X**		**X**	
Shiff	**X**		**X**	

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

   ▶ ( ) Yes. Describe them ▶

   ▶ (**X**) No.

Animals Requested

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

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Dog (mongrel)	**M/F**	**Adult dogs (over 9 mos. old)**	[redacted]	**SPF**

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

USDA Category B

Procedures▶						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category B TOTAL**

USDA Category C

Procedures▶						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category C TOTAL**
Dog	**40**	**40***	**40***			**40***
*** A total of 40 dogs will be used, including those now enrolled. Those listed under Years 2 & 3 are inclusive of those in Year 1.**						

USDA Category D

Procedures▶						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category D TOTAL**

USDA Category E

Procedures▶						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category E TOTAL**

TOTALS over all Categories

Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**GRAND TOTAL**
Dog	**40**	**40***	**40***			**40***
*** A total of 40 dogs will be used, including those now enrolled. Those listed under Years 2 & 3 are inclusive of those in Year 1.**						

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

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

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

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

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

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

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

▶

Veterinary Care and Husbandry

L. **Veterinary Support.**

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

[redacted]

2. Veterinary consultation during the planning of this protocol.

Name of the laboratory animal veterinarian consulted▶ [redacted]
Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ▶ [redacted]

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

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

a. Species	b. Type of housing*		c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)		e. Estimated maximum number of housing units needed at any one time
Dog	**X**	**Standard**	**One or two**	**X**	**Yes**	**34**
		Departures from the Guide			**No**	

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

▶ (X) **Standard (See SOP)—Enter SOP in the table in Item Y.**
▶ ( ) **Standard (not covered by a SOP)**
▶ **Describe:**

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

► ( ) **N/A: Animals will be housed in stable pairs or groups.**
► (**X**) Animals will be housed singly:
►Provide justification: **Group housing of compatible dog pairs will be used. Group housing is not possible for dogs wearing jackets because sensors and external insulin pumps need to be protected. These dogs will be housed in single dog runs, but are able to see and hear each other. In addition, dogs will be allowed to socialize in small compatible groups in the outside exercise area (or in the housing room outside of their cages as allowed by the Exercise SOP) whenever feasible.**

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

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

a. Species	b. Description of Enrichment*		c. Frequency	
Dog	**X**	**TBD by VMO**	**X**	**TBD by VMO**
		Non-standard enrichment (describe and justify below)		**Other**

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

► (**X**) **Standard (TBD by VMO)**
► ( ) **Non-standard**
Description on non-standard enrichment and justification:

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

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.

►

► ( ) This ACORP does NOT include use of any animals that will require customized routine husbandry. If checked, go to item N.

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

► ( ) **N/A**
► (X) **Describe: Dogs will frequently have multiple subcutaneous glucose sensors and an insulin catheter inserted subcutaneously. Both of these devices are used clinically in the same manner utilized in this protocol. Sensors and catheters will be inserted using aseptic techniques to reduce the chances of infection at the insertion site. Human diabetic patients self-insert these devices with nothing more than an alcohol swipe.**

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

► ( ) **N/A**
► ( ) **Describe:**

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

► **(X)** Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU. If it will be in the VMU, just indicate West LA VMU or Sepulveda VMU.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]	**TBD by VMO**	**X**	

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table. **(NOTE TO REVIEWERS: This space is for NON VA Facilities, like USC and UCLA. Dr. Martin can assist on AAALAC status).**

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

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

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

Special Features

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

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".
► **(X)** NO animals on this protocol will be used in the production and harvesting of antibodies.

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

► **(X)** This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".
► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

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

Procedure	Surgical?		Bldg/Room Number	Requires transport between the VMU and the laboratory, or transport between laboratories?	
	Yes	No		No	If Yes – describe method of discreet transport
Clamp testing		**X**	[redacted]		**Dogs are walked to the laboratory using a leash.**
Blood sampling		**X**			**Dogs are walked to the laboratory using a leash.**

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

Body Fluid, Tissue, or Device to be Collected	Collected AFTER Euthanasia	Collected BEFORE Euthanasia		
		Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
Blood samples				**X**
Glucose sensors				**X**
Insulin pump catheters				**X**

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

► **(X*)** Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".
► ( ) NO animals on this protocol will undergo surgery.

*** Although the insertion of glucose sensors, insulin infusion catheters, and IV catheters are not considered surgeries, the Surgery Appendix has been completed to describe the use of local anesthesia and aseptic preparation of insertion sites.**

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved).

In addition, specify how often the animals will be weighed to be sure weight loss does not exceed 10%.

► **The dogs are monitored daily, and the physical and psychological well-being of each dog is a priority. For that reason, the dogs (diabetics and non-diabetics) are capable of participating in this protocol for many years. The dogs are habituated to different situations (lying on a table without restraint for several hours, daily blood sampling, etc.), and it takes several months of training before these dogs can be used for the procedures described. If the health and status of the dog allow it, we plan to keep each dog up to 10 years of age, after which we will try to find homes for the dogs following established methods, or they will be euthanized if deemed appropriate.**

Body weight will be measured once per month. The Purina Body Condition Scoring System will be used to estimate body fat/muscle mass. Inappetence, signs of distress, and other abnormal health conditions will be monitored daily. Endpoint criteria will be reached if any animal loses more than 10% of their normal body weight (as determined at their last annual physical exam), has a Body Condition Score falling below 4 on a scale of 1-9, or displays signs of inappetence, distress, or any other abnormal health condition. If a dog meets any of these criteria, the veterinary staff will be consulted and treatment will be provided.

Some dogs display mild-to-moderate localized skin irritation when adhesive tape (used to anchor gauze pads overlying glucose sensors) is removed, even when using adhesive solvent. Significant irritation will be treated with diphenhydramine (Benadryl) (125 mg qd po or 1-2% bid topical for three days) to provide comfort and protection from scratching.

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

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

► **Dogs that meet endpoint criteria will be withdrawn from the study until they have been rehabilitated. Rehabilitation will be attempted in consultation with the veterinary staff. Dogs may rejoin the study protocol once they have been successfully rehabilitated (i.e., weight loss <10% and a Body Condition Score of 4 or more), and with the approval of a veterinarian. Dogs that do not respond to treatment will be euthanized at the discretion of the veterinarian.**

Any medical condition that seriously affects the well-being of a dog will cause removal of that dog from the study protocol, and that medical condition will be treated by the veterinary staff. Any dog that responds to treatment will be allowed to rejoin the study once the veterinary staff determines that the underlying medical condition has resolved. Any dog that remains seriously

ill and does not respond to treatment after a reasonable amount of time will be euthanized at the discretion of the veterinarian.

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

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►				
X	Anesthetic overdose Agent► **Pentobarbital** Dose► **100 mg/kg** Route of administration► **IV** Method for verifying death► **Cessation of heartbeat and respiration**	**Dog**	**X**		
	Decapitation under anesthesia Agent► Dose► Route of administration►				
	Exsanguination under anesthesia Agent► Dose► Route of administration► Method for verifying death►				
	Other (Describe) ► Method for verifying death►				

	Other (Describe) ► Method for verifying death►				

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

   ► (X) **N/A**
   ► ( ) **Justification:**

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

   ► (X) **N/A**
   ► ( ) **Justification:**

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.

   ► ( ) **N/A**
   ► **Only trained veterinary staff will perform euthanasia.**

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.

      ► (X) **According to Biocontainment SOP.**
      ► ( ) **Not according to Biocontainment SOP:**
      ►**Justification and description (must review first with VMO):**

   b. Describe how the PI's staff should be contacted.

      ► (X) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
      Name► [redacted]
      Contact Information► [redacted]

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

Name►
Contact Information►

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

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
Feeding schedule		Items:	(**X**) **
Diabetic dog care		Items:	(**X**) **
Bathing		Items:	(**X**) **
Prolonged recumbency		Items:	(**X**) **

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

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

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

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

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ► (**X**) **N/A**
   ► ( ) **Painful or distressing procedures:**

   ►

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

PI should run at least one search on the ALTBIB website for animal use alternatives. Please use the link http://toxnet.nlm.nih.gov/altbib.html

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
ALTBIB	**12/02/16**	**2000 - 2016**	**N/A**	**diabetes model**	**X**			
PubMed	**12/02/16**	**1980 - 2016**	**N/A**	**(diabetes) AND (dog) AND (glucose sensor)**		**X**	**X**	**X**
PubMed	**12/02/16**	**1980 - 2016**	**N/A**	**(diabetes) AND (dog) AND (insulin pump)**		**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.

► Based on our literature searches, we determined that these procedures cannot be replaced by a computer model or be conducted *in vitro*. The physiological reaction of a diabetic dog or human patient to a glucose sensor cannot be simulated by computers. Neither can the *in vivo* reaction of tissue and cells to the glucose sensor be simulated by a computer or *in vitro*. Preliminary sensor testing is done *in vitro* with glucose solutions, but the influence of other substances in the body and the body's reaction to the sensor cannot be reproduced in an *in vitro* model.

Smaller animals are too small for most of our devices and their blood volume is not large enough to allow frequent blood glucose measurements without negative health consequences. Based on our literature searches and on our own extensive experience in this field, dogs were chosen because the model is widely used in metabolic studies and comes closest to approximating the human diabetic condition. The dog was also chosen because they are highly cooperative and easily handled. Blood volume and glucose response characteristics allow for fast and easy manipulation of blood glucose levels, and dogs have a large area for subcutaneous application of glucose sensors. Dogs do not react adversely to the insertion of a sensor or catheters. As discussed in Section D, other large animals such as pigs and sheep have been tried and were far less suitable than dogs for this work.

In addition, the main purpose of these experiments is to collect preclinical data to support the safety and efficacy of glucose sensors, glucose pumps, and the algorithms used to control the rate of insulin infusion. One of the most important factors used by the FDA to evaluate the suitability of a preclinical animal model is precedent. There are no known non-mammalian models that have been used to collect the aforementioned preclinical data, and therefore, non-mammalian species would be unsuitable for the present protocol.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.

   ► **The number of experiments conducted is the minimum necessary to properly test new sensors and glucose-sensing/insulin-infusion algorithms. Also, reuse of dogs multiple times in these relatively non-invasive procedures reduces the total number of dogs needed.**

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

   ► **The dog diabetes model does not produce excessive distress, pain, or suffering, and should not exceed that experienced by humans with diabetes. In our experience, the dogs do not develop many of the problems seen in human diabetic patients (e.g., diabetes-related kidney failure, blindness, foot gangrene, etc.). Furthermore, our dogs are much slower to develop cataracts than pet dogs with diabetes. We attribute this to our careful blood-glucose control. Based on our literature searches and on our own extensive experience in this field, further refinement of our experimental protocol is not possible and would not improve the study results or the treatment of the dogs.**

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

   ► **Based on our literature searches and our own extensive experience in this field, this project does not duplicate any previous work. New algorithms will be tested, as will different glucose sensor and insulin pump variations. Testing these new sensors, and sensor/pump variations, is necessary to develop new clinical products for the treatment of diabetes.**

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

► **(X*) N/A (Go to Question 2).**
*** Pentobarbital for euthanasia will be stored and used by VMU staff only.**

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

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Phar-macy	Non-VA
		( )*	TBD				
		( )*					
		( )*					

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

► ( ) **N/A**
► ( ) **Justification:**

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

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

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

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".
► (**X**) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

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

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".
► (**X**) No explosive agent(s) will be used as part of this protocol.

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

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

► (**X**) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (**X**) Appendix 3, "Biosafety"

► (**X**) Appendix 4, "Ante-mortem Specimen Collection"
► (**X**) Appendix 5, "Surgery"
► (**X**) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"
► ( ) Appendix 10, "Overnight housing"

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

Item	SOP		Approval Date
	Title	ID	
C.2.c	This needs to be pre-populated		
M.1			
M.2			
U.4.a			
U.4.b			
V			

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

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

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

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

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

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
   - Changing any procedure in any way that has the potential to increase the pain/distress category

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

I further certify that:

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

Principal Investigator	PI Signature	Date
[redacted]		[redacted]

b. **Certification by IACUC Officials.**

We certify that:

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

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

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

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

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted]		[redacted]

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

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

3. **Appendix 3. Biosafety.**

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

   We certify that:

   - Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

   - All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Principal Investigator	Signature	Date
[redacted]		[redacted]
Name of Institutional Veterinarian	Signature	Date
[redacted]		[redacted]
Name of IACUC Chair		Date
[redacted]		[redacted]

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

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

   - The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;

- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

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

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

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

Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee	Signature	Date

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:
  - Identification of each animal such that care for individual animals can be documented.
  - Daily postoperative medical records for each animal, that include documentation of daily

evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
- Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
- Daily records covering at least the period defined as "post-operative" by local policy.
- The signature or initials of the person making each entry.

Principal Investigator	PI Signature	Date
[redacted]		[redacted]

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

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

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

Principal Investigator	PI Signature	Date

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

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

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

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

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

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

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

   I further certify that:

   - Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
   - All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
   - Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;

- Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

Principal Investigator	PI Signature	Date

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

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of VISN Regional Safety Officer	Signature	Date

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

10. **Appendix 10. Certification by Principal Investigator is on the Appendix.**

Last Name of PI▶
Protocol No. Assigned by the IACUC▶
Official Date of Approval▶

ACORP Appendix 1
ADDITIONAL LOCAL INFORMATION
VERSION 4 V2 6/17/2015
(Required for all protocols)

(See ACORP App. 1 Instructions, for more detailed explanations of the information requested.)

Species covered by this Appendix: **Dog**

This protocol involves the following (check all that apply):

☐ Breeding ☐ Tumor Formation ☐ Hazardous agents used in animals
☐ Multiple survival surgery ☐ Food and/or Fluid Restriction
☐ Antibody/Ascites Formation ☐ Hazard to VMU Personnel
☐ Prolonged Restraint (> than 15 minutes) ☐ Tumor formation

a.VA project # **0013**	b. Protocol # **08075-02** [redacted]

[redacted]

h. Protocol title: **Glucose Sensing and Physiologic Insulin Delivery** [redacted]

p. Animals taken to lab? ☒ Yes ☐ No [redacted]

q. Animals taken to lab and then returned to vivarium (*VMU return room only*) ☒ Yes ☐ No If yes, provide a scientific justification here: [redacted]

r. Animals housed in the lab for 12 or more consecutive hours? ☐ Yes ☒ No
If yes, Bldg: and Room(s): and fill out part B below.

s. Is wire-floored caging required? ☐ Yes ☒ No

t. Do animals need to be exempted from the environmental enrichment program?
☐ Yes ☒ No If yes, provide a scientific justification here:

u. Maximum allowable body weight loss (10% unless scientifically justified): **10%**

v. Hazards used in animals (check all that apply): ☒ None ☐ Toxic ☐ Infectious
☐ Biological ☐ Radioactive ☐ Other (list):

w. Will VMU personnel be exposed to any of these hazards? (This includes animals housed in labs since VMU staff check them, wash the cages, etc.) ☐ Yes ☒ No
If yes, list which hazards:

x. Body fluid, tissue and/or device collection? ☐ None ☒ Live ☐ Dead ☐ Both

y. Surgery? ☒ None ☐ Minor ☐ Major ☐ Both ☐ Non-survival
Multiple survival surgeries? ☐ Yes ☒ No If there are multiple survival surgeries, list surgery types:

z. Anesthetics/analgesics used (excluding euthanasia)? ☒ Yes ☐ No If yes, list: **acepromazine, lidocaine**
aa. Euthanasia methods (must include anesthetic plus physical method unless scientifically justified): **pentobarbital overdose**
bb. All controlled substances used: **pentobarbital (from VMU)**
cc. List any other drugs from Appendix 5 (surgery appendix): **None**

Delegation of Authority: Complete this section for every employee in this study, starting with the PI, specifying which procedures each is allowed to perform. All should be listed in the ACORP main body. Everyone listed must also have current employment status (VA or WOC) and be up-to-date with all required training and medical clearances.

Please note: There must always be at least one person responsible for task codes A, D, and H.

Species: Dog

Last name, first name, degree(s):	Task codes (use the list below):
[redacted]	**D, J**
	A, D, F
	A, F
	A, F
	A, F
	A, F

Task codes

A = Routine daily care of animal	I = Performs in vivo procedures other than sample collection or surgery, such as behavioral studies
B = Performs survival surgery	J = Other work with animals, please specify: **Administrative oversight**
C = Performs non-survival surgery	K = PI with no animal contact. The PI must be listed in section E of the ACORP Main Body. If the PI does have animal contact, list them with the appropriate task codes.
D = Evaluates endpoint criteria	L = Non-PI with no animal contact. This person does not need to be listed in section E of the ACORP.
E = Collects samples with anesthesia	
F = Collects samples without anesthesia	
G = Collects or works with samples postmortem	
H = Euthanizes animal subjects	

Last Name of PI▶
Protocol No. Assigned by the IACUC▶
Official Date of Approval▶

ACORP APPENDIX 3
BIOSAFETY
VERSION 4 V2 6-17-2015

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

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

Material Identify the specific materials including radioisotopes, chemicals, drugs (standard clinical agents as well as test agents), controlled substances, infectious agents, biomaterials, prosthetic devices, minipumps, special diets, and cells, tissues, or body fluids. *(Do not list drinking water and the standard food from the VMU)*	**Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors)	**Nature of Material**							
		Toxic Agent (such as mutagens, carcinogens, teratogens, neurotoxins, Select Agents, ect. - Item 4)	Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, or 2*)	Biological Agent (Item 6)	Radioactive Agent (Item 7)	Contains Recombinant Nucleic Acid (Item 8)	Routine Pre- or Post-Procedural Drug	Euthanasia agent	Other
Glucose sensors	**Medtronic**		BSL-						**X**
Insulin catheter	**Medtronic**		BSL-						**X**
Venous catheter	**Medtronic**		BSL-						**X**
Glucose	**Pharmacy**		BSL-						**X**
Insulin	**Medtronic**		BSL-						**X**
Liraglutide	**Pharmacy**		BSL-						**X**
Glucagon	**Pharmacy**		BSL-						**X**
Bacitracin, neomycin, polymyxin B (antibiotic cream)	**Pharmacy**		BSL-						**X**
Diphenhydramine (oral)	**Pharmacy**		BSL-						**X**
Diphenhydramine (topical)	**Pharmacy**		BSL-						**X**
Acepromazine	**Pharmacy**		BSL-						**X**

Lidocaine	**Pharmacy**		BSL-						**X**
Pentobarbital	**Pharmacy**		BSL-					**X**	

Only BSL 1, 2 or 2* work is permitted at VA-GLA. No BSL 3 or 4 work.

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in Item 1 above. For each item, note if it is USP grade, FDA approved, a fixative, or a special diet.

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify "Main Body" or "App #", and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)
Glucose sensors	**N/A**	**N/A**	**N/A**	**Up to 14 days**	**To measure subcutaneous glucose levels**	**App. 5**	**N**
Insulin catheter	**N/A**	**N/A**	**N/A**	**Up to 14 days**	**To deliver insulin**	**App. 5**	**N**
Venous catheter	**N/A**	**N/A**	**N/A**	**5 hrs**	**To measure blood glucose levels**	**App. 5**	**N**
Glucose	**5-20 mg/ kg/min**	**Sterile, pharma-ceutical grade saline**	**IV or oral**	**1-5 hrs**	**To reduce hypoglycemia or produce hyperglycemia**	**App. 6**	**N**
Insulin	**2-50 units/day**	**N/A**	**SC or oral**	**Daily, lifelong treatment**	**To reduce hyperglycemia**	**App. 6**	**N**
Liraglutide	**25-100 µg/kg**	**N/A**	**SC**	**Daily**	**To stabilize blood-glucose levels and minimize hyperglycemia**	**App. 6**	**N**
Glucagon	**0.05 units/hr**	**N/A**	**SC**	**1-5 hrs**	**To reduce hyperglycemia**	**App. 6**	**N**

Bacitracin, neomycin, polymyxin B (antibiotic cream)	**N/A**	**N/A**	**Topi-cal**	**PRN**	**To treat minor skin abrasions**	**App. 5**	**N**
Diphenhydramine	**125 mg**	**N/A**	**PO**	**Daily**	**To treat skin irritation**	**App. 6**	**N**
Diphenhydramine	**1-2%**	**N/A**	**Topi-cal**	**BID**	**To treat skin irritation**	**App. 6**	**N**
Acepromazine	**0.3 mg/kg**	**N/A**	**PO**	**Once**	**To reduce anxiety**	**App. 5**	**N**
Lidocaine	**2%**	**N/A**	**SC**	**Once**	**To prevent minor suture pain**	**App. 5**	**N**
Pentobarbital	**100 mg/kg**	**N/A**	**IV**	**Once**	**Euthanasia**	**Main Body**	**N**

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm
or animals http://www.accessdata.fda.gov/scripts/animaldrugsatfda/

Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, fill out tables 2a and 2b below to explain why the use of a non-pharmaceutical grade formulation is necessary, and to describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)

Table 2a

List all items from table 2 that are not USP grade, FDA approved, a fixative, or a special diet	Why the use of a non-pharmaceutical grade formulation necessary? *Please put an X in the appropriate column, and add rows as needed.*			
	No FDA approved version exists	The FDA approved injectable forms are too dilute or have the wrong diluents for this study*	The FDA approved versions are only in pills or other forms that aren't suitable for this study	Other (please explain)

**Note: Injectables that are too concentrated can usually be diluted with saline.*

Table 2b

List all items from table 2 that are not USP grade, FDA approved, a fixative, or a special diet	How it will be ensured that the material is suitable for use? *Please put YES, N/A, or an explanation in each column, and add rows as needed.*					
	Purity/grade/pyro-genicity	Sterility	Osmolality	Stability	Formulation and pharmacokinetics	pH
	The certificate of analysis from the manufacturer will be examined to ensure the material is suitable.	If the drug does not come as a sterile solution, it will be sterile filtered before use.	Sterile USP grade isotonic diluents will be used, such as USP grade normal saline.	The supplier's guidelines on storage and stability will be followed.	The literature has been consulted to determine the appropriate formulation and that the pharmacokinetics are suitable	The pH of the solution will be tested (with pH paper or a meter) before injection

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

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

   b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.
   ▶ Administration of the materials/drugs is not distressing or painful for the dogs. Administrative methods in dogs are similar to those used clinically in humans without anesthesia.

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

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

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

Name of agent ▶

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

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

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

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

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

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

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

Name of agent ►

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

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

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

Name of Biological Agent	Screening for Infectious Agents

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

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

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

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

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

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

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

   a. Complete the table below.

Name of Hazardous Agent	Approving Committee or Official	Institution (VA or affiliate)	Names of Animal Facility Staff Members at Risk (for VA-GLA put "to be determined")

   b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents. The following Agent Management Plan is provided for the VMU staff and others who come into contact with these animals.

AGENT MANAGEMENT PLAN
11. Names and strains (if applicable) of agent(s), chemical(s) or toxin(s) covered in this plan:
12. ☐ Yes ☐ No: Is baseline screening of serum required for VMU animal care workers? If you are unsure if this is necessary, please contact the RBSO via email at Jerry.Dungan@va.gov
13. Discuss appropriate PPE to be worn by PI, PI's staff, and VMU staff:
14. Identify any risks associated with bedding, and precautions/handling measures to be observed by VMU staff. This includes cages/bedding returned to the VMU from work conducted in the laboratory.
15. Indicate any particular instructions/precautions regarding potential hazards for project and/or VMU staff for handling animal food and water.
16. Discuss any special medical treatment guidelines that the project and/or VMU staff need to be aware of in case of animal medical emergency. For instance, are there specific compounds or circumstances that cannot be used or should be avoided?

17. Discuss specific health risks relative to the agent/chemical/toxin addressed in this Plan for persons who come in contact with animals/returned carcasses covered under this PSP, to include routes/mechanisms of transmission. Describe initial signs and symptoms of exposure.			
18. Discuss precautions/protocols that should be taken in case of escape of a live animal to which the agent/chemical/toxin addressed in this Plan has been administered.			
19. **Check all cage card requirements:**			
☐ Carcinogens	☐ Infectious Agents	☐ Neurotoxins	☐ Mutagens/Teratogens
☐ Biologic Toxins	☐ Human Cells and/or Cell Lines	☐ Other (specify):	

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

Last Name of PI▶
Protocol No. Assigned by the IACUC▶
Official Date of Approval▶

ACORP Appendix 4

ANTEMORTEM SPECIMEN COLLECTION
VERSION 4 V2 6-17-2015

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

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

Specimen Collected	Site and Method of Collection	Anesthesia (Yes/No)	Amount Collected Each Time	Volume Replacement (Yes/No/NA)	Total Number of Collections per Animal	Time Intervals Between Successive Collections
Blood	**Cephalic or saphenous vein for daily treatment of diabetic dog (venipuncture) (location rotated to reduce fibrosis) (blood is cooled during transport to lab). Blood-sample sites will be shaved and wiped with alcohol.**	**No**	**0.2-0.5 ml**	**No**	**2-5 times/ day (no more than 3 draws will be from the same vein/day)**	**Daily**
Blood	**Cephalic or saphenous vein for clamp or closed-loop tests (via an acutely placed catheter) (location rotated to reduce fibrosis). Blood-sample sites will be shaved and wiped with alcohol.**	**No**	**0.2-0.5 ml**	**No**	**35-140 samples/ test**	**At least one day**

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

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

      (1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain). **► Blood collection will be performed either with a needle and syringe (for daily blood samples) or a venous catheter (for clamp tests). The dogs are accustomed to the method and do not need to be restrained for blood collection. In none of these experiments will**

more than 10% of the total blood volume be taken within a two-week period to prevent anemia or hypovolemia.

(2) Completely describe any method of physical restraint that may be used.
► **None**

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

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) and volume (ml)	Route of administration	Frequency of administration

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

a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.
► **The volume of blood collected is not sufficient to require volume replacement. Volume replacement may also skew the blood glucose readings.**

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

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).
► **Dogs will not be anesthetized and the small volume of blood collected will not require monitoring. Pressure will be applied to the blood sampling site when needles or catheters are removed to reduce bleeding and/or hematoma formation.**

Last Name of PI▶
Protocol No. Assigned by the IACUC▶
Official Date of Approval▶

ACORP Appendix 5

SURGERY
VERSION 4 v2 6-17-2015

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

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

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	**IV catheter insertion**	**No**	**X**		( )*
2	**Subcutaneous glucose sensor insertion**	**No**	**X**		( )*
3	**Subcutaneous insulin catheter insertion**	**No**	**X**		( )*

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

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

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

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

Note: The following procedures are not considered surgeries, but are described here in order to provide important details regarding the preparation of insertion sites, and the insertion techniques for placement of IV catheters, subcutaneous glucose sensors, and subcutaneous insulin delivery catheters.

Surgery 1 ▶ **Two catheters will be placed in separate locations of the cephalic and/or saphenous veins (one for blood collections, and the other for glucose/insulin injections). After prepping the insertion site (described below), each catheter will be inserted into the vein and fixed in place with tape. The catheters will contain a three-way valve to allow blood collection from one port, and flushing of the catheter through the other port. After flushing, the valve will be closed. IV catheters will only remain in place during clamp tests, and will be removed at the end of the test.**

Surgery 2 ▶ **Glucose sensors will be inserted on the lateral portion of the thorax, each spaced approximately two inches apart and the intended locations marked. Lidocaine will be injected**

into the dermis at a 45-degree angle with a 27-gauge needle and syringe until a small bubble of fluid is visible under the skin. This will be repeated for all insertion and suture sites. Lidocaine is not administered when these sensors are used in humans, but has been added to the dog protocol to reduce any pain involved.

Using aseptic technique, glucose sensors will be inserted into the skin according to the manufacturer's instructions. The sensors consist of a small strip of sensor material (approximately the size of a hypodermic needle) that is inserted into the skin using a rigid insertion cannula that is approximately 1 cm in length. Once inserted, each sensor will be sutured in place with non-absorbable coated vicryl using aseptic techniques. This will be repeated for each sensor. The transmitting unit for each sensor will then be connected, the area will be covered with sterile gauze, and taped in place using medical-grade tape. A dog jacket will be used to prevent the sensors from becoming dislodged.

Surgery 3 ► **An insulin catheter will be inserted subcutaneously on the lateral portion of the thorax using exactly the same methods as for the placement of glucose sensors. These catheters will be replaced at least every 14 days. When replaced, they will be moved at least two inches from the previous infusion site.**

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

Name	Surgery #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	All	X*			
	All	X*			
	All	X*			
	All	X*			
	All	X*			

[redacted]

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

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
[redacted]		**All**	**No**	**( )***	**(X)***

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

► **These procedures are not considered surgeries, so a dedicated surgical facility is not necessary.**

5. **Pre-operative protocol.**

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

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

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

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Lidocaine (at location of sensor insertion/suturing)	**All**	**0.1-0.3 mg/kg of a 2% solution**	**Intradermal**	**Once per insertion site**	**10-15 minutes before procedure**
Acepromazine	**All**	**0.3 mg/kg (0.03 ml of a 10 mg/ml solution)**	**SC**	**Once (only given if needed to reduce anxiety)**	**10-15 minutes before procedure**

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

Surgery 1 ▶ **The skin over the cephalic and/or saphenous catheter insertion sites will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

Surgery 2 ▶ **The skin over the lateral thorax will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

Surgery 3 ▶ **The skin over the lateral thorax will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

6. **Intra-operative management.**

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

NOTE: If saline is being administered, it must be warmed to body temperature first.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
None	( )*				

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.

▶

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).

▶ **Dogs will be lying on a thick, soft mat to keep them comfortable while they are on the table.**

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.

▶ **Dogs are awake throughout the placement of sensors and catheters.**

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

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

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

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

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

Surgery 1 ► **None necessary.**

Surgery 2 ► **None necessary.**

Surgery 3 ► **None necessary.**

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

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1	**None**				
2	**None**				
3	**None**				

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

► **None of these insertion procedures produce anything more than minor momentary pain, so no analgesics are necessary. Human diabetic patients self-insert these items several times a week with nothing more than an alcohol swipe.**

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

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1	**None**				
2	**None**				
3	**None**				

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

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**None**		
2	**None**		
3	**None**		

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

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**Daily**	**Indefinite**	[redacted]
2	**Daily**	**Indefinite**	
3	**Daily**	**Indefinite**	

f. Post-operative consequences and complications.

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

Surgery 1 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

Surgery 2 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

Surgery 3 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

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

Surgery 1 ► **Refer to the endpoint criteria described in the Main Body.**

Surgery 2 ► **Refer to the endpoint criteria described in the Main Body.**

Surgery 3 ► **Refer to the endpoint criteria described in the Main Body.**

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

► **Any drug can be used for emergency treatment at the discretion of a VMU veterinarian.**

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

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	[redacted]		X	
2			X	
3			X	

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

Last Name of PI►
Protocol No. Assigned by the IACUC►
Official Date of Approval►

ACORP Appendix 5

SURGERY
VERSION 4 V2 6-17-2015

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

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

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	**IV catheter insertion**	**No**	**X**		( )*
2	**Subcutaneous glucose sensor insertion**	**No**	**X**		( )*
3	**Subcutaneous insulin catheter insertion**	**No**	**X**		( )*

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

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

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

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

Note: The following procedures are not considered surgeries, but are described here in order to provide important details regarding the preparation of insertion sites, and the insertion techniques for placement of IV catheters, subcutaneous glucose sensors, and subcutaneous insulin delivery catheters.

Surgery 1 ► **Two catheters will be placed in separate locations of the cephalic and/or saphenous veins (one for blood collections, and the other for glucose/insulin injections). After prepping the insertion site (described below), each catheter will be inserted into the vein and fixed in place with tape. The catheters will contain a three-way valve to allow blood collection from one port, and flushing of the catheter through the other port. After flushing, the valve will be closed. IV catheters will only remain in place during clamp tests, and will be removed at the end of the test.**

Surgery 2 ► **Glucose sensors will be inserted on the lateral portion of the thorax, each spaced approximately two inches apart and the intended locations marked. Lidocaine will be injected**

into the dermis at a 45-degree angle with a 27-gauge needle and syringe until a small bubble of fluid is visible under the skin. This will be repeated for all insertion and suture sites. Lidocaine is not administered when these sensors are used in humans, but has been added to the dog protocol to reduce any pain involved.

Using aseptic technique, glucose sensors will be inserted into the skin according to the manufacturer's instructions. The sensors consist of a small strip of sensor material (approximately the size of a hypodermic needle) that is inserted into the skin using a rigid insertion cannula that is approximately 1 cm in length. Once inserted, each sensor will be sutured in place with non-absorbable coated vicryl using aseptic techniques. This will be repeated for each sensor. The transmitting unit for each sensor will then be connected, the area will be covered with sterile gauze, and taped in place using medical-grade tape. A dog jacket will be used to prevent the sensors from becoming dislodged.

Surgery 3 ► **An insulin catheter will be inserted subcutaneously on the lateral portion of the thorax using exactly the same methods as for the placement of glucose sensors. These catheters will be replaced at least every 14 days. When replaced, they will be moved at least two inches from the previous infusion site.**

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

Name	Surgery #(s) (see Item 1)	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	All	X*			
	All	X*			
	All	X*			
	All	X*			
	All	X*			

*** Since these aren't surgeries, "Surgeon" is probably not the appropriate term, but all staff will be authorized to perform these procedures.**

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

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
[redacted]		**All**	**No**	**( )***	**(X)***

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

► **These procedures are not considered surgeries, so a dedicated surgical facility is not necessary.**

5. **Pre-operative protocol.**

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

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

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

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Lidocaine (at location of sensor insertion/suturing)	**All**	**0.1-0.3 mg/kg of a 2% solution**	**Intradermal**	**Once per insertion site**	**10-15 minutes before procedure**
Acepromazine	**All**	**0.3 mg/kg (0.03 ml of a 10 mg/ml solution)**	**SC**	**Once (only given if needed to reduce anxiety)**	**10-15 minutes before procedure**

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

Surgery 1 ► **The skin over the cephalic and/or saphenous catheter insertion sites will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

Surgery 2 ► **The skin over the lateral thorax will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

Surgery 3 ► **The skin over the lateral thorax will be shaved, then scrubbed with three alternating wipes of chlorhexidine and isopropyl alcohol, each performed in a circular fashion starting at the planned insertion site and working outward. The last wipe will be performed with isopropyl alcohol and allowed to dry.**

6. **Intra-operative management.**

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

NOTE: If saline is being administered, it must be warmed to body temperature first.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
None	( )*				

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.

►

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).

► **Dogs will be lying on a thick, soft mat to keep them comfortable while they are on the table.**

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.

► **Dogs are awake throughout the placement of sensors and catheters.**

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

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

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

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

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

Surgery 1 ▶ **None necessary.**

Surgery 2 ▶ **None necessary.**

Surgery 3 ▶ **None necessary.**

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

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1	**None**				
2	**None**				
3	**None**				

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

▶ **None of these insertion procedures produce anything more than minor momentary pain, so no analgesics are necessary. Human diabetic patients self-insert these items several times a week with nothing more than an alcohol swipe.**

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

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1	**None**				
2	**None**				
3	**None**				

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

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**None**		
2	**None**		
3	**None**		

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

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**Daily**	**Indefinite**	[redacted]
2	**Daily**	**Indefinite**	
3	**Daily**	**Indefinite**	

f. Post-operative consequences and complications.

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

Surgery 1 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

Surgery 2 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

Surgery 3 ► **The only possible complication would involve infection of the insertion sites. If the insertion site shows any signs of infection (e.g., heat, redness, swelling, and pain), a VMU veterinarian will be consulted for treatment.**

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

Surgery 1 ► **Refer to the endpoint criteria described in the Main Body.**

Surgery 2 ► **Refer to the endpoint criteria described in the Main Body.**

Surgery 3 ► **Refer to the endpoint criteria described in the Main Body.**

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

► **Any drug can be used for emergency treatment at the discretion of a VMU veterinarian.**

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

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	[redacted]		X	
2			X	
3			X	

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

Last Name of PI►
Protocol No. Assigned by the IACUC►
Official Date of Approval►

ACORP APPENDIX 6

SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4 V2 6-17-2015

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

Special Procedure		Features							
Number	Brief Description	Special Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Work	Irradiation	Imaging	Other**
1	**Daily care**	X							
2	**Monthly care**	X							
3	**Diabetes monitoring/treatment**	X							
4	**Long periods of recumbency**	X							
5	**Wearing of jackets**	X							

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.

►

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

Special Procedure 1 ► **Daily care: Dogs are not food deprived, but are normally given a fixed amount of food once daily at the same time each morning to ensure predictable blood-glucose excursions throughout the day. All dogs are fed dry kibble mixed with canned dog food. Upon the recommendation of a VMU veterinarian, some dogs may be placed on a special prescription diet and/or fed twice daily to promote weight gain. Dogs will be observed daily for signs of inappetence, distress, or any other abnormal health condition (such as skin lesions). Logs of these daily observations will be kept in the laboratory and made available for inspection. Any abnormal findings will be reported to a VMU veterinarian for treatment.**

Special Procedure 2 ► **Monthly care: Dogs will be thoroughly bathed once per month (shampooed, rinsed, and dried), and jackets replaced. To ensure they are receiving proper nutrition, the amount of body fat/muscle mass will be evaluated when jackets are removed prior**

to bathing using a scale of 1-9 on the Purina Body Condition Scoring System. Dogs falling below a score of 4 (out of 9) will be reported to a VMU veterinarian for examination. Dogs will also be weighed at this time. Dogs experiencing a weight loss of more than 10% as compared to their last annual exam weight will be reported to a VMU veterinarian for examination. Weights and body-condition scores will be documented on treatment sheets kept in the laboratory, and also in a log book kept in the VMU.

Special Procedure 3 ► **Diabetes monitoring/treatment: Approximately one-third of the dogs used in this project have been made diabetic by pancreatectomy or chemical induction by the** [redacted] **under an approved IACUC protocol and by veterinarians trained to perform these procedures prior to arrival. These dogs must receive careful daily care, including frequent blood-glucose measurement and insulin injections/infusion. The food for these dogs will be treated with pancreatic enzymes (Panakare Plus, Viokase, or Pancrezyme) to improve digestion and nutrient absorption in the intestinal tract. Several blood samples will be taken daily from the saphenous or cephalic vein (~0.5 ml/sample). The location of blood sampling site will be changed daily to prevent fibrosis, the area will be cleansed before each sample is taken with 70% isopropyl alcohol, and no more than three blood draws will be taken from the same vein per day. Blood samples are cooled when transported from the kennel to the laboratory, where they will be analyzed to determine blood-glucose levels. Blood-glucose levels will be maintained between 80-180 mg/dl. Diabetic dogs will either be given manual insulin injections or continuous insulin infusion via an external insulin pump (FDA approved and used clinically). If the latter, dogs will wear a jacket in which the external insulin pump will be placed, and a subcutaneous infusion catheter will be used to deliver the insulin. When the infusion catheter is changed, the skin status under the jacket will be monitored and jackets will be replaced as needed. If an insulin pump cannot be used for some reason, insulin treatment will be provided by manual injections. Liraglutide may be injected once per day in all diabetic dogs at a dose of 25-100 µg/kg SC to help stabilize blood glucose levels. When necessary to maintain safe blood-glucose levels, lab staff must force-feed some diabetic dogs by placing food into their mouths and gently holding their mouths closed until they swallow the food.**

Special Procedure 4 ► **Long periods of recumbency: Dogs will be placed on a soft, thick mat on a table during the clamp and closed-loop tests. They have to remain on the table for the duration of each test (2-9 hours), but are allowed to freely change position as needed for their comfort. The dogs are unrestrained and are well-adapted to this type of procedure. Most dogs sleep for the duration of the testing and enjoy being petted and being around their caretakers. The dogs will be under constant supervision.**

Training for recumbency: New dogs will be trained to sit on the table for gradually increasing periods of time prior to enrollment into the study over a period of 1-3 months. Food will be used as a reward. No experiment will be performed during this habituation training.

Special Procedure 5 ► **Wearing of jackets: In order to secure glucose sensors and/or insulin catheters, the thorax is wrapped with gauze and tape, then covered with a thick vinyl jacket. Without this precaution, the dogs will remove these devices. Occasionally, neck collars are used to prevent dogs from chewing on their jackets. Some dogs display mild-to-moderate localized skin irritation when adhesive tape (used to anchor gauze pads overlying glucose sensors) is removed, even when using adhesive solvent. Significant irritation will be treated with diphenhydramine (Benadryl) (125 mg qd po or 1-2% bid topical for three days) to provide comfort and protection from scratching.**

b. Explain why each of these special procedures is necessary:

Special Procedure 1 ▶ **Described above.**

Special Procedure 2 ▶ **Described above.**

Special Procedure 3 ▶ **Described above.**

Special Procedure 4 ▶ **Described above.**

Special Procedure 5 ▶ **Described above.**

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

Procedure Number (see Item 1)	Responsible Individual(s)	
	Carrying Out Procedure	Monitoring the Animals
1	**Any lab staff**	**Any lab staff**
2	**Any lab staff**	**Any lab staff**
3	**Any lab staff**	**Any lab staff**
4	**Any lab staff**	**Any lab staff**
5	**Any lab staff**	**Any lab staff**

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

Procedure Number (see Item 1)	Expected Potential Pain and/or Distress			
	No	Yes		
		Description	To Be Relieved	Not to Be Relieved
1	**X**		( )[a]	( )[b]
2	**X**		( )[a]	( )[b]
3	**X**		( )[a]	( )[b]
4	**X**		( )[a]	( )[b]

5		**Some dogs display mild-to-moderate localized skin irritation when adhesive tape (used to anchor gauze pads overlying glucose sensors) is removed, even when using adhesive solvent. Significant irritation will be treated with diphenhydramine (Benadryl) (125 mg qd po or 1-2% bid topical for three days) to provide comfort and protection from scratching.**	(**X**)[a]	( )[b]

a. For each procedure for which potential pain and/or distress is expected, but WILL be prevented or alleviated by administration of the analgesic(s) or stress-relieving agents, complete the table below:

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1					
2					
3					
4					
5	**Diphenhydramine**	**125 mg or 1-2%**	**po or topical**	**Daily or bid**	**3 days**

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ▶

Special Procedure 2 ▶

Special Procedure 3 ▶

Special Procedure 4 ▶

Special Procedure 5 ▶

b. For each procedure for which potential pain and/or distress is expected and will NOT be prevented or alleviated, provide the scientific justification for this:

Special Procedure 1 ▶

Special Procedure 2 ▶

Special Procedure 3 ▶

Special Procedure 4 ▶

Special Procedure 5 ▶

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be

removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

Procedure Number (see Item 1)	Monitoring Methods	Endpoint Criteria
1	**Described above**	**Described above**
2	**Described above**	**Described above**
3	**Described above**	**Described above**
4	**Described above**	**Described above**
5	**Described above**	**Described above**

5. **Animal restraint.** Complete the table below for each special procedure in which animals are put under restraint for more than 15 minutes.

Prolonged Restraint (defined by the IACUC as over 15 minutes)						
Method of restraint	Species	Approved Duration of Restraint	Method of acclimatization	Monitoring	Criteria for removing animals that do not adapt or acclimate	Provision of veterinary care for animals with adverse clinical consequences
None						

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Greater Los Angeles, CA - 691	[redacted]	Glucose Sensing and Physiologic Insulin Delivery

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 was submitted as a triennial review and describes ongoing research using canines to develop and/or refine subcutaneously implanted devices, which measure glucose levels and administer an appropriate dose of insulin as needed. The investigators notes that "a number of glucose sensors tested in this study have gone on to clinical trials and are now available to diabetic patients." The investigator and his research team are well-qualified through experience and training to perform the purposed study. Commendable aspects of the protocol include the clear justification for the canine model, measures taken to limit the number of dogs used, and the detailed health monitoring plan. Some 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.
Items A, C.2, and I	In Item A, the investigator states " During the last three-year period, we used a total of 31 dogs to obtain these results, most of which were carried over from the previous three-year approval period, and all of the current 24 dogs will continue into the next three-year approval period." The investigator notes in item C.2.a, normal and diabetic dogs will be used in the protocol at approximately a 2:1 ratio. In regard to the justification of the group sizes and total numbers of animals requested, the investigator cites "…two unknown factors make it difficult to determine the number of dogs to be used 1) the number of sensor types to be tested in the three-year approval period, 2) the number of times an individual sensor must be tested to achieve an acceptable insulin-infusion algorithm." Item I lists a total of 40 category C dogs to be used for this three year period. Based on the above information, it

(cont.)

	appears the 16 new dogs will be normal controls; is this correct? If some of the new dogs are diabetic, when and where was the pancreatectomies performed? Although, it is understood that the two unknown factors referenced previously complicate calculating the number of dogs to be used; the basic rationale for how the number of dogs was derived should be explained.
Items C.2, item R, and Appendix 4	In regard to devices used for the clamp tests, the investigator in item C.2 indicates the following: • Hyperglycemic-clamp test (normal dogs only) – glucose sensors will remain in place for 4-7 days. • Closed-loop test (diabetic dogs only) – At the end of the test, the sensors are removed. • Bi-hormonal closed-loop test (diabetics dogs only) – At the end of the test, the IV catheters, sensors, and the glucagon pump and catheter will be removed. Item R notes that the collection of blood samples, glucose sensors and insulin pump catheters is addressed in Appendix 4. Appendix 4 only describes blood collection. Please address the following: • Is the glucagon pump an externally worn device similar to the external insulin pump? • If up to six glucose sensors are subcutaneously implanted in a dog (item C.2.c) for a hyperglycemic-clamp test, are all the six sensors removed at once or is removal time depend on how many times an individual sensor must be tested to achieve an acceptable insulin-infusion algorithm? • Depending on the response to the question shown above, what is the maximum length of time an individual glucose sensor will remain implanted? • How are the glucose sensors removed?
Item T and Appendix 6	Maintaining the dogs at an appropriate weight is important for the animals to continue participating in the study; dogs are weighed monthly. Measures will be taken to improve body weight, if a weight loss of more than 10% of normal body weight occurs. As opposed to the method listed, has the investigator considered offering more frequent meals and/or feeding a higher calorie diet?
Appendices 3 and 6	Appendix 6 notes that diabetic dogs will be treated with pancreatic enzymes, please add to Appendix 3.

(cont.)

Literature search Los Angeles [redacted]

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

The focus of this research is developing an easy to use glucose sensor placed under the skin that will automatically measure blood glucose and communicate wirelessly with a wearable insulin pump. Insulin will then be automatically delivered as needed to the patient with no effort or "finger sticks" on their part.

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*). Easier and better methods for glucose control would be a major benefit to this large group of Veterans.

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	Last 5 years	glucose sensor, insulin pump, closed-loop	77

This is an ongoing product development/testing project funded by a medical equipment company. This is a rapidly evolving area, as evidenced by the many papers published in just the last five years. The company is continually testing new sensors, algorithms, and insulin pumps and is not duplicating published work. The "closed-loop" term refers to the direct connection between the sensor and the pump – no patient intervention needed.

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</u>	3/11/18	All available years	glucose sensor, insulin pump, closed-loop	0

Alternatives as the main topic				
ALTBIB animal alternatives search strategy - all citations	3/11/18	2000-present	glucose sensor, insulin pump, closed-loop	10

An ALTBIB search for “alternatives to using animals” for this study yielded no papers at all.

A second ALTBIB search that included all relevant citations since 2000 brought up 10 papers, of which 8 were computer models, one was a review article, and one studied individual pancreatic beta cells.

Although computer models are attractive, the goal of this project is new products that will be approved by the FDA, and the FDA requires an established pre-clinical animal model.

It should be noted the sensors are first tested in vitro at various glucose concentrations to be sure they work in that simple situation before they are tested in vivo.

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?
PubMed filtered for “other animals”	3/11/18	2000-present	glucose sensor, insulin pump, closed-loop	2

A PubMed search set to filter for “other animals” brought up only two papers. One looked at individual beta cells, and the other was from 40 years ago describing a very early glucose sensor.

The sensor and pump are sized for humans and are too large to test in small animals such as rats, mice, rabbits, or non-mammalian models such as zebrafish. The protocol also requires frequent blood tests to compare with the sensor readings, and their blood volume is not large enough to allow for such frequent blood draws without negative health consequences.

Pigs have been tried as test subjects and found to have a number of disadvantages: 1) their skin is too tough for the sensors; 2) their subcutaneous fat interferes with the sensors; and 3) they literally rub the sensors and pumps off against the wall. Sheep and other ruminants have not been well-characterized as a diabetes model, probably because of the profound differences in their digestive system compared to humans and dogs.

Dogs have been found to be the most suitable model for this work: 1) they are the model most used in diabetes research and come closest to approximating the human diabetic condition; 2) they are highly cooperative and easily handled; 3) they have a large area for subcutaneous testing of glucose sensors; and 4) their blood volume and glucose response characteristics allow for fast and easy manipulation of blood glucose levels.

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?
PubMed	3/11/18	All available years	Diabetes with glucose sensor and insulin pump	diabetes AND dog AND glucose sensor AND insulin pump	5

The dog diabetes model used does not produce excessive distress, pain, or suffering, and should not exceed that experienced by humans with well-controlled diabetes. This group practices very careful blood-glucose control with the dogs, and the dogs do not develop many of the problems seen in human diabetics (diabetes-related kidney failure, blindness, foot gangrene, etc.). Furthermore, thanks to the careful glucose control the dogs are far slower to develop cataracts than pet dogs with diabetes.

For the testing the sensors, algorithms and pumps, the dogs rest unrestrained on soft mats in a quiet room with familiar staff members. The skin is anesthetized with lidocaine before the glucose sensors are inserted (human patients typically insert the sensors with no anesthetic). Based on their extensive experience and their familiarity with the literature this group does not believe their procedures could be made any less painful or distressing for the animals.