Protocol 42593 Amendment 3.1

Approval date	03/18/2024
Expiration date	08/11/2026

1. Basic Information

1. Elements ID

   For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

2. eACUC Number (Automatically Assigned)

   42593

3. Principal Investigator

   Pitts, Teresa Greene

   Job title PROF, ASOC
   Department Speech Lang & Hearing Sci
   Division Health Sciences
   Business unit University of MO-Columbia

4. Protocol Title

   1) Regulation of swallow and the effects of high cervical spinal cord injury and 2) Influence of Opioids on the Brainstem Respiratory Network Central and Peripheral Regulation of Laryngeal Adduction

5. Triennial Re-write

   Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

   ○ Yes ● No

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

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Species	Strain/ Stock/Breed	Age/ Weight	Pain/Distress Category	Authorized	Ordered	Received	Adjustment	Available
Cat	Any breed	adult	USDA Category D	91				91
Total Cats:				91	0	0	0	91
Guinea Pig	any breed	adult	USDA Category D	300				300
Total Guinea Pigs:				300	0	0	0	300

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

○ Yes ● No

3. Proposal Overview

1. Purpose

Purpose of the study:

The long-term goal of this project is to determine the role of tracheal, pharyngeal, and spinal sensory feedback in the regulation of cough and swallow.

Our central hypothesis is that an aspiration event produces a series of coughs and swallows which are expressed in various behavioral interaction patterns, and that there are decipherable rules that regulate the various patterns of expression. Cough and swallow are airway protective behaviors. The pharyngeal phase of swallow prevents aspiration of oral material (saliva, food and liquid), by epiglottal movement, laryngeal adduction, and clearing the mouth and pharynx. Cough is an aspiration-response behavior that clears material from the airway. Coordination of these behaviors is vital to protect the airway from further aspiration-promoting events, such as a swallow occurring during the inspiratory phase of cough. The peripheral inputs, operational characteristics, and primary strategies that coordinate cough and swallow are unknown. This lack of information impedes understanding of the deficits in airway protection, with co-occurrence of dystussia and dysphagia, which occurs with diseases such as Parkinson's disease and Alzheimer's disease.

The Specific Aims of the project are: 1) Identify the operational principles that govern the coordination of cough and swallow motor patterns following activation of tracheal and pharyngeal afferents during an

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aspiration event; 2) Develop a predictive computational distributed network model of the central influence of tracheal sensory pathways on the expression of cough and swallow; 3) Identify the role of neurons in the reticular formation in processing tracheal and pharyngeal afferent feedback on cough and swallow; 5) Identify the effects of cervical spinal cord disruption on the swallow motor pattern; and 6) Determine the therapeutic efficacy of 5-HT1A agonists on recovery of swallow function after spinal cord injury.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

A variety of neuromuscular/ neurotraumatic diseases result in impaired airway protection including breathing, swallow and/or swallow function.

Impairment of these airway protective behaviors results in an increase in pulmonary infections due to aspiration. Pulmonary complications related to inadequate airway defense are the leading cause of death in patients with spinal cord injuries and Parkinson's disease. The long-term goal of this project is to determine brainstem and spinal mechanisms that control the regulation of airway protection. Our central hypothesis is the brainstem is reliant on feedback from the spinal cord to regulate airway protective behaviors. Loss of this information creates mis-timed swallows, laryngeal dysregulation and increases aspiration risk. The cat and guinea pig models are essential to the study. These animals exhibit the same cough and swallow reflex as humans, even when they are anesthetized.

Currently there are no effective treatments for swallow disorders beyond the limited effects of exercise. This work provides the foundation to translate experimental models into clinical effects.

3. Lay Term Description of Experimental Design

To put something in layman’s terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This section should be written so that someone with a **10th grade science education can easily understand the project.**

There are several disorders that can hurt a person's ability to swallow properly and to coordinate swallow with breathing. The airway is normally protected by several reflexes, which include swallow and airway closure. When the airway is not properly protected, food and liquids can invade, and can enter the lungs to cause breathing distress and dangerous infections (pneumonia). Such complications are the leading cause of death in patients with spinal cord injuries and Parkinson's disease. The long-term goal of this project is to determine the processes in the lower brain and spinal cord that control the regulation of airway protection. Our main hypothesis is that the lower brain relies on input from the spinal cord to regulate airway protection. Loss of this information creates mis-timed swallows, disorder of muscles at the opening of the airway, and increases risk of food and liquids entering the lungs. It is necessary for us to use cats or guinea pigs in these experiments because, unlike rodents, they have the all of the same cough and swallow reflexes as humans, even when they are anesthetized. Currently there are no effective treatments for swallow disorders beyond the limited effects of exercise. This work provides the foundation to translate animal work into strategies that can help humans.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

The substances used for the general procedure are as follows: Sevoflurane or Isoflurane will initially be administered through box-induction then transitioned into mask inhalation after loss of consciousness. The

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animals will then be weaned onto sodium pentobarbital through a forelimb radial vein intravenous injection (no catheter). Later, as part of the experimental protocol, the femoral vein will be catheterized, and anesthetic doses and (supplemental doses as needed) will be given through the femoral IV catheter. Atropine sulfate will be given as needed to maintain mucous secretion. Decrease in total blood pressure or a reduced pulse pressure (from outside the normal physiological range) will prompt the use of lactated ringers solution into the femoral IV line. Arterial blood gas showing a metabolic acidosis (secondary to surgery, etc) will be treated with administration of sodium bicarbonate through the femoral IV line. Heparin pork will be placed in saline to clean the femoral IA line after blood collection for blood gas measurement. Lidocaine will be used before placement of the ear bars only one time during the experiment. Doxapram HCL, Baclofen, Kynurenic Acid, Isoguvacine, Lidocaine, Cobalt Chloride, Substance P, Glutamate, Allostatin, Saporin, capsaicin, citric acid, acetic acid, and Codeine will be used as needed to suppress cough and/or swallow depending on the experimental protocol.

1) Modulation of airway reflexes -- terminal electrophysiology experiments: Adult cats or guinea pigs may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats or guinea pigs will be anesthetized to a surgical level of anesthesia. Electromyographic recordings of swallow and breathing muscle activity will be made during stimulation of airway reflexes including cough and swallow. Crainiotomy and/or laminectomy will be performed. Stimulations will be performed again. Microinjection and/or infusion of experimental compounds may be administered, then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e.g., 1 hour, 2 hours, 3 hours and 4 hours). In some animals we may elect to stimulate the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. In some animals an acute spinal cord injury will be performed and then the stimulations will be repeated (as described above). At the end of the experiment, anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo perfusion. In some animals, cats or guinea pigs will be given buprenorphine prior to the terminal electrophysiology procedure. Condition A: 40 cats and 110 guinea pigs will undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition B: 30 cats and 70 guinea pigs will undergo an acute spinal cord injury and undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition A (40) + Condition B (30) = 70 cats - 25 cats already completed = a total of 45 cats requested. Condition A (110) + Condition B (70) = 180 guinea pigs requested (none yet completed).

2) Neuron Recording -- terminal electrophysiology experiments: Adult cats or guinea pigs may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats or guinea pigs will be anesthetized to a surgical level of anesthesia. Electromyographic recordings of swallow and breathing muscle activity will be made during stimulation of airway reflexes including cough and swallow. Crainiotomy and/or laminectomy will be performed. Recording electrode/s will then be placed in targeted areas of the brainstem and/or spinal cord to extracellularly record from neurons of interest. Then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e.g., 1 hour, 2 hours, 3 hours and 4 hours). In some animals we may elect to stimulate the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. In some animals an acute spinal cord injury will be performed and then the stimulations will be repeated (as described above). At the end of the experiment, anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo

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perfusion. This protocol will use 50 cats - 4 animals already completed = a total of 46 cats requested, and 120 guinea pigs requested (none yet completed).

4. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

In vivo, there are multiple neural circuits and large variety of cells, muscles, and nerves which contribute to the control of airway protective behaviors. The cat and guinea pig are models used for assessing coordination of breathing and airway behaviors, allowing us to make relevant comparisons within the field. Similarly, the airway protective muscles exhibit a number of functional and anatomical similarities to humans. We will work to reduce the number of animals by assessing multiple behaviors and completing multiple protocols on each animal used. No suitable alternatives have been identified to replace the use of live animals for the studies associated with this protocol. The lack of suitable alternatives was determined by a continual review of the literature on a weekly bases.

2. Justify Animal Species

Justify the choice of species for your study.

Adult cats and guinea pigs are used for three reasons: (a) While swallow has been assessed using videofluoroscopy in various rodent models of disease, aspiration has never been seen. Russell, et al. (Dysphagia, 28; 95-104; 2013) thoroughly discussed how the anatomical specifics of the rodent do not make aspiration or its use as an outcome measure possible. (b) The cat has been used extensively as a model system for studying vertebrate motor control, including cough, which is a critical respiratory-related defense with similarities to swallow. The extensive literature on feline motor control during breathing and cough, including our own studies, supports the importance of this model for translation. Relative to other species, a large amount of information exists on the physiology and pharmacology of airway protection and vagal afferents in the cat and guinea pig. Further, because the rat lacks airway protective behaviors such as cough and/or vomiting, use of cats and guinea pigs affords greater translational potential for the current study. (c) The complex instrumentation during some of the terminal electrophysiology procedures requires that larger animals be used.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total numbers should match the requested numbers in the species section.

- Animal Numbers Justification
- The Logical Determination of “N” in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

This protocol is for 91 cats and 300 guinea pigs altogether.

Modulation of airway reflexes - terminal electrophysiology:
Three conditions will be needed to complete this specific aim. Condition A: 40 cats and 110 guinea pigs will undergo airway reflex testing and modulation with administration of compounds via infusion or

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microinjection. Condition B: 30 cats and 70 guinea pigs will undergo an acute spinal cord injury and undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition A (40) + Condition B (30) = 70 cats. Condition A (110) + Condition B (70) = 180 guinea pigs.

We have completed 15 cats from Condition A and 10 cats from condition B. These have been removed removed from the cat sample size calculation. For a total of 45 cats. These sample size calculations are based on % change in our previous studies in EMG, with an alpha level of p< 0.05 and a power of 0.9.

Neural Recording - terminal electrophysiology:
The historical yield for these types of experiments is that 5% of total neuron pairs produce features that suggest functional interactions between the neurons. Given the complex nature of this network, as well as the fact that we will study a population of neurons about which there is little published information, we anticipate approximately 1700 pairs with evidence of functional interactions. Therefore, we estimate that the minimum number of neuron phenotypes that we will encounter in these experiments is 19, representing (19)(18)/2=171 possible combinations. Allowing for at least ten examples of each yields a total of 3,400 pairs with significant functional interactions. This number represents 5% of the total pairs required, which is 3,400/0.05=68,000 pairs. We anticipate a yield of approximately 350 pairs per cat or 68,000 pairs/350 pairs per cat=194 total cats for the project. Yields will be lower for guinea pigs due to the smaller area available for placing electrode arrays. Fifty cats and 120 guinea pigs will be used to complete this protocol. Should our yields per animal be higher than anticipated, fewer than 50 cats and 120 guinea pigs will be used. We have completed 4 cats from this experiment, for a total of 46 cats and 120 guinea pigs needed.

5. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

	Facility
1	[redacted]
2	[redacted]
3	[redacted]

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

○ Yes ● No

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

● Yes ○ No

A. Description of Transportation

Describe how animals will be moved, including caging/transport carriers used (covered microisolator cages, dog/cat carrier, etc.), type of transport used (i.e. personal car or van), and projected transport time. (Note: Animals should be transported in a temperature controlled environment.)

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Animals will be transported in commercial cat carrier cages using personal cars in which temperature is controlled. Projected transport time is less than 10 minutes.

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

○ Yes
○ No
● Overnight only

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

☐ Single housing of social species
☐ Wire-bottom cages
☐ Special diet/water
☐ Extended time to weaning
☐ Extended time between cage changes
☐ Alternative light cycles
☐ Out of range temperatures
☐ Cage-size exceptions
☐ Other

6. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

● Yes ○ No

A. Sample Type

Type of sample(s):

Blood

B. Sample Volume

Volume of sample(s):

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1 mL per sample for cat; 0.15 mL per sample for guinea pig: arterial blood gas sampling during terminal electrophysiology experiments

C. Sampling Frequency and Duration

Frequency of collection and for how long:

Every 60-90 minutes for the duration of the experiment (1-18 hrs)

D. Sampling Method

Method of collection:

Femoral artery catheter

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

	Procedure	Description of procedure	Building name	Room number or area
1	Laryngeal Imaging	In some animals we will choose to image their vocal folds (larynx). Animals will be anesthetized and a laryngoscope will be placed to lower the epiglottis to view the vocal folds. Then a flexible endoscope with video capabilities will be run along the laryngoscope. Video will be taken on the larynx during breathing, and some animals will alter respiratory state (as described above). Video will be recorded through out the procedure. In some animals we may give buprenorphine IM ~ 1hour before beginning the evaluation. Animals will be returned to the Veterinary Medicine facility after the procedure.	[redacted]	Room not yet assigned - will be done in a lab

7. Substances Used in Animals

1. Substances Used in Animals

List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

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	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
1	Doxapram HCL	0.001-30mg/ Kg	IV, IA (femoral or vertebral artery), Microinjection	1-10x per hour; 1-3 hours	No	No
2	Baclofen	0.03-1,000ug/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
3	Codeine	0.001-30mg/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
4	Cobalt Chloride	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
5	Isoguvacine	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
6	Capsaicin	0.016-50 µM	IV, IA (femoral or vertebral), Microinjection, aerosolized in ethanol and saline and placed in the upper and or lower airways	1-10x per hour; 1-3 hours	No	Yes
7	Citric Acid	0.125-32%	IV, IA (femoral or vertebral), Microinjection, aerosolized in ethanol and saline and placed in the upper and or lower airways	1-10x per hour; 1-3 hours	No	Yes
8	Substance P	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
9	Glutamate	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
10	Allostatin	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
11	Saporin	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No

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	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
12	Kynurenic Acid	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
13	8-OH-DPAT	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour, or via infusion to maintain a steady concentration; 1-18 hours (duration of experiment)	No	No
14	WAY-100635	0.03-3000ug/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour, or via infusion to maintain a steady concentration; 1-18 hours (duration of experiment)	No	No
15	Buspirone	1-10mg	PO	Up to 3 doses per day (i.e. 1hr before feeding or assessment); or 1 dose prior to terminal experiment	No	Yes
16	Artificial Cerebrospinal Fluid	0.01-1mL	CNS microinjection or intravertebral artery	1-10x per hour; 1-3 hours	No	Yes
17	Saturated KCl	3 cc	IV	1 dose; 1x per procedure	No	Yes
18	Acetic Acid (vinegar)	1-5%	Topical on mucosa in the upper and/ or lower airway	1-10x per hour; 1-3 hours	No	Yes
19	Thrombin (bovine)	10-1000 units/ mL	Applied to surface of skin, muscle or bone	To control bleeding when cauterization is not an option; applied until bleeding is controlled	No	No
20	Buprenorphine	0.01-0.05 mg/ kg	IM	For an opioid study: buprenorphine IM at a dose of 0.02 mg/kg or 0.03- 0.04 mg/kg ~q12 hours for ~48 hours. May be repeated after 1 week with the lower/ higher dose.	No	Yes

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	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
21	Carbon dioxide	up to 10%	Inhalation	once initially, repeated as indicated by hypercapnic challenge protocol	No	No
22	Sodium Nitrite	0.1-1% solution / 1 cc of 1% solution	IV	Used only once during terminal perfusion	No	No
23	Paraformaldehyde	4% solution / 3-4 L	Intracardial perfusion	Used only once during terminal perfusion	Yes	No

2. Non-Pharmaceutical Grade Substances

For those substances that are marked “no” as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

Only agents with certification of purity will be used, artificial cerebrospinal fluid or saline will be used to ensure physiologic compatibility, and formulations will be filtered and pH adjusted to 7.4.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non- survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

We have been unable to access pharmaceutical-grade thrombin, but thrombin is rarely used in these terminal experiments.

Sodium Nitrite and paraformaldehyde are used in a deeply sedated, non-responsive animal within 1-3 minutes of death. Thus, we use chemical grade solutions that we mix in the laboratory. We use an overdose of Sodium Nitrite to vasodilate the vasculature immediately prior to introduction of the transcardial perfusion solution of 4% Paraformaldehyde in a buffer system (pH 7.4). A sterile option for Paraformaldehyde is not available.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., “All substances” or “non-hazardous substances”) if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

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	Substance	Gloves	Eye Protection	Lab Coat	Face Mask	Fume hood	Biosafety cabinet	Double-Gloves	Other	Other PPE
1	All substances	☑	☐	☑	☐	☐	☐	☐	☐	
2	Paraformaldehyde	☑	☑	☑	☑	☑	☐	☐	☐	

Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

8. Hazardous Materials

1. Will you use any Biological Hazards?

○ Yes ● No

2. Will you use any Chemical Hazards?

● Yes ○ No

A. Chemical Hazards

List all chemical hazards that will be used in live animal work.

	Agent or type of hazard	Receiving species	Dose	Route/Volume of Admin.	Frequency of Admin.	Other
1	Paraformaldehyde	cat	4% solution / 3-4 L	Intracardial perfusion	Used only once during terminal perfusion	
2	Paraformaldehyde	guinea pig	4% solution / 1-3 L	Intracardial perfusion	Used only once during terminal perfusion	

B. Is this an FDA Approved Drug?

○ Yes ● No

C. Chemical Hazard - Anticipated Effect(s)

List any anticipated effect(s) of hazardous chemical on animal.

This is used near the time of death during terminal perfusion.

D. Chemical Hazard Housing/Procedure Sites

	Agent	Receiving Species	Building	Room or area	Housing	Procedure
1	Paraformaldehyde	cat	Dalton (DCRC)	310	☐	☑
2	Paraformaldehyde	guinea pig	Dalton (DCRC)	310	☐	☑

E. Chemical Hazard - Animal Identification

Explain how animals treated with a chemical hazard will be identified (ex. cage card, ear tag, etc.)

☐ Cage Card

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- ☐ Chip
- ☐ Door Sign
- ☐ Other

F. Chemical Hazard - By-Products/Presence

- ☐ None
- ☐ Feces/Urine/Bedding
- ☐ Saliva
- ☐ Blood
- ☐ Aerosols
- ☐ Animal Bite/Scratch
- ☐ Animal Carcasses/Tissues
- ☐ Surgical Site Wound or Sore
- ☐ Other

G. Chemical Hazard - Personal Protection Equipment (PPE)

PPE to be worn when handling chemical hazards include: (ex. safety glasses, surgical mask, etc.)

	Chemical Agent	Gloves	Eye Protection	Lab Coat	Double-Gloves	Face Mask	Fume hood	Other	Other PPE
1	Paraformaldehyde	☑	☑	☑	☐	☑	☑	☐	

H. Additional Information

List additional information, i.e., special precautions for pregnant women, immunocompromised individuals, special handling, or storage, etc.

3. Will you use any Radiation Hazards?

○ Yes ● No

9. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

● Yes ○ No

2. Preanesthetic Agent(s)

List preanesthetic agents here

No Preanesthetic Agents...

3. Anesthetic Agent(s)

List anesthetic agents here. **Do not list isoflurane here, it will be listed later in the form.**

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	Species	Agent	Controlled Substance	Dose/ Volume	Route	Frequency of Admin.
1	Cat	Sevoflurane	No	0.1-5%	Inhalation	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment)
2	Cat	Sodium pentobarbital (Nembutal or pharmaceutical grade generic)	Yes	35 mg/ kg	IV	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment). Initial dose is 35 mg/kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose will always be given
3	Cat	Lidocaine HCl 2%	No	2-4 mg/ kg	Topical, subcutaneous	Subcutaneous: once injected around each ear for nerve block; Topical: anesthesia of the larynx or trachea; 30 minute duration of effect.
4	Guinea Pig	Sevoflurane	No	0.1-5%	Inhalation	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment
5	Guinea Pig	Sodium pentobarbital (Nembutal or pharmaceutical grade generic)	Yes	20 mg/ kg	IV	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment). Initial dose is 20 mg/kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose will always be given
6	Guinea Pig	Lidocaine HCl 2%	No	2-4 mg/ kg	Topical, subcutaneous	Subcutaneous: once injected around each ear for nerve block; Topical: anesthesia of the larynx or trachea; 30 minute duration of effect.

4. Isoflurane Use

A. Will you use isoflurane?

● Yes ○ No

B. What species?

cat, guinea pig

C. How will it be administered?

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- [x] Vaporizer with nose cone
- [ ] Vaporizer with endotracheal tube
- [x] Induction box connected to vaporizer
- [ ] Open drop method (bell jar, etc.)
- [ ] Other

5. Non-Pharmaceutical Grade Anesthesia

If non-pharmaceutical grade anesthesia must be used, strong scientific justification must be provided. In addition, describe how the solution will be made and stored. Also, describe how pH, osmolarity, and sterility will be kept in acceptable physiological ranges. Please see ACUC policies for Anesthesia and Non-pharmaceutical Anesthesia and ACUC Guidelines for pentobarbital, avertin, inactin, and urethane/chloralose.

6. Monitoring and Life Support

Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:

Body temperature measurement and support using temperature-measuring probe.
Use of intra-procedural fluids.
Anesthetic depth checked at intervals no less than 15 minutes.
Anesthetic depth verified by withdrawal reflex (toe/tail pinch, eye blink, and/or jaw tone).
Monitoring heart and respiratory rates.

7. Post Anesthetic Recovery

Complete description of post-anesthetic recovery monitoring and care:

Animals will be anesthetized with isoflurane for laryngeal imaging tests. After the testing and withdrawal of anesthesia, animals will be monitored until they are fully alert and ambulatory, prior to returning to housing.

8. Pharmaceutical Analgesia

○ Yes ● No

9. Non-pharmacologic control of pain

○ Yes ● No

10. Paralytic Agents

○ Yes ● No

11. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

● Yes ○ No

12. Antibiotics and Other Agents

List other agents such as antibiotics and other emergency drugs

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	Species	Agent	Dose/ Volume	Route	Frequency of Admin.
1	Cat	Lactated Ringers Solution +/- 5% sucrose	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
2	Cat	Normal saline	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; also used to flush IV and IA lines; 1-18 hrs (duration of experiment)
3	Cat	Atropine Sulfate	0.01-10 mg/ kg	IV or IM	Before tracheostomy and as needed based on blood pressure measurement; 1-18 hours (duration of experiment)
4	Cat	Sodium Bicarbonate	0.2 x kg x base deficit	IV	As need based on base deficit from blood gas measurements; 1-18 hours (duration of experiment)
5	Cat	Heparin Pork	1MU/mL	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
6	Guinea Pig	Lactated Ringers Solution +/- 5% sucrose	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
7	Guinea Pig	Normal saline	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; also used to flush IV and IA lines; 1-18 hrs (duration of experiment)
8	Guinea Pig	Atropine Sulfate	0.01-10 mg/ kg	SC, IV, or IM	Before tracheostomy and as needed based on blood pressure measurement; 1-18 hours (duration of experiment)
9	Guinea Pig	Sodium Bicarbonate	0.2 x kg x base deficit	IV	As need based on base deficit from blood gas measurements; 1-18 hours (duration of experiment)
10	Guinea Pig	Heparin Pork	1MU/mL	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment

10. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

● Yes ○ No

2. Surgery: Pre-surgical Prep

Describe pre-surgical preparation of the animals. Include information about fluid/food restriction, skin, and instrument prep.

Food will be withheld the morning of the surgery (4-6 hrs). The requirements for terminal surgery will be followed, including shaving the surgical site and using clean instruments.

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3. Surgical Procedures

List surgical procedures (include incision location and size, tissue(s) manipulated, and closure methods and materials).

Uncovered by a White Coat Waste investigation

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
1	Alteration in Respiratory State	While under anesthesia, in some animals we will choose to observe how changes in the breathing pattern will effect the EMGs or neurons of interest. We will hyperventilate, (hyperventilation is the method in which we create a hypocapnic condition in the animal) the animal by putting them on a ventilator and increasing the respiratory rate (we will then run an arterial blood gas to insure the hypercapnia has been reached then conduct the cough protocol. We will also be subjecting these animals to a hypercapnic or hypoxic condition to observe this modified physiological state . This is done by supplementing the spontaneously breathing animal with up to 10% CO2.	Terminal	[redacted]	[redacted]
2	Blood collection	Every 60-90 minutes 1cc of blood will be collected from the femoral artery cannula and run for blood-gas measurement. The line will be flushed with heparinized saline. A total of 8- 22cc's of blood. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
3	Cough stimulation	Cough will be stimulated by placement of a 4-5 inch piece of small tubing caudal into the trachea to the level of the carina or rostral into the larynx and slowly rotating. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
4	Cough-swallow stimulation	The described cough-swallow stimulation will be overlapped to produce cough and swallow in sequence. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
5	Craniotomy	An occipital craniotomy will be performed and the caudal portion of the cerebellum will be removed to allow access to the brainstem. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
6	Electrical Simulation of the superior laryngeal nerve	In some animals we may elect to stimulate the superior laryngeal nerve (SLN) or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. A bilateral electrode will be placed around one or both of the SLN or on the skin without cutting/ damaging the nerve. Stimulation will be at <4V and at a range from 5-500 Hz. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
7	Electromyography placement	Parasternal intercostal, diaphragm (costal and crural), laryngeal, abdominal, and pharyngeal muscles will be exposed and electrodes placed for EMG recordings of muscle activity during cough and swallow. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]

Uncovered by a White Coat Waste investigation

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
8	Microinjection into the brainstem and/or spinal cord	In some animals we will microinject compounds that are known antitussives or alter areas known to participate in the control of cough and swallow into the areas of interest in the brainstem or spinal cord. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
9	Multi-electrode array	Up to three multi-electrode arrays will be placed in the brainstem or spinal cord. Electrodes will be advanced until the number of simultaneously recorded neurons is maximized for each array. Once the number of well-isolated neurons is maximized, recordings will be obtained during breathing for approximately 30 minutes. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
10	Placement of catheters	An incision will be made in the medial aspect of the right hindlimb and catheters (made of polyethylene tubing) will be placed in the artery and vein for additional venous access for fluid maintenance, supplemental anesthesia and arterial pressure observation. An incision will be made through the left armpit, the axillary artery is exposed and cannulated to allow for infusion of compounds into the vertebral artery. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
11	Placement of ear bars	In only non-survival experiments, while the animal is under general anesthesia, a local anesthetic will be infiltrated into the skin around the ear canal for analgesia. Ear bars will be placed bilaterally and the head affixed to the stereotaxic apparatus.	Terminal	[redacted]	[redacted]
12	Swallow stimulation	A 3 cc syringe will be attached to a 1 inch piece of PE-90 polyethylene tubing. 3 cc's of a water bolus will be injected into the mouth. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
13	Acute Spinal Cord Injury	The spinal column will be exposed by making an incision through the skin and then the muscle overlying the vertebra. A bilateral laminectomy will be performed to expose the underlying spinal level. The dura will be slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. The spinal cord will either be: completely severed, over severed, or under severed, using iridectomy scissors or a midline incision will be made. More than one cut may be made. Any fibers left adhering to the ventral or lateral dura may be gently lifted with suction and cut with iridectomy scissors. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]

Uncovered by a White Coat Waste investigation

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
14	Distension	In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended by a balloon to test the effects of mechanoreceptors activity on cough and swallow. This may be combined with the above described cough and swallow protocols. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
15	Swallow/cough suppression	In some animals we may bathe the trachea, esophagus, larynx, and/or pharynx in capsaicin, citric acid, and/or acetic acid. This may be combined with the above described cough and swallow protocols. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
16	Laminectomy	Removal of bone covering spinal segment of interest. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
17	Perfusion	Trans or intra-cardial perfusion may be performed. Initially, animals will be anesthetized with isoflurane or sevoflurane. Once anesthetized, Sodium Pentobarbital (IP, IV or IA) will be given. As needed, animals will be given one or more supplemental doses of sodium pentobarbital (typically 25% of initial dose) to achieve or maintain a deep plane of anesthesia (IA, IP or IV). They will then be given 1 cc of a blood thinner (e.g. 10% heparin, IV) followed ~20 minutes later by a vasodilator (e.g. ~1 cc of 1% sodium nitrite). Immediately following administration of sodium nitrite, they may be perfused transcardially with 0.9% saline typically followed by a fixative (eg. 4% paraformaldehyde and/or 1.25%-4% glutaraldehyde in a buffer solution (pH 7.4)). It is critical that the heart continue beating until the perfusion of fluids has begun in order to prevent collection of blood in the vascular system which interferes with post-mortem histological procedures. This is why Nembutal is the drug of choice and other euthanasia options are not good options as they rapidly stop the heart. By providing a bolus of Nembutal IP after the animal is initially anesthetized, a deep plane of anesthesia is easily maintained and the heart continues beating for a long time which allows completion of the transcardial perfusion steps required. The tissues of interest (including spinal cord, brain, leg bones, muscles, etc) will be removed after perfusion. If the heart is not beating strongly enough, an option may be exercised to introduce the blood thinner and/or vasodilator directly into the left ventricle immediately prior to starting the intracardial perfusion of saline.	Terminal	[redacted]	[redacted]

4. Surgery: Post-operative Care

Describe post-operative care (include **both short and long-term care**; monitoring, surgical wound care including suture removal, and list drugs and doses anticipated to be used).

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N/A

5. Surgery: Special Needs

Special needs of the animals following surgery:

N/A

6. Surgery: Length of Time Alive

Length of time animals will be kept alive following surgery:

N/A

11. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

Non-survival experiments: Surgical procedures include placement of catheters, tracheal cannulation, EMG placement, craniotomy, and alterations of respiratory state. Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps will be taken to re-established a surgical plane of anesthesia and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end- tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone. This will be monitored at minimum every 15 minutes. Should the adverse event occur, the response will be to halt the procedure and deliver additional anesthetic or euthanize the animal.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

Anesthesia will be maintained during all procedures to eliminate pain/distress. Lidocaine will be used to minimize the sympathetic response during placement of the ear bars.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

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○ Yes ● No

12. Disposition of Animals

1. Animal Disposition

Check all that apply

- ☐ Adoption (See MU adoption policy)
- ☐ Market
- ☑ Euthanasia
- ☑ Transfer to different project, PI, or another institution
- ☐ Returns to breeding colony, herd, source, owner, or wildlife site
- ☐ Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, "Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue."

A. Method of Euthanasia

Select the method of euthanasia

- ☐ Inhalant agent
- ☐ Physical Method without Anesthesia
- ☑ Physical Method with Anesthesia
- ☑ Noninhalent Pharmaceutical Agent

B. Euthanasia Descriptions

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	Species	Agent/Method	Dose/Volume	Route
1	Cat	Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
2	Cat	General anesthesia as described for experiments, followed by an adjunctive physical method such as bilateral thoracotomy, exsanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.	Doses according to general anesthesia with sodium pentobarbital, sevoflurane, isoflurane, Beuthanasia aka Euthanasia III Solution	IV or inhaled
3	Cat	General anesthesia as described for experiments, followed by intravenous or intracardial injection of potassium chloride (KCl, at least 75-150 mg/kg).	3 cc of saturated KCl following general anesthesia	IV
4	Cat	General anesthesia as described for experiments, followed by Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
5	Guinea Pig	Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.	Initial dose is 20 mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
6	Guinea Pig	General anesthesia as described for experiments, followed by an adjunctive physical method such as bilateral thoracotomy, exsanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.	Doses according to general anesthesia with sodium pentobarbital, sevoflurane, isoflurane, Beuthanasia aka Euthanasia III Solution	IV or inhaled

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	Species	Agent/Method	Dose/Volume	Route
7	Guinea Pig	General anesthesia as described for experiments, followed by intravenous or intracardial injection of potassium chloride (KCl, at least 75-150 mg/kg).	1-2 mEq/kg (equivalent to 75-150 mg KCl/ kg) following general anesthesia	IV
8	Guinea Pig	General anesthesia as described for experiments, followed by Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

D. Scientific Justification for Use

- [x] AVMA Approved Method
- [ ] Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

- [x] Bilateral pneumothorax
- [ ] Cervical dislocation
- [ ] Decapitation
- [x] Exsanguination
- [x] Removal of vital organs

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☐ Other

13. Project Information

1.

Associate	Role	Responsibilities	OHSP Training	Animal Care & Use	Survival Surgery	P&D Training
Pitts, Teresa Greene [redacted]	Principal Investigator Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ Mar 3, 2023	☑ Mar 3, 2023	☑ Mar 3, 2023	⊖
[redacted]	Co-Investigator Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ May 1, 2023	☑ May 1, 2023	⊖	⊖
[redacted]	Key Personnel Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ Feb 6, 2024	☑ Feb 5, 2024	☑ Feb 6, 2024	⊖
[redacted]	Key Personnel Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ May 30, 2023	☑ May 26, 2023	☑ May 26, 2023	⊖
[redacted]	Key Personnel Access to view cages	Surgery Euthanasia P&D assessment	☑ Mar 6, 2024	☑ Mar 6, 2024	☑ Mar 6, 2024	⊖

2. Training and Qualifications

Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	Pitts, Teresa Greene	> 15 years experience with cats	Dr. Pitts will perform all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone	Dr. Pitts has over fifteen years of experience using the cat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats in acute and chronic experiments. She has continued to work extensively with anesthetized cats and rats. She has successfully completed the all online module trainings.	Full-time employee

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
2	[redacted]	Board-certified ABVP canine feline practice (since 2003) with > 30 years experience with cats and other animals	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/ or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone	[redacted] is veterinarian that is AVBP certified in canine feline practice. He has over thirty years of experience with animals including cats, and has used the cat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. He is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats and rats in acute and chronic experiments. He has continued to work extensively with anesthetized cats and rats. He has successfully completed the all online module trainings.	Part-time employee

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
3	[redacted]	> 10 years experience with cats	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/ or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone.	[redacted] has over ten years of experience using the rat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats and rats in acute and chronic experiments. She has continued to work extensively with anesthetized cats and rats. She has successfully completed the all online module trainings.	Full-time employee

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
4	[redacted]	>15 years with mammals	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotactic System, Spinal Cord Injury (SCI), Euthanasia via Transcardial Perfusion, Tissue Collection, Lung Volume Determination, Agonist/Antagonist IV Injection Protocol, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Brainstem and /or Spinal Cord Microinjection, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal/ Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Electrical Stimulation, and Oral Administration of Buspirone	[redacted] has over fifteen years of experience using rodents as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia in acute and chronic experiments. She has continued to work extensively with anesthetized rodents, and will also perform the same procedures with cats. She previously served on an institutional biosafety committee. She has successfully completed the all online module trainings.	Full-time employee

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
5	[redacted]	Still in training	[redacted] will initially assist with procedures and will be supervised until she can perform them independently. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotactic System, Spinal Cord Injury (SCI), Euthanasia via Transcardial Perfusion, Tissue Collection, Lung Volume Determination, Agonist/Antagonist IV Injection Protocol, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Brainstem and /or Spinal Cord Microinjection, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal/ Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Electrical Stimulation, and Oral Administration of Buspirone	[redacted] will initially assist with procedures and will be supervised until she can perform them independently. She has successfully completed the all online module trainings.	Undergraduate student

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- [x] PHS (NIH, CDC, FDA, NSF, NASA)
- [ ] DoD
- [ ] VA
- [ ] AHA
- [ ] USDA
- [ ] Foundation/Industry
- [ ] Internal

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☐ Other

14. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

● Yes ○ No

2. USDA Covered Species

Does this protocol utilize animals covered by the Animal Welfare Act or assigned to Category E? (AWA covered species include all warm blooded animals except birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research, horses not used for research purposes, and other farm animals.)

● Yes, includes USDA covered species or Category E ○ No

3. Includes USDA covered species or Category E

Search for Animal Alternatives

In the literature search and in the written narrative, replacement by non-animal systems, reduction in numbers of animals and refinement of experimental methods (the three R's) must be addressed.

Provide at least two sources of information: one of these sources must be a scientific literature database; documented expert consultation may be used as one source of information.

If you are in the School of Medicine and need assistance with this item, please contact Rachel Alexander, HSL Research Support Librarian, at AlexanderRL@health.missouri.edu. Others can contact the Zalk Veterinary Medical Library, at MU CVM VetMed Library for help.

See also:

https://www.nal.usda.gov/awic/sample-searches
https://library.missouri.edu
Literature Search Help

A. Source 1: Literature Database

Complete the information below:

Uncovered by a White Coat Waste investigation

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	05/17/2023	MEDLINE	1966-present	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, buspirone, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare
2	2/20/2024	MEDLINE	1966-present	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, guinea pig, cavies, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, guinea pig, cavies, laminectomy, hemilaminectomy / laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, buspirone, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare

B. Source 2: Literature Database

For the second source you may use a literature database search or an expert consultation (see following question).

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	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	05/17/2023	PubMed	All dates	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare)
2	2/20/2024	PubMed	all years	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, guinea pig, cavies, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, guinea pig, cavies, laminectomy, hemilaminectomy / laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare)

C. Source 2: Expert Consultation (alternative)

For the second source you may use a literature database search or an expert consultation. Documented expert consultation may be used as one source of information.

No Sources...

D. Animal Alternatives Narrative

Based on the information from the sources above, provide a written narrative of alternatives to procedures that may potentially cause more than momentary or slight pain or distress. The narrative should be such that the ACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

If a possible alternative was identified or is known, but will not be employed, discuss why.

Non-survival experiments include surgical procedures including: placement of catheters, tracheal cannulation, EMG placement, craniotomy, and alterations of respiratory state. Anesthesia will be maintained during all procedures to eliminate pain/distress. Lidocaine will be used to minimize the

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sympathetic response during placement of the ear bars.

We have chosen to use the cat model due to our expertise in this model, the specificity of established locomotor tests in the cat and the existing literature on SCI, locomotion and reflex in the cat. Airway protection has been extensively studied in the guinea pig model, as rodents do not possess the full complement of airway protective reflexes, particularly cough. Our previous work and the existing literature give us interpretive power that is not possible in less sentient species and allow us to directly compare any new work to our previous data and the data of others in our field. The cat is an important translational model for issues involving SCI in humans. These include its spinal size, more similarity between recovery in cat and human than rat and human, elegant motor control, similarity of spinal anatomy to the human, and seminal work regarding task specific training. There are multiple importance and significant benefits of the animal models to be used and these currently cannot be achieved in other animal or non-animal models.

RESULTS OF LITERATURE SEARCHES:

We directly deal with pain associated with surgical manipulations through the consistent use of pain medication following any surgical procedure. We also included the option in our protocol to extend the pain medication time frame in the unlikely event that we see pain behaviors after completion of the standard medication regimen. We also carefully monitor the animals' depth of anesthesia during all surgical procedures to assure that there is no perception of the procedure as it is occurring. Drugs used during all procedures are carefully chosen to adequately support the animal as well as assure that the research is translationally relevant.

The conclusions we have drawn from literature searches indicate the importance and significant benefits of the surgical procedures to be used, that these currently cannot be achieved with other approaches, and that our drug regimen is the most effective for the studies. As indicated above under two specific search engines, no suitable alternatives to the surgical procedures /approaches involving spinal cord injuries have been identified for the studies associated with this protocol. The lack of suitable alternatives was determined by continual literature review (typically a weekly basis). Searches used a variety of databases including Pub Med (which searches articles back to 1966) and current contents (broader, but searches only back to the early 90's), as well as search engines (i.e. google, yahoo) and the Johns Hopkins ALTWEB site. In addition to frequent searches and discussions with local colleagues, I, and members of my research group, attend meetings and give presentations at other scientific institutions (academic and private companies). During these meetings/trips, we
have professional discussions with colleagues that cover many topics including surgical approaches, pain control and alternative, viable approaches. Several of my colleagues are either veterinarians or have appointments in a Veterinary School of Medicine.

HUMANE ENDPOINTS:

For terminal surgical procedures: Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps will be taken to re-established a surgical plane of anesthesia and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end-tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone; this will be observed at least every 15 minutes; Additional anesthetic will be delivered or animal will be euthanized.

15. Investigator Assurances

1. ABSL-2 Assurance

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I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

- ○ Yes, I will meet the requirements of this statement.
- ○ No, I will not meet the requirements of this statement.
- ● Not Applicable

2. Investigator Assurances

- ☑ 1. The information provided herein is accurate to the best of my knowledge.
- ☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
- ☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.
- ☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.
- ☑ 5. I assure that I am not unnecessarily duplicating previous experiments.
- ☑ 6. I have considered alternative methods to using animals.
- ☑ 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

2024-06-13 10:23:29 -0500

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Protocol 29642 Amendment 2.0

Approval date	10/10/2022
Expiration date	11/15/2024

1. Basic Information

1. Elements ID

For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

29642

2. eACUC Number (Automatically Assigned)

29642

3. Principal Investigator

Cohn, Leah Ann

Job title	INTERIM ASSOCIATE DEAN FOR ACADEMIC & STUDENT AFFAIRS AND PROFESSOR VETERINARY MEDICINE & SURGERY
Department	Veterinary Medicine & Surgery
Division	Veterinary Medicine
Business unit	University of MO-Columbia

4. Protocol Title

Canine and Feline UMC-VHC Blood Donor Colonies

5. Triennial Re-write

Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

● Yes ○ No

A. Historical Protocol Number

What is the ACUC number this protocol was previously approved under?

Protocol 20981

B. 3 Year Progress Report

Give a brief summary of the work completed on the historical protocol listed above.

This is not a research project but rather a protocol to cover the use of live-in blood donor dogs and cats. These animals provide blood for transfusion to patients of the Veterianry Health Center. At the end of the use period, they are adopted to permanent pet homes. While they are here, they are also used as stress relief for veterinary students by attending student study sessions, exams, or meetings. They are sometimes used for demonstrations such as the vet hospital open house, or for school demonstrations. They have also taken part in pet therapy programs after individual evaluation for temperment (Dr. Cohn

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is one of two vets at MU that can certify pets for Pet Assisted Love and Support, aka, PALS pet therapy program). No major problems have come up with any of the donor animals or the donor program.

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

Species	Strain/ Stock/ Breed	Age/Weight	Pain/ Distress Category	Authorized	Ordered	Received	Adjustment	Available
Cat	Domestic (Cat)	adult (typically young adult to middle aged) cats over 8 pounds will be chosen as donors. Every effort will be made to use cats 9 pounds or more.	USDA Category C	15		10		5
Total Cats:				15	0	10	0	5
Dog	Any (Dog)	Adults over 55 pounds (typically young adult to middle aged). Every effort will be made to use only dogs over 60 pounds.	USDA Category C	20				20
Total Dogs:				20	0	0	0	20

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

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○ Yes ● No

3. Proposal Overview

1. Purpose

Purpose of the study:

The animal's primary use will be as a ready source of blood and blood products for transfusion to sick or injured pet animals. The UMC-VHC maintains a blood donor colony of both dogs and cats to allow for time-efficient collection and administration of blood products from a safe source. By using colony-maintained animals, we can screen for a number of potentially important infectious diseases and then limit exposure of the donor animals to either sources of infection or parasites that may transmit infections. In addition, we can screen blood donor blood types to insure that appropriately matched blood products are available for hospital patients. Dogs and cats each have a variety of blood types, and attaining an appropriate match for the animal receiving the blood is crucial.

Although there are commercial animal blood banks, the volume of blood required for our own hospital use easily justifies maintenance of our own donor colony. Blood products have a finite shelf life, but when they are required, the need is often urgent. By maintaining our own donors, the needs of our patients can be met quickly, effectively, and safely.

Although the separation of feline blood into components is not practically feasible at this time, we do routinely collect blood from our dogs for separation and short-term storage. By separating canine blood into plasma (that can be frozen) and packed red blood cells (that can be refrigerated), we minimize the need for fresh whole blood transfusions and therefore minimize the number of donor animals needed on the premises at any one time.

An additional use of these donors is to facilitate learning for professional degree students in the CVM. The donors are cared for by students on a daily basis, allowing them to practice physical examination skills, observational skills, animal husbandry skills, etc.

Finally, these animals are used to improve human well being by serving as therapy animals and animal ambassadors to a variety of groups including vet students and others as part of demonstrations or therapy sessions. Only those animals judged to have the temperment necessary to participate in such activities take part. Animals are always under supervision during such activities and they participate only after an invitation has been extended and they are welcome to be in the situation.

AMENDMENT: we have moved toward an all volunteer canine donor program but will leave open the option of going back to live in donors. As of August 2022, we have no more live in donor dogs.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

This is not a research study, but rather directly provides for the good of animals in the care of the UMC-VHC. Client- owned dogs and cats will literally have their lives saved by blood obtained from these donor animals.

3. Lay Term Description of Experimental Design

To put something in layman's terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This

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section should be written so that someone with a **10th grade science education can easily understand the project.**

These dogs and cats will live in the VHC for a period of time, and will be used, at most, once every 3 weeks to donate a unit of blood for transfusion to sick pets. The dogs are walked twice daily and cats are given attention daily while they live here. At the end of their time as donors, they are adopted to homes as pets. More information can be found on the donor website at http://vhc.missouri.edu/small-animal-hospital/blood-bank/, or on the blood donors Facebook page.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

This is not an experimental study, but the animals are used as a source of blood for transfusion to sick patients in the teaching hospital. They are cared for in a standard fashion and are adopted out at the end of their time here. These dogs and cats will live in the VHC for a period of time, and will be used, at most, once every 3 weeks to donate a unit of blood for transfusion to sick pets. The dogs are walked twice daily and cats are given attention daily while they live here. At the end of their time as donors, they are adopted to homes as pets. More information can be found on the donor website at http://vhc.missouri.edu/small-animal-hospital/blood-bank/, or on the blood donors Facebook page.

4. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

Blood transfusion is species specific. Thus, dogs are needed to donate blood for dogs and cats are needed to donate blood for cats. For most blood products, there is no alternative source of material. Although synthetic hemoglobin products have been approved for use in dogs, these products are no longer commercially available. Additional, when they were available those products could substitute only for the oxygen carrying capacity of red blood cells, and provide none of the other life-saving components of whole blood. The VHC must have a ready and immediate source of blood product for transfusion to our patients. While we do purchase some blood products, in order to function as an emergency hospital, secondary, and tertiary care center we must maintain a blood bank. By keeping blood donors on the premises rather than using a volunteer system, we ensure adequate access to animals and appropriate on-going care such as parasite prevention.

2. Justify Animal Species

Justify the choice of species for your study.

Because of species specificity of blood transfusion, there are no other choices for treatment of dogs and cats requiring blood; dogs must recieve dog blood and cats must recieve cat blood.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total

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numbers should match the requested numbers in the species section.

- Animal Numbers Justification
- The Logical Determination of "N" in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

Many years of experience maintaining the blood donor colony suggests that these numbers of animals are sufficient for our hospital needs, and provide some flexibility so that we can occasionally provide blood products for local veterinarians in urgent need of such products. We do not plan to maintain the maximum number of animals we are requesting permission to use. Rather, these numbers allow us to have some period of overlap as new donors are brought into the colony and screened for disease while the retiring donors are still available for use. Dogs and cats are generally adopted out of the colony after 2 years, meaning that there is a somewhat frequent turnover of animals. Most times we will have 5 to 6 cats (most with blood type A and one with blood type B) and 6 to 8 dogs. In recent years, we have had fewer dogs due to difficulty in finding new donors.

AMENDMENT: We no longer have live in donor dogs but continue to have cats. Cats are now housed outside of Clydesdale Hall and in the laboratory animal facilities in the Veterinary Medicine building instead. They are either collected in the work room in that facility or transported across the parking lot for blood collection in Clydesdale Hall then returned when fully awake.

5. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

	Facility
1	[redacted]
2	[redacted]

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

○ Yes ● No

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

● Yes ○ No

A. Description of Transportation

Describe how animals will be moved, including caging/transport carriers used (covered microisolator cages, dog/cat carrier, etc.), type of transport used (i.e. personal car or van), and projected transport time. (Note: Animals should be transported in a temperature controlled environment.)

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Transport may be by walking the dogs (they are leash walked at least twice a day all around campus), or by private vehicle if the distance is too far to walk. When the dogs are walked in public they wear basket muzzels and are leashed. The are very used to this type of walking. We do not allow children to control the leash. Animals are kept in kennels when driven by private vehicle.
AMENDMENT: now that cats are housed in the [redacted], they are sometimes transported across the parking lot to [redacted] for blood collection. They are kept in carriers for this purpose and are returned to their usual housing facility as soon as they are fully awake.

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

○ Yes
● No
○ Overnight only

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

- ☐ Single housing of social species
- ☐ Wire-bottom cages
- ☐ Special diet/water
- ☐ Extended time to weaning
- ☐ Extended time between cage changes
- ☐ Alternative light cycles
- ☐ Out of range temperatures
- ☐ Cage-size exceptions
- ☑ Other

i. Explain other

Dogs will recieve oral multivitamins with iron and their teeth are brushed regularly. They are walked on leash twice daily at a minimum of 30 minutes each walk, and wear basket muzzles during walks to prevent them from eating debris they may find on campus.

i. Explain non-standard husbandry and list the length of time the animal will undergo non-standard husbandry.

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Dogs will recieve oral multivitamins with iron and their teeth are brushed regularly. They are walked on leash twice daily at a minimum of 30 minutes each walk, and wear basket muzzles during walks to prevent them from eating debris they may find on campus.

6. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

● Yes ○ No

A. Sample Type

Type of sample(s):

Blood will be collected for transfusion

B. Sample Volume

Volume of sample(s):

From cats, up to 55 ml is collected. From dogs, 450 ml is collected.

C. Sampling Frequency and Duration

Frequency of collection and for how long:

For dogs no more than once every 3 weeks, for cats no more than once a month. Once animals are no longer used for donation they are adopted to homes, so duration of sampling is their entire stay.

D. Sampling Method

Method of collection:

Jugular veinipuncture

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

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	Procedure	Description of procedure	Building name	Room number or area
1	dog - blood withdrawal	Dogs - an area over the jugular vein will be clipped and prepped with disinfectant solution. A standard human blood collection device will be used, and with the dog in lateral recumbence the needle inserted into the vein. Blood will be allowed to flow until the unit is full (as monitored by constant weighing of the collection bag). The needle is then withdrawn, and a light bandage placed on the neck. For a single dog, sedation will be used until the nervous dog can be appropriately acclimated to blood donation. Sedation will be with dexmedetomidine.	[redacted]	[redacted] is used for the vast majority of blood draws.
2	Cat blood withdrawal	Cats - the cat will be sedated with ketamine (generally 30 to 40 mg/cat) and midazolam (generally 2 mg/cat for either) intravenously. Once sedate, an area over the jugular vein will be clipped and prepped with disinfectant solution. The tubing from a 20 gauge butterfly needle is attached to a 60 cc syringe containing 5 ml of anticoagulant. Using gentle suction, up to 55 ml of blood is withdrawn and then the needle removed. Gentle pressure is applied for a sufficient time to prevent hematoma formation. The cat is monitored until sternal and at least somewhat ambulatory.	[redacted]	[redacted] is used for the vast majority of blood draws.
3	animals with prior VAP	The blood donor program might occasionally acquire by transfer animals that already have vascular access ports (VAP) in place. Rather than use veinipuncture to obtain blood from these animals, we will take advantage of the VAP. This will negate the need for sedation for blood draws and minimize any discomfort to the cat or dog. Care of the VAP is described here. A 22 or 20 g Huber needle is placed through the center of the VAP diaphragm. One to two ml of blood is collected and discarded. The catheter is then flushed with 3 ml of saline. Then 2 ml of blood is collected and saved (syringe 2). The blood sample is collected and then the blood in syringe 2 is placed back in the cat. The VAP is flushed with 5 ml of saline and then heplocked by injecting 1ml of heparin (100 U/ml) while withdrawing the needle. please note that we currently do not have any animals with a VAP, and do not intend to place VAPs. We would only use them if they come into the program with a VAP in place.	[redacted]	[redacted] is used for the vast majority of blood draws.
4	physical examination	Use of animals in teaching includes physical examination by students, or examination of external structures (eg, identification of landmarks). Student brush the dogs teeth daily, and give them weekly baths.	[redacted]	[redacted] for students to practice some aspect of the physical examination

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	Procedure	Description of procedure	Building name	Room number or area
5	exercise/ socialization	Dogs are walked outside for half an hour twice daily while wearing basket muzzles; occasionally walks may be shorter due to inclement weather or if the animal becomes lame, and often walks are longer or more frequent. Cats are allowed to play not only in their group housing unit, but are often brough out into rooms during teaching rounds to play and interact with veterinary students. They are also sometimes taken to other rooms to "hang out" with vet students taking exams, or with clients meeting with our social worker during grief counseling.	[redacted]	various rooms of vet school campus

7. Substances Used in Animals

1. Substances Used in Animals

List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

No Substance used in animals...

2. Non-Pharmaceutical Grade Substances

For those substances that are marked “no” as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., “All substances” or “non-hazardous substances”) if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

No Test Substances PPEs...

Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

8. Hazardous Materials

1. Will you use any Biological Hazards?

○ Yes ● No

2. Will you use any Chemical Hazards?

○ Yes ● No

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3. Will you use any Radiation Hazards?

○ Yes ● No

9. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

● Yes ○ No

2. Preanesthetic Agent(s)

List preanesthetic agents here

No Preanesthetic Agents...

3. Anesthetic Agent(s)

List anesthetic agents here. **Do not list isoflurane here, it will be listed later in the form.**

	Species	Agent	Dose/Volume	Route	Frequency of Admin.
1	Cat	ketamine	40 mg/CAT;	IV	once
2	Dog	ketamine	0.4 ml (100 mg/ml) solution	IV	once
3	Cat	midazolam	2 mg/CAT;	IV	once
4	Dog	midazolam	0.4 ml; 5 mg/ml solution	IV	once
5	Cat	dexmedetomidine	5 microgram/kg;	IV	once
6	Dog	dexmedetomidine	5 microgram/kg; 0.4 ml	IV	once

4. Isoflurane Use

A. Will you use isoflurane?

○ Yes ● No

5. Non-Pharmaceutical Grade Anesthesia

If non-pharmaceutical grade anesthesia must be used, strong scientific justification must be provided. In addition, describe how the solution will be made and stored. Also, describe how pH, osmolarity, and sterility will be kept in acceptable physiological ranges. Please see ACUC policies for Anesthesia and Non-pharmaceutical Anesthesia and ACUC Guidelines for pentobarbital, avertin, inactin, and urethane/chloralose.

6. Monitoring and Life Support

Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:

Cats are closely watched during blood donation for respiratory rate and depth of anesthesia/sedation. The pulse is also checked at the start of the procedure and upon completion. Dogs are seldom sedated, but would be montiored in a simillar fashion.

7. Post Anesthetic Recovery

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Complete description of post-anesthetic recovery monitoring and care:

Cats are watched continuously until they are sternal and awake, which is typically very shortly after blood donation is complete. They are not returned to teh cat donor ward until they are 100% recovered from sedation and can interact with the other cats appropriately. Dogs are usually not sedated, but if they were they would be monitored during recovery in a similar fashion.

8. Pharmaceutical Analgesia

○ Yes ● No

9. Non-pharmacologic control of pain

○ Yes ● No

10. Paralytic Agents

○ Yes ● No

11. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

○ Yes ● No

10. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

○ Yes ● No

11. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

The routine use of these animals (as blood donors and subjects of physical exam) has little potential for adverse consequences. The potential consequences include anemia, hypovolemia, or adverse reactions to sedative agents. All donors will have PCV checked prior to each donation, and will not be used if found to be anemic. Bleeding a maximum on once every 3 weeks allows sufficient time for regeneration of lost blood. Iron supplementation helps assure the ability to regenerate lost hemoglobin. Dogs and cats recieve once weekly physical exams by vet students, and periodic eams by veterinrians. Any health issue (eg, heart

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murmur) would be fully investigated prior to continued use as a donor, reducing the risk of hard to both the donor or the blood recipient.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

Physical examinations are not painful, and in fact, the animals enjoy the attention. Collection of blood involves very little pain past the insertion of the needle. The dogs routinely become acclimated so that they are eager to jump onto the table to give blood as they seem to anticipate a food reward afterward. Cats are sedated to avoid any stress or discomfort as they are restrained in lateral recumbence during the ~10 minute blood collection period.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

○ Yes ● No

12. Disposition of Animals

1. Animal Disposition

Check all that apply

- ☑ Adoption (See MU adoption policy)
- ☐ Market
- ☑ Euthanasia
- ☐ Transfer to different project, PI, or another institution
- ☐ Returns to breeding colony, herd, source, owner, or wildlife site
- ☐ Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, "Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue."

A. Method of Euthanasia

Select the method of euthanasia

- ☐ Inhalant agent
- ☐ Physical Method without Anesthesia
- ☐ Physical Method with Anesthesia

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☑ Noninhalent Pharmaceutical Agent

B. Euthanasia Descriptions

	Species	Agent/Method	Dose/Volume	Route
1	Cat	Fatal plus (barbiturate)	1 ml/10 lb	IV
2	Dog	Fatal plus (barbiturate)	1 ml/10 lb	IV

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

No animals are euthanized unless it is medically necessary (an extremely rare occurence as we generally provide any medical therapy required to return donors to good health and allow for continued use or adoption to a pet home).

D. Scientific Justification for Use

☑ AVMA Approved Method

☐ Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

☐ Bilateral pneumothorax

☐ Cervical dislocation

☐ Decapitation

☐ Exsanguination

☐ Removal of vital organs

☑ Other

i. Please specify:

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Will assure death by asucultation of heart.

13. Project Information

1.

Associate	Role	Responsibilities	OHSP Training	Animal Care & Use	Survival Surgery	P&D Training
Cohn, Leah Ann [redacted]	Principal Investigator Authorized to order animals	Euthanasia	☑ Aug 29, 2022	☑ Nov 16, 2020	☑ Nov 1, 2002	⊖
[redacted]	Key Personnel		☑ Oct 23, 2020	☑ Jan 26, 2022	⊖	⊖
[redacted]	Key Personnel Authorized to order animals		⊖	☑ Feb 11, 2020	☑ Sep 25, 2013	⊖
[redacted]	Key Personnel		☑ Feb 2, 2021	☑ Jul 12, 2021	⊖	⊖
[redacted]	Key Personnel Access to view cages		☑ Oct 26, 2020	☑ Oct 27, 2020	⊖	⊖
[redacted]	Key Personnel		☑ Jun 14, 2022	☑ Oct 18, 2021	⊖	⊖
[redacted]	Key Personnel		☑ Jan 20, 2022	☑ Jan 20, 2022	⊖	☑ Mar 15, 2022
[redacted]	Key Personnel		☑ Jan 20, 2022	☑ Jan 20, 2022	⊖	⊖
VHC, Residents [redacted]	Key Personnel		⊖	☑ Jun 21, 2021	⊖	⊖
[redacted]	Protocol Creator		☑ Jan 14, 2021	☑ Mar 18, 2022	☑ Mar 16, 2022	☑ Nov 5, 2021

2. Training and Qualifications

Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	Cohn, Leah Ann	liscenced DVM, specialist in small animal internal medicine	oversee's entire donor program	liscenced DVM, specialist in small animal internal medicine	
2	[redacted]	liscenced vet tech with over 20 years experience	day-to-day animal oversight, blood collection, animal adoption and ordering	liscenced vet tech with over 20 years experience	
3	VHC, Residents	DVM's	Ohter veterinary residents may need to provide medical care, or to obtain blood on an emergency basis on occasion.	DVM's	
4	[redacted]	experience with [redacted] lab	play with the cats for environmental enrichment	Same thing was done for [redacted] protocols	Volunteer/non-employee
5	[redacted]	Unsure but lab manager for [redacted] lab	play with cats for environmental enrichment	Has done so with [redacted] lab	Full-time employee
6	[redacted]	experience with [redacted] lab	Play with cats for environmental enrichment	Has done so with [redacted] lab	Volunteer/non-employee
7	[redacted]	experience with [redacted] lab	Play with cats for environmental enrichment	Has done so with [redacted] lab	Volunteer/non-employee
8	[redacted]	experience with [redacted] lab	Play with cats for environmental enrichment	has done so with [redacted] lab	Volunteer/non-employee
9	[redacted]	experience with [redacted] lab	Play with cats for environmental enrichment	Has done so with [redacted] lab	Volunteer/non-employee
10	[redacted]	experience with [redacted] lab	Play with cats for environmental enrichment	has done so with [redacted] lab	Volunteer/non-employee

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- ☐ PHS (NIH, CDC, FDA, NSF, NASA)
- ☐ DoD
- ☐ VA

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- ☐ AHA
- ☐ USDA
- ☐ Foundation/Industry
- ☑ Internal
  - i. Specify funding source

    UMC-VHC
- ☐ Other

14. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

○ Yes ● No

15. Investigator Assurances

1. ABSL-2 Assurance

I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

○ Yes, I will meet the requirements of this statement.
○ No, I will not meet the requirements of this statement.
● Not Applicable

2. Investigator Assurances

☑ 1. The information provided herein is accurate to the best of my knowledge.
☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.
☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.
☑ 5. I assure that I am not unnecessarily duplicating previous experiments.
☑ 6. I have considered alternative methods to using animals.

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☑ 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

2022-11-29 09:44:27 -0600

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University of Missouri

Animal Care Quality Assurance
McReynolds Hall
Columbia, MO 65211
PHONE 573-882-1746
EMAIL ACUC@Missouri.edu
WEB research.missouri.edu/acqa/

November 29, 2022

Subject: IACUC APPROVAL

Dear Dr. Cohn,

Your MU animal use protocol Protocol 29642 Amendment 2.0 entitled "Canine and Feline UMC-VHC Blood Donor Colonies" was approved by the IACUC on October 10, 2022 and will expire on November 15, 2024.

Sincerely,

Scott Rector, Ph.D.
Chair, Animal Care and Use Committee
Division of Research, Innovation & Impact
[redacted]

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Principles of transfusion medicine in small animals

Anne Lanevschi, K. Jane Wardrop

Abstract — The purpose of this review was to provide the reader with an updated overview of small animal transfusion medicine, and an approach to integrating it into private practice, based on a review of the veterinary and human literature spanning the last 3 decades. Electronic, online databases that were searched included CAB International and Medline; multiple keywords or subject headings were searched that were appropriate to each of the sections reviewed: canine and feline blood groups, blood-typing and crossmatching, donors, blood collection, storage, blood components, blood transfusion, blood component therapy, blood substitutes, and adverse reactions. The safe use of blood component therapy requires knowledge of blood groups and antibody prevalence, and knowledge of the means to minimize the risk of adverse reactions by including the use of proper donors and screening assays that facilitate detection of serological incompatibility. The 2 assays available to the practitioner are crossmatching, which is readily done in-house, and blood typing. Blood typing is available in the form of a commercial testing kit, through use of purchased reagents, or via a request to an external laboratory. The risk of potentially fatal adverse reactions is higher in cats than in dogs. The decision to transfuse and the type of product to administer depend on several factors, such as the type of anemia and the size of the animal. In conclusion, transfusion medicine has become more feasible in small animal practice, with improved access to blood products through either on-site donors, the purchase of blood bank products, external donor programs, or the availability of blood component substitutes.

Résumé — Principes de la médecine de transfusion chez les petits animaux. Le but de cette revue était de fournir aux lecteurs une vue d'ensemble à jour de la médecine de transfusion chez les petits animaux et une approche d'intégration en pratique privée basée sur une revue de la littérature tant vétérinaire qu'humaine s'étendant sur trois décennies. Les bases de données informatiques retenues comprennent CAB International et Medline; les mots-clés multiples ou les rubriques appropriées à chaque section analysée ont été recherchés : groupes sanguins canins et félins, groupes sanguins et compatibilité croisée, donneurs, prélèvements, entreposage, composantes du sang, transfusion sanguine, thérapie par composantes du sang, substituts du sang et réactions indésirables. L'utilisation sécuritaire des thérapies par composantes du sang requiert la connaissance des groupes sanguins et de la prévalence des anticorps ainsi que les moyens pour minimiser les risques de réactions indésirables dont le choix des bons donneurs et les tests de dépistage permettant la détection de l'incompatibilité sérologique. Les deux analyses disponibles pour le praticien sont la compatibilité croisée, facilement réalisable sur place, et la détermination des groupes sanguins. La détermination des groupes sanguins peut se faire par utilisation d'une trousse comprenant des réactifs disponibles dans le commerce ou par un laboratoire spécialisé. Les risques de réactions indésirables mortelles sont plus élevés chez les chats que chez les chiens. La décision de transfuser et le type de produit à administrer dépend de nombreux facteurs tels que le type d'anémie et la grosseur de l'animal. En conclusion, la médecine de transfusion est maintenant plus accessible en pratique des petits animaux à cause d'une meilleure disponibilité des produits sanguins reliée à la présence de donneurs sur place, à l'achat de produit provenant de banques de sang, à l'accès à des donneurs externes ou à la disponibilité de substituts aux composantes du sang.

(Traduit par Docteur André Blouin)

Can Vet J 2001;42:447–454

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Address correspondence and reprint requests to Dr. Anne Lanevschi.

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Introduction

The purpose of this review is to provide the reader with an updated overview of small animal transfusion medicine, and an approach to integrating it into private practice, based on a review of the recent veterinary and human literature. Transfusion medicine has gradually become more feasible in small animal practice, with improved access to blood products through either on-site donors, the purchase of blood bank products, external donor programs, or the availability of blood component substitutes. However, the safe use of blood component therapy requires knowledge of blood groups and antibody prevalence, and knowledge of the means to minimize the risk of adverse reactions by including the use of proper donors and screening assays that facilitate detection of serological incompatibility. This review covers a brief history of transfusion medicine, an update on canine and feline blood groups and known blood incompatibilities, laboratory testing for blood type or compatibility, donor selection and blood collection, storage of blood components, blood component and blood substitute therapy, and adverse reactions in small animal transfusion medicine.

Literature search

The veterinary literature was emphasized, and the human literature was cited to provide support for specific concepts and guidelines in veterinary transfusion medicine for those areas in which extensive veterinary research has not yet been performed. The 2 main, electronic, online databases used were CAB International (Commonwealth Agricultural Bureaux International, Wallingford, Oxon, UK) (1973–2000), for the veterinary literature, and Medline (National Library of Medicine, Bethseda, Maryland, USA) (1966–2000), for the human literature. Multiple keywords or subject headings were searched appropriate to each of the sections reviewed: canine and feline blood groups, bloodtyping and crossmatching, donors, blood collection, storage, blood components, blood transfusion, blood component therapy, blood substitutes, adverse reactions. Search terms or subject headings included transfusion medicine, history, canine, feline, blood groups, alloantibodies, crossmatching, bloodtyping, diagnosis, donors, adverse effects, therapeutic use, plasma, coagulation factors, toxicity, incompatibility, blood components, platelets, storage, blood substitutes, and thrombocytopenic purpura.

History

The history of transfusion medicine dates back to the Early Modern Period immediately following the Renaissance, with landmark discoveries, such as William Harvey's theory of circulation (1628), making advances in this field possible. It was not until the 19th century that transfusion became a more common occurrence, albeit as a high-risk procedure, in women suffering from postpartum hemorrhage. The 20th century saw several major breakthroughs that made this practice safer and more widespread, including the discovery of anticoagulants and preservatives for blood products, the description of human blood groups, and the development of compatibility assays (1). The tragic occurrence of World War II enhanced developments in transfusion medicine, including large-scale blood-banking under the Red Cross. In the veterinary field, transfusion medicine emerged as a practice from the 1950s onwards (2). Recently, research on substitutes for oxygen transport led to the approval in 1998 by the United States Food and Drug Administration of a hemoglobin-based oxygen carrying solution for use in dogs.

Blood groups

Erythrocytes possess particular antigens (glycoproteins or glycolipids) on the surface of their cell membranes that allow their classification into blood groups. A characteristic of these antigens is their ability to trigger a reaction caused by circulating anti-erythrocyte antibodies in the opponent host or donor. These antibodies may occur naturally or be induced following a previous transfusion. A severe, and potentially life-threatening, situation is one in which the interaction leads to the destruction by hemolysis of red blood cells (RBCs). In type B cats receiving type A blood, the rapid destruction of RBCs may be mediated by IgM and complement fixation, as well as the release of potent vasoactive compounds. This may cause shock and generally occurs when the patient possesses antibodies towards the transfused RBCs. In other instances, the antibody-red cell antigen interaction is less severe, and the most important outcome is that the transfusion loses its efficacy as the mean survival time of transfused RBCs is drastically reduced.

Canine blood groups — The terminology for canine blood groups has changed over time. Currently, the prefix DEA ("dog erythrocyte antigen") is used, and antigens from 5 blood groups can be characterized by using commercial antisera. The most important blood groups are DEA-1 (1.1 and 1.2) and DEA-7. The DEA-1 group has 4 alleles. A dog may be DEA-1-negative, or DEA-1.1- or DEA-1.2-positive. Recently, DEA-1.3 has been described (3). Transfusion of DEA-1.1 type erythrocytes in a DEA-1-negative recipient results in the formation of anti-DEA-1.1 alloantibodies, and as a result, the lifespan of the transfused RBCs is decreased (4). More importantly, subsequent transfusions in the same patient and with the same blood type will result in an acute hemolytic crisis. Similar to DEA-1.1 incompatibility, transfusion of DEA-1.2 type erythrocytes in a DEA-1-negative recipient that possesses circulating anti-DEA-1.2 alloantibodies results in a half-life of transfused erythrocytes of 0.5 d (5). In contrast, DEA-7 is a soluble, nonerythroid antigen that adsorbs to the red cell surface. Dogs that are DEA-7 negative may possess naturally occurring anti-DEA-7 alloantibodies, but their importance is debated (4,6,7). For these reasons, ideal canine donors should at least be DEA-1.1 and -1.2 negative. In screening for candidate donors, DEA-1.1-negative dogs should be further tested for DEA-1.2. Other canine blood groups are infrequent, and incompatible transfusions with these blood groups do not generally result in a clinically apparent transfusion reaction, although the lifespan of transfused RBCs may be diminished (8,9).

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Table 1. Crossmatching assay procedure

1. Centrifuge (1000 × *g* or 3400 rpm) whole blood from a red-top (no anticoagulant) tube (Vacutainer; Becton-Dickinson, Franklin Lakes, New Jersey, USA) in order to obtain serum, and whole blood from a purple-top (EDTA) tube (Vacutainer; Becton-Dickinson) in order to obtain RBCs from both the patient and donor.

2. Washing RBCs: resuspend 0.25 mL of RBCs in 2 to 4 mL of saline; centrifuge 1 min, remove supernatant, and repeat the procedure twice; remove supernatant.

3. Resuspend 0.1 to 0.2 mL of RBCs in approximately 4.8 mL saline in order to obtain a 2% to 4% RBC solution.

4. In 3 tubes identified "Major," "Minor," and "Control" add the following:

	"Major" crossmatch	"Minor" crossmatch	Control
Patient	2 drops serum	1 drop RBC solution*	1 drop patient RBC solution* + 2 drops patient serum
Donor	1 drop RBC solution*	2 drops serum	1 drop donor RBC solution* + 2 drops donor serum

5. Incubate tubes 15 min at 37°C.

6. Centrifuge tubes 15 s.

7. Reading results: Note serum color and record any hemolysis. Then gently resuspend the red cell button into the overlying serum layer, noting the presence of agglutinating clumps. Next, place a drop of resuspended RBCs on a slide, apply coverslip, and read at 100X and 400X. If the crossmatch is compatible, the RBCs should be individually distributed. Hemolysis (compared to control) or agglutination is seen with an incompatible crossmatch.

8. Rouleaux, a physiological plasma-related phenomenon, may sometimes be observed. In order to distinguish this from agglutination, centrifuge the tubes again for 15 s, remove serum, and add 2 drops of saline; then centrifuge the tubes once more and reexamine the RBC suspensions.

* — saline solution containing 2 to 4% RBCs; EDTA — ethylenediaminetetraacetic acid

Feline blood groups — Cats have an AB blood group system, and their RBCs may be A, B, or, rarely, AB. A and B are alleles in the same locus, where A is dominant over B. The mode of inheritance for AB has not yet been described (10).

The frequency of feline blood types varies according to breed and geographic location, but most cats (> 95%) have type A RBCs. Less than 5% of the cat population possesses the type B RBCs, and 1% or less of domestic shorthair or longhair cats have type B blood. The frequency of type B blood is higher in certain breeds and may be as high as 77% in the British shorthair (11–13). Most type B cats have powerful naturally occurring anti-A alloantibodies, such that transfusion of type A blood into a type B cat will result in an acute hemolytic reaction. The half-life of transfused RBCs following such transfusions is approximately 1 h. Approximately 30% of type A cats will possess weakly agglutinating anti-B antibodies, such that only a minor reaction occurs when a type A cat is transfused with type B blood, rendering the transfusion inefficient. The half-life of the transfused RBCs in these transfusions is approximately 2 d (14). Type AB cats do not possess anti-A or anti-B antibodies and can be safely transfused with type A blood (15). Although a type AB cat could technically receive RBCs from a type A or type B cat, anti-A alloantibodies in plasma from a type B cat could cause a reaction. Ideally, 2 types of feline donors are desired: a type A cat, at the very least, and an available type B cat, whenever possible.

Laboratory testing: blood-typing and crossmatching

Blood typing enables the practitioner to identify the blood group of the patient or a candidate donor. This assay is based on a hemolytic or agglutinating reaction in which the reagent or antibody reacts with the RBCs of the test subject. The crossmatching test does not identify the blood group but, instead, detects serological incompatibility between a candidate donor and the patient. Reasons for testing, using either of these methods, prior to transfusion include the need to avoid an acute hemolytic reaction during or following transfusion, an assurance of an optimal lifespan of the transfused RBCs, the prevention of incompatible blood transfusions in the future, and the prevention of neonatal isoerythrolysis.

Blood-typing cards are available commercially for the cat and the dog (Rapid Vet-H feline and canine blood-typing cards; DMS Laboratories, Flemington, New Jersey, USA). The test is simple and quick to perform; it involves the mixing of a drop of blood from the animal to be tested with a drop of the antibody reagent and then noting the presence of agglutination. For the dog, the reagent is a murine monoclonal anti-DEA-1.1 antibody; for the cat, the reagents are an anti-B reagent (a wheat germ lectin) and natural anti-A antibody (16). Controls may be included with the cards. However, care should be taken to ensure that these controls contain stable cells and that hemolysis of the controls has not occurred. False positive reactions have been reported with commercially

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available DEA-1.1 typing cards (17). An alternative for blood typing in dogs is to submit samples to an external laboratory. Blood typing in dogs is particularly indicated for screening candidate donors and for emergency situations in which there can be no delay in waiting for a result from an external laboratory. It should be noted that the possibility of false positive reactions by using the DEA-1.1 typing cards could lead to inadvertent transfusion of DEA-1.1-positive blood to a DEA-1.1-negative recipient that falsely tested DEA-1.1-positive. This animal will be sensitized and react to subsequent transfusions with DEA 1.1-positive blood. For this reason, it is preferable to test donors rather than recipients. For cat blood typing, in-house typing other than with the commercially available typing cards is feasible, if both type A and type B cat donors are available. Heat-inactivated serum from the type B cat can be used as anti-A reagent; for detection of type B blood, a wheat germ lectin (*Triticum vulgaris*) solution can be used (*Triticum vulgaris* lectin, 2-mg vial [product no. L9640; Sigma Chemical Company, St. Louis, Missouri, USA], reconstituted with 20 mL of phosphate-buffered saline for a 100-µg/mL solution, stored at 4°C) (14). Blood from donors of known blood type can serve as a control for the assay.

Crossmatching requires that centrifuged RBCs from the donor or recipient be washed several times with saline and prepared as a 2% to 4% suspension before being incubated at 37°C with serum from the recipient or donor, respectively (Table 1). The only pieces of equipment required are a centrifuge and a temperature-regulated hot water bath or incubator.

Screening tests to detect serologic incompatibility are strongly recommended in dogs, even in the event of a first transfusion where the risk of a serious adverse reaction is low. Testing becomes essential if the animal has already received a transfusion, especially if the donor is not known to be DEA-1.1 or DEA-1.2-negative (4). The risk of blood incompatibility is much higher in the cat, especially in purebred cats (except for Siamese) in which the frequency of B-cats is higher. Blood typing or crossmatching is essential to minimize this risk, although the risk is lower in shorthairs. It is important to note that a negative result from crossmatching blood from a donor and a patient does not mean that 2 subjects have the same blood type. Also, crossmatching does not provide a guarantee of leukocyte, protein, or platelet compatibility, and, in some cases, it may be too insensitive to detect anti-erythrocyte antibodies (18).

Table 2. Advantages and disadvantages of in-house donors versus an external donor program

In-house donors	
Advantage	• donor is readily available
Disadvantage	• higher maintenance cost • a donor may not fulfill the practice's need for blood products
External donor program	
Advantage	• less costly • multiple donors provide a greater supply of blood product • collection may be scheduled at a convenient time • sensitizes owner to the value of veterinary medicine in society
Disadvantage	• requires advance planning • requires storage of blood products

Donor selection and blood collection

Several approaches are available in order to obtain blood products. One approach is to purchase products as the need arises from a blood bank. There are several drawbacks with this option, such as delays in obtaining blood components, limitations as to the availability of certain blood components when requested, and blood components that are not produced by a given blood bank. Another approach is to have access to donors either as in-house donors or from an external donor program. There are advantages and disadvantages to both options, and the choice should take into account the practice's needs (Table 2) (19–21). For an external donor program, a list of reliable donors has to be established. As the list grows, the blood collection interval can be increased. However, too many donors increase yearly health screening costs. With either on-site or external donors, a donor log should be kept in addition to the donor's medical file. The log can contain the name, blood type, scheduled dates for yearly or twice yearly screening tests, blood collection dates, and the owner's contact information, if it is an external donor. A transfusion log should also be kept with information on each transfusion, including the date of transfusion, donor, type and volume of blood component used, recipient, diagnosis, and problems encountered, such as adverse reactions. In addition, a note that the recipient has received a blood transfusion should be included in a visible location in the recipient's medical file (20).

The ideal canine donor should have the following characteristics: weigh more than 30 kg, have taut neck skin that permits easy access to the jugular vein, have a packed cell volume that is at least 0.40 L/L, have demonstrated a good temperament and be in fit physical condition, have no previous history of transfusion or pregnancy, be DEA-1.1 and DEA-1.2-negative, and possess adequate levels of von Willebrand factor (vWF).

The ideal feline donor should have the following characteristics: weigh more than 4.5 kg, have a packed cell volume that is at least 0.35 L/L, have demonstrated a good temperament, and be in fit physical condition.

Proper maintenance of donors requires up-to-date vaccinations; fecal floatation every 6 mo if there is contact with new animals; a yearly hemogram, clinical chemistry profile, and screen for infectious diseases; and, in the dog, preventative heartworm therapy in regions where it is appropriate. At each blood collection, the donor's weight, temperature, and packed cell volume should be checked (22–24).

Blood collection usually should not exceed 15–20 mL/kg bodyweight (BW) every 3 wk in dogs, and 10–15 mL/kg BW or a total of 60 mL every 3 wk in cats. This schedule may require iron supplementation. An increased interval is recommended, if the packed cell volume or plasma protein drops significantly after blood collection (20,24).

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Anticoagulants and preservatives

Heparin and citrate are anticoagulants that will not contribute to cell preservation during long-term storage and whole blood collected in these anticoagulants should be used within 24 h. Commonly used preservatives include acid citrate dextrose (ACD), citrate phosphate dextrose (CPD and CP2D), and citrate-phosphate-dextrose-adenine (CPDA-1), in which the added dextrose, phosphate, and adenine favor the viability of RBCs, permitting their storage for up to 3 to 5 wk, depending on the preservative (25–27). Additive solutions (Adsol, Fenwal Division of Baxter Healthcare Corporation, Deerfield, Illinois, USA; Nutricel, Cutter Biological, Division of Miles Laboratories, Emeryville, California, USA; Optisol, Terumo Medical Corporation, Somerset, New Jersey, USA) can be added to blood from which plasma has been removed. These solutions contain factors, such as dextrose, adenine, mannitol, and the sodium chloride, needed by RBCs to maintain their energy metabolism and viability during storage. Whole blood may be collected using ACD, CPD or CPDA-1. For dogs, a standard 450 mL CPDA-1 bag enables the collection of one unit of blood. If sedation is required, it is best to avoid acepromazine, as it may alter platelet function. Butorphanol (0.1 mg/kg BW, IV), administered 10–15 min before collection, has been recommended in the dog (28). In the cat, ketamine may be used as needed: 2 to 4 mg/kg BW, IV, plus 0.1 to 0.2 mg/kg BW diazepam, IV, works well. In the cat, blood can be collected via jugular vein venipuncture into a syringe containing either ACD, CPD, or CPDA-1 (1 mL/9 mL of blood), or heparin (5 units/mL of blood) by using a 19- to 20-gauge needle or butterfly. It can be stored in a small blood bag, as long as a preservative solution is used (29).

Blood product indications

The choice of blood components to be used cannot be based solely on the packed cell volume; the rate, as well as the quantity and type of components lost or missing, influences the clinician's choice. Whole blood is indicated in a patient that requires several blood components or has acutely lost more than 50% of its total blood volume, in order to replace both oxygen-carrying capacity and oncotic activity. For example, fresh whole blood containing labile coagulation factors would be indicated in a patient suffering from hemorrhage due to thrombocytopenia, hemophilia, liver insufficiency, or disseminated intravascular coagulation (DIC), whereas whole blood containing stable coagulation factors would be indicated in vitamin K antagonist rodenticide toxicosis (30). The calculation of a patient's total blood volume is 85–90 mL/kg BW in the dog and 65–75 mL/kg BW in the cat (31). Whole blood administration attains 2 goals: expansion of the blood volume and tissue reoxygenation. Platelets gradually lose their viability following refrigeration, such that they will not contribute significant procoagulant activity when previously refrigerated whole blood is used (32,33). However, whole blood collected within the previous 24 h still contains much of its labile coagulation factor activity (factors V and VIII), as well as its stable coagulation factor activity. After this time, its labile coagulation factor activity is gradually lost (34–36). Whole blood is not the ideal product where tissue reoxygenation is specifically required and there is little or no need for plasma volume expansion. Examples of this are acute hemorrhage with less than 50% of total blood volume loss, in which the volume expansion needed may be provided by crystalloid solutions and tissue reoxygenation can be supplied by packed RBCs. Other examples include chronic hemorrhage, hemolytic anemia, or nonregenerative anemia. Packed red cell transfusion is preferable to whole blood transfusion in these situations, since whole blood transfusion can lead to hypervolemia in these patients (34). An exception is with the treatment of small dogs or cats, where it may be more difficult to prepare small volumes of packed RBCs.

Packed RBCs are prepared by removing 200 to 250 mL of plasma from 450 mL (1 unit) of whole blood after centrifugation. The packed cell volume of the RBC preparation is approximately 0.70 to 0.80 L/L, and the RBCs can be resuspended in a protein-poor additive solution, such as Adsol, to a packed cell volume of 0.55 to 0.65 L/L. Shelf life depends on the preservative solution used (37–39). The transfusion of packed RBCs is indicated for tissue reoxygenation and is ideal for a normovolemic, anemic patient.

Plasma contains 2 labile coagulation factors: V and VIII. If freshly collected human plasma is not frozen, half of the procoagulant activity associated with factor VIII (but not factor V) is lost within the first 24 h, whereas other coagulation factors remain stable (40). Fresh frozen plasma (FFP) is plasma that has been separated from RBCs and frozen at -18°C within 8 h of collection (if preserved with CPDA-1, CPD, or CP2D; 6 h if preserved with sodium citrate or ACD); plasma frozen after this period is referred to as frozen plasma (FP). One unit of FFP has an approximate volume of 200 to 250 mL and contains labile and stable coagulation factors. The main indications for FFP administration are a lack of coagulation factors associated with hepatic insufficiency, DIC, vitamin K deficiency (rodenticide toxicosis, liver insufficiency, biliary tract obstruction, malassimilation syndrome, chronic antibiotic use), a need for plasma volume expansion, or a massive blood loss within a few hours. Other indications include a congenital or a hereditary deficiency in coagulation factors, such as hemophilia A, B, or von Willebrand's disease. On the other hand, FP, which lacks labile coagulation factor activity, may be used to treat conditions in which stable coagulation factors are needed, such as rodenticide toxicosis or hemophilia B. As much as possible, FFP should be reserved for patients requiring labile coagulation factors (DIC, hemophilia A, and von Willebrand's disease), and FP used for other disorders. Plasma (FP or FFP) is not indicated as a long-term source of protein for patients suffering excessive protein loss (enteropathy, nephropathy, exudative dermatitis) or suffering from inadequate intake, but it may be used initially in the emergency management of such patients (41,42). Enteral or parenteral nutritional support may be more effective in patients suffering protein loss, as it may favor hepatic albumin synthesis. An instance in which plasma is indicated for hypoproteinemia due to protein loss is acute albumin loss, such as occurs in burn victims (43).

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Special clinical considerations for transfusion of whole blood or packed RBCs

Choosing the proper blood component or components and calculating the amount to administer to the patient must be based on a case-by-case evaluation. In acute hemorrhage, packed cell volume may be a poor indicator of the degree of blood loss. Since all blood components are lost in hemorrhage, signs of shock may be seen initially when the packed cell volume is within reference limits. The packed cell volume in such patients will gradually drop over the 72 h following the initiating incident, as extravascular fluid is redistributed and enters the intravascular space, assuming that there has been no volume replacement with crystalloids, in which case the packed cell volume will drop more quickly. The potential for survival depends mainly on 2 factors: reestablishment of the blood volume and tissue reoxygenation. Initial fluid therapy with crystalloids or colloids is essential to reestablish blood volume. This will be sufficient for losses that do not exceed 20% of the patient's total blood volume. For losses exceeding 20%, whole blood or packed red cell transfusion is indicated. Losses between 20% and 50% of blood volume require crystalloids and packed RBCs (44). Whole blood is unnecessary, as there is no need for colloids, coagulation proteins, or platelets. Losses between 50% and 100% of total blood volume require replacement of RBCs and a correction of colloidal oncotic pressure. In such an instance, whole blood or, alternatively, packed RBCs combined with either plasma or an equivalent product (dextran-70, pentastarch, or hetastarch) that will favor blood volume expansion should be administered; whole blood is not essential, as there is no need for coagulation proteins or platelets. Losses exceeding 100% of total blood volume (trauma) require a replacement of all blood components; therefore, whole blood transfusion is necessary. Hemostasis imbalance (hemophilia, von Willebrand's disease, rodenticide toxicosis, liver insufficiency, DIC, thrombocytopenia) may require multiple blood components or fresh whole blood (45).

Patients that suffer from nonregenerative anemia are normovolemic and may develop pulmonary edema due to hypervolemia following whole blood transfusion. For these patients, packed RBC transfusion is the treatment of choice.

Among other blood components that may occasionally be indicated, cryoprecipitate and platelet-rich plasma are worthy of mention. Cryoprecipitate is rich in vWF, factors VIII, XIII, fibrinogen, and fibronectin. It is especially useful in vWF-deficient patients either suffering from blood loss or for whom surgery (such as elective orthopedic surgery) is anticipated. The advantage of cryoprecipitate is that it enables the replacement of one or several coagulation proteins with the administration of a small volume of plasma. Cryoprecipitate is obtained by slowly thawing fresh frozen plasma at 4°C until some crystals remain in suspension. After this gradual thaw, the bag is centrifuged and the supernatant is removed. Cryoprecipitate thus obtained can be frozen at -18°C for up to 1 y. Other indications for using cryoprecipitate are bleeding hemophilia A patients, or patients suffering hypo- or dysfibrinogenemia (46,47).

The use of platelet-rich plasma is occasionally indicated in veterinary practice. The main limitations with platelet-rich plasma therapy are threefold: the difficulty in obtaining a sufficient volume of platelet-rich plasma to be of therapeutic value in a patient; the short platelet lifespan requiring the use of platelet-rich plasma as soon as possible after its preparation (however, platelet concentrates may be stored at 22°C either on an agitator or manually mixed once per 24 (48)); the risk of alloimmunization following a first transfusion, leading to ineffective subsequent transfusions and the possibility of adverse reactions (47). Frozen platelet concentrates are an alternative to platelet-rich plasma and are currently available through one animal blood bank (Midwest Animal Blood Services, Stockbridge, Michigan, USA, www.midwestabs.com/VetInfo/platelet_concentrate.htm). Platelet-rich plasma may be more useful in small-sized animals; for larger dogs, it is difficult to obtain a sufficient volume to boost the platelet count effectively. Platelet concentrates, if available, may increase the platelet count more effectively (49). Spontaneous bleeding may occur with platelet counts below 50×10^9/L to 75×10^9/L, but patients with counts as low as 10×10^9/L may not show signs of spontaneous bleeding (28). Spontaneous bleeding may also occur with platelet dysfunction at higher platelet counts. In immune-mediated thrombocytopenia, transfused platelets will be rapidly destroyed, the transfusion of several units may be required, and the risk of alloantibody formation is increased (47).

Alternatives to blood products for expansion of plasma volume include different types of colloids, such as dextrans and hetastarch. A drawback of dextrans and hetastarch is that they may alter hemostasis (50,51). A hemoglobin-based oxygen carrier (Oxyglobin; Biopure Corporation, Cambridge, Massachusetts, USA), approved for use in the dog in 1998, is useful in that it can be used as a blood substitute in emergency situations when there is no time to prepare blood products or carry out compatibility testing.

Adverse transfusion reactions

The risk of an adverse reaction is minimized under the following conditions: the administered product has been properly collected, processed, and stored; the donors are healthy animals of known blood group type (dogs that are negative for the DEA-1.1, and DEA-1.2); and proper screening tests have been performed (crossmatching). In veterinary medicine, one study determined that up to 13% of dogs developed an adverse reaction, but all animals survived (52). There are 2 types of adverse reaction, an immediate reaction that occurs during or within 1 to 2 h following transfusion or a delayed reaction that may occur days, months, or years later (53). The severity of an adverse reaction varies from mild (fever) to severe (death).

The most serious transfusion reaction that the practitioner can prevent is an acute hemolytic reaction. This is an immunological reaction that takes place when the patient has circulating natural or acquired antibodies towards donor erythrocytic antigens. Clinical signs are due to the intravascular hemolysis that results in hemoglobinuria, vasoconstriction, renal ischemia, and DIC,

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which itself can lead to ischemia in several organ systems and complement activation, resulting in shock. Clinical signs in dogs include fever, tachycardia or bradycardia, hypotension, dyspnea, cyanosis, excessive salivation, tearing, urination, defecation, vomiting, collapse, opisthotonos, cardiac arrest, hemoglobinemia, and hemoglobinuria. The severity of the signs in dogs depends on the volume administered. A clinician presented with an acute hemolytic reaction should interrupt the transfusion immediately and treat for shock, if present. He or she should also verify the blood product being used and retrace the steps that led to the transfusion, including a repetition of the crossmatch procedure (53,54). The acute hemolytic reaction is rare in the dog, due to the low prevalence of naturally occurring anti-erythrocytic antibodies in this species. Non-DEA-7 type dogs may have anti-DEA-7 antibodies, but these antibodies are unlikely to produce a marked hemolytic reaction. The risk is greater in an animal that has previously been transfused, particularly if DEA-1 type blood is administered a second time to a DEA-1-negative dog. In cats, an acute hemolytic reaction is very likely in a type B cat receiving type A blood. The above risks are minimized if DEA-1.1- or DEA-1.2-negative dogs are used and blood is screened for incompatibility, especially in the cat.

Other adverse reactions include nonhemolytic fever in the hour following transfusion; this occurred in approximately 5% of transfusions performed at a veterinary teaching hospital (K.J. Wardrop, unpublished observations). This reaction is most likely associated with circulating antileukocyte antibodies in the recipient. It is important to note that fever may also occur in an acute hemolytic reaction in association with septicemia, if contaminated blood products have been inadvertently administered. Anaphylactic shock (not associated with an acute hemolytic reaction) following transfusions has not been documented in veterinary medicine. Following plasma administration, vomiting or diarrhea may occasionally be observed. Urticaria is not uncommon but seldom poses a danger to the patient and may be treated with antihistamines, with or without glucocorticosteroids. It is probably caused by antibodies against soluble proteins in the blood product administered (plasma). Hypervolemia may result when whole blood is administered to a normovolemic patient; with the rapid administration of a large volume of a blood component; transfusion in cats or small-sized dogs, which, presumably, are more likely to receive an excessive volume of blood; and in patients suffering from cardiovascular, pulmonary, renal, or hepatic compromise. One possible outcome of hypervolemia is pulmonary edema. Clinical signs associated with hypervolemia include cough, tachypnea, dyspnea, or cyanosis. Treatment includes stopping the transfusion, administering diuretics (furosemide) to reduce pulmonary edema, and providing oxygen support (28,54).

Delayed adverse transfusion reactions include delayed hemolytic reaction, transmission of infectious disease, and posttransfusion purpura (55–57). There are possibly lesser known antigens that may provoke antibody production in a patient following transfusion. This may lead to a delayed hemolytic reaction in the days or weeks following a transfusion or may shorten the half-life of transfused RBCs (19). Clinical signs, if present, vary from fever to icterus. An unexplained drop in packed cell volume may be observed (58) and the Coomb's test may become positive. If a delayed hemolytic reaction is suspected, urine output should be monitored, but treatment is usually unnecessary. Crossmatching prior to transfusion may enable detection of naturally occurring anti-DEA-3, -5, or -7 alloantibodies and prevent an associated delayed transfusion reaction. However, crossmatching will not detect an incompatibility for antigens that trigger sensitization in a recipient, since the antibodies are only produced following transfusion. For this reason, the use of DEA-1.1- and DEA-1.2-negative donor dogs as well as cats of the proper blood type, minimizes the risk of such a reaction. Posttransfusion purpura has been reported in the dog, characterized by the appearance of severe thrombocytopenia in the week following a second transfusion (57). Posttransfusion purpura is caused by the production of antiplatelet antibodies in the patient. Spontaneous remission generally resolves this problem within 1 to 6 wk after the thrombocytopenic episode (59).

In conclusion, the medical decision to transfuse and the product selected depend on several factors. Blood products can be obtained in several ways, depending on the particular requirements of a given practice, either through the purchase of blood products as needed, or through in-house or external donors. The risk of potentially fatal adverse transfusion reactions is higher in cats than in dogs. This risk can be minimized in both species by using known donors and screening assays that permit detection of incompatibility, such as blood typing or crossmatching. These assays are easy to perform either as in-house assays or via a request to an external laboratory. CVJ

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Uncovered by a White Coat Waste investigation

Changes to the MU VHC Blood donor program

After running the live-in dog and cat blood donor program in essentially the same way for 20 years, there were major changes as of summer 2022.

We have moved to a volunteered canine donor program under a separate ACUC application/approval. We are not discontinuing the live in ACUC for now just in case change is needed, but for now we do not anticipate using any live-in donor dogs in the program.

We continue to have a live-in donor cat program. However, rather than housing cats in the VHC and providing routine care through our veterinary clinical students and faculty, we will house cats in the Veterinary Medical building animal housing facilities with routine care provided by paid staff and routine veterinary care by the lab animal veterinarians. We will work closely with those faculty and staff to assure great care for these cats. Blood will be collected as always either in the small work room that is located within the animal housing unit, or cats will be transported across the parking lot to Clydesdale Hall for blood collection as has always been done before. Cats would be returned to the Veterinary Medical building housing once they are fully awake. Cats would be transported in carriers.

We have also added students to the protocol who have worked with these cats as part of [redacted] protocol. The only purpose for adding them is so that they can continue to play with the cats that they are attached to, thereby providing environmental enrichment for the cats. They will not collect blood from the cats.

Leah Cohn

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA
[redacted]

Patient 7537924 - Mike
Feline
Domestic Shorthair (DSH)
Male 5.40 kg
Nov 01, 2018

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:19 PM

Local History

History

This young adult cat was acquired from the Jefferson city Animal Shelter. He is apparently healthy and will be used as a blood donor.
There is no further information on his background, but he will undergo rigorous screening to ensure good health before use as a donor.

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA
[redacted]

Patient 7537924 - Mike
Feline
Domestic Shorthair (DSH)
Male 5.40 kg
Nov 01, 2018

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:19 PM

Local Physical

Physical Summary

The OAR does the admitting physical exam and is recorded in their system. There are no abnormalities noted on any physical parameter..

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001973
Reference Number: 2824180
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 6 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Lithium Heparin - Whole Blood

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood			
Sample Condition	CLEAR			
Glucose	104	mg/dL	80-155	01/29/2025 11:09 AM
Urea Nitrogen	20	mg/dL	19-35	01/29/2025 11:09 AM
Creatinine	1.6	mg/dL	0.9-2.0	01/29/2025 11:09 AM
Sodium	154	mEq/L	148-154	01/29/2025 11:09 AM
Potassium	3.3	mEq/L	3.1-4.6	01/29/2025 11:09 AM
Chloride	118	mEq/L	112-123	01/29/2025 11:09 AM
Bicarbonate	22	mEq/L	13-23	01/29/2025 11:09 AM
Anion Gap	17	mEq/L	13-26	01/29/2025 11:09 AM
Albumin	2.9	g/dL	2.8-3.7	01/29/2025 11:09 AM
Total Protein	7.2	g/dL	6.1-8.1	01/29/2025 11:09 AM
Globulin	4.3	g/dL	2.5-5.2	01/29/2025 11:09 AM
Calcium	9.2	mg/dL	8.6-10.4	01/29/2025 11:09 AM
Phosphorus	4.5	mg/dL	2.3-5.0	01/29/2025 11:09 AM
Magnesium	1.9	mg/dL	1.7-2.6	01/29/2025 11:09 AM
Cholesterol	154	mg/dL	80-258	01/29/2025 11:09 AM
Total Bilirubin	0.1	mg/dL	0.1 - 0.2	01/29/2025 11:09 AM
ALT	43	U/L	28 - 84	01/29/2025 11:09 AM
ALP	18	U/L	13 - 50	01/29/2025 11:09 AM
GGT	<3	U/L	0-3	01/29/2025 11:09 AM
CK	1930 H	U/L	65 - 574	01/29/2025 11:09 AM
Resulted by	[redacted]			

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001975
Reference Number: 2824179
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 6 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Urine

Clinical Pathology

Complete Urinalysis

Animal Id	Mike
Specimen Description	Urine
Collection	Cystocentesis
Color	Yellow
Clarity	Cloudy
Urine Volume	5.0 mL
Specific Gravity	1.061
Glucose	Negative
Bilirubin	Negative
Ketones	Trace
Heme	Negative
pH	7.0
Protein - Dipstick	2+
Urobilinogen	0.2 E.U./dL
WBC/hpf	Rare
RBC/hpf	Rare
Bacteria/hpf	None Seen
Transitional Cells/lpf	None Seen
Squamous Cells/lpf	None Seen
Casts/lpf	None Seen
Crystals/lpf	Few Struvite
Testing Completed On	January 29, 2025
Resulted by	[redacted]

Uncovered by a White Coat Waste investigation

This report supersedes a preliminary report issued for this case on 1/29/2025 at 12:40 PM

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001974
Reference Number: 2824178
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 6 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Edta - Whole Blood

Clinical Pathology

CBC, Small Animal

Specimen Description	Edta - Whole Blood					
WBC		8.15		x10^3/uL	4.25 - 18.32	01/29/2025 10:50 AM
RBC		8.45		x10^6/uL	5.91 - 10.69	01/29/2025 10:50 AM
HGB		12.5		g/dL	9.2 - 16.1	01/29/2025 10:50 AM
HCT		35.4		%	28.9 - 44.8	01/29/2025 10:50 AM
MCV		42.0		fL	36.3 - 49.1	01/29/2025 10:50 AM
MCH		14.8		pg	12.8 - 17.3	01/29/2025 10:50 AM
MCHC		35.2		g/dL	34.1 - 38.8	01/29/2025 10:50 AM
RDW		16.1		%	13.7 - 17.3	01/29/2025 10:50 AM
Abs Reticulocyte		24.0		x10^3 /uL	6.4 - 58.9	01/29/2025 10:50 AM
Platelet Count		310		x10^3/uL	127 - 450	01/29/2025 10:50 AM
MPV		11.6		fL	10.1 - 24.7	01/29/2025 10:50 AM
PCT		0.36		%	0.10 - 0.52	01/29/2025 10:50 AM
Segmented Neutrophils	(61%)	4.97		x10^3 /uL	2.50 - 13.92	01/29/2025 10:50 AM
Band Neutrophils	(2%)	0.16	H	x10^3 /uL	0.00 - 0.07	01/29/2025 10:50 AM
Lymphocytes	(30%)	2.45		x10^3/uL	1.07 - 6.02	01/29/2025 10:50 AM
Monocytes	(2%)	0.16		x10^3 /uL	0.00 - 0.67	01/29/2025 10:50 AM
Eosinophils	(5%)	0.41		x10^3/uL	0.10 - 1.36	01/29/2025 10:50 AM
Basophils	(0%)	0.00		x10^3/uL	0.00 - 0.08	01/29/2025 10:50 AM
Other	(0%)	0.00		x10^3 /uL	No Ref Interval	01/29/2025 10:50 AM
NRBC/100 WBC		0.0			0.0 - 1.0	01/29/2025 10:50 AM
Absolute nRBC		0.00		x10^3/uL	No Ref Interval	01/29/2025 10:50 AM
Morphology	NORMAL RBCS					
Resulted by	[redacted]					

Uncovered by a White Coat Waste investigation

IDEXX Reference Laboratories
Division of IDEXX Laboratories
www.idexx.com

One IDEXX Drive
Westbrook, Maine 04092
United States

IDEXX Reference Laboratories
Customer Support
888 433 9987

IDEXX REFERENCE LABORATORY TEST REPORT

MIKE UMC BLOOD DONOR SA

PET OWNER:	UMC BLOOD DONOR SA
PATIENT ID:	7537924
SPECIES:	FELINE
BREED:	SHORTHAIR, DOMESTIC
GENDER:	MALE NEUTERED
AGE:	5Y6M14D
MICROCHIP #:	

UMC VET CLIN PATH LAB
[redacted]

ACCOUNT #:	76576
ORDERED BY:	LEAH COHN

ACCESSION #	4611694601
REQUISITION #:	235879475
DATE OF COLLECTION:	05/10/2024
DATE OF RECEIPT:	05/14/2024
DATE OF REPORT:	05/15/2024

IDEXX SERVICES: **26354 FELV QUANT REALPCR™ TEST**

MOLECULAR DIAGNOSTICS

TEST	RESULT	UNITS
FeLV Quant RealPCR [a]	NEGATIVE	

NOTES

MOLECULAR DIAGNOSTICS

a A NEGATIVE FeLV PCR result indicates that FeLV proviral DNA was not detected in the sample submitted. If FeLV antigen by ELISA test result is also negative, FeLV infection is very unlikely. Occasionally, the FeLV antigen test by ELISA may be positive in a cat with a negative FeLV PCR result. This combination is most consistent with a regressive or focal FeLV infection. Less commonly, the proviral DNA in the sample may be below the limit of detection, the sample may contain an uncommon strain that is not detected by PCR or the antigen test was falsely positive. Learn more at www.idexx.com/FeLVQuant.

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011539
Reference Number: 2735270
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 5 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Urine

Clinical Pathology

Complete Urinalysis

Animal Id	Mike
Specimen Description	Urine
Collection	Cystocentesis
Color	Yellow
Clarity	Slightly Cloudy
Urine Volume	5.0 mL
Specific Gravity	1.073
Glucose	Negative
Bilirubin	Negative
Ketones	Negative
Heme	Negative
pH	6.5
Protein - Dipstick	1+
Urobilinogen	0.2 E.U./dL
WBC/hpf	Rare
RBC/hpf	None Seen
Bacteria/hpf	None Seen
Transitional Cells/lpf	None Seen
Squamous Cells/lpf	Rare
Casts/lpf	None Seen
Crystals/lpf	None Seen
Testing Completed On	May 9, 2024
Resulted by	[redacted]

Uncovered by a White Coat Waste investigation

This report supersedes any previous reports issued for this case prior to 12/02/2025 at 12:40 PM

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011540
Reference Number: 2735269
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 5 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Lithium Heparin - Whole Blood

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood			
Sample Condition	CLEAR			
Glucose	108	mg/dL	80-155	05/09/2024 02:22 PM
Urea Nitrogen	23	mg/dL	19-35	05/09/2024 02:22 PM
Creatinine	1.1	mg/dL	0.9-2.0	05/09/2024 02:22 PM
Sodium	154	mEq/L	148-154	05/09/2024 02:22 PM
Potassium	3.9	mEq/L	3.1-4.6	05/09/2024 02:22 PM
Chloride	119	mEq/L	112-123	05/09/2024 02:22 PM
Bicarbonate	22	mEq/L	13-23	05/09/2024 02:22 PM
Anion Gap	17	mEq/L	13-26	05/09/2024 02:22 PM
Albumin	3.6	g/dL	2.8-3.7	05/09/2024 02:22 PM
Total Protein	7.3	g/dL	6.1-8.1	05/09/2024 02:22 PM
Globulin	3.7	g/dL	2.5-5.2	05/09/2024 02:22 PM
Calcium	9.6	mg/dL	8.6-10.4	05/09/2024 02:22 PM
Phosphorus	3.9	mg/dL	2.3-5.0	05/09/2024 02:22 PM
Magnesium	1.9	mg/dL	1.7-2.6	05/09/2024 02:22 PM
Cholesterol	171	mg/dL	80-258	05/09/2024 02:22 PM
Total Bilirubin	0.1	mg/dL	0.1 - 0.2	05/09/2024 02:22 PM
ALT	37	U/L	28 - 84	05/09/2024 02:22 PM
ALP	20	U/L	13 - 50	05/09/2024 02:22 PM
GGT	<3	U/L	0-3	05/09/2024 02:22 PM
CK	391	U/L	65 - 574	05/09/2024 02:22 PM
Resulted by	[redacted]			

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone 573-882-6811
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011541
Reference Number: 2735268
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 5 Y
Animal ID: Mike
Case Ref #: 7537924
Specimen: Edta - Whole Blood

Clinical Pathology

CBC, Small Animal

Specimen Description		Edta - Whole Blood				
WBC		9.14		x10^3/uL	4.25 - 18.32	05/09/2024 02:47 PM
RBC		7.76		x10^6/uL	5.91 - 10.69	05/09/2024 02:47 PM
HGB		12.5		g/dL	9.2 - 16.1	05/09/2024 02:47 PM
HCT		36.0		%	28.9 - 44.8	05/09/2024 02:47 PM
MCV		46.4		fL	36.3 - 49.1	05/09/2024 02:47 PM
MCH		16.1		pg	12.8 - 17.3	05/09/2024 02:47 PM
MCHC		34.7		g/dL	34.1 - 38.8	05/09/2024 02:47 PM
RDW		14.7		%	13.7 - 17.3	05/09/2024 02:47 PM
Abs Reticulocyte		13.0		x10^3 /uL	6.4 - 58.9	05/09/2024 02:47 PM
Platelet Count		385		x10^3/uL	127 - 450	05/09/2024 02:47 PM
MPV		11.0		fL	10.1 - 24.7	05/09/2024 02:47 PM
PCT		0.42		%	0.10 - 0.52	05/09/2024 02:47 PM
Segmented Neutrophils	(64%)	5.85		x10^3 /uL	2.50 - 13.92	05/09/2024 02:47 PM
Band Neutrophils	(0%)	0.00		x10^3 /uL	0.00 - 0.07	05/09/2024 02:47 PM
Lymphocytes	(22%)	2.01		x10^3/uL	1.07 - 6.02	05/09/2024 02:47 PM
Monocytes	(6%)	0.55		x10^3 /uL	0.00 - 0.67	05/09/2024 02:47 PM
Eosinophils	(7%)	0.64		x10^3/uL	0.10 - 1.36	05/09/2024 02:47 PM
Basophils	(1%)	0.09	H	x10^3/uL	0.00 - 0.08	05/09/2024 02:47 PM
Other	(0%)	0.00		x10^3 /uL	No Ref Interval	05/09/2024 02:47 PM
NRBC/100 WBC		0.0			0.0 - 1.0	05/09/2024 02:47 PM
Absolute nRBC		0.00		x10^3/uL	No Ref Interval	05/09/2024 02:47 PM
Morphology		FEW REACTIVE LYMPHOCYTES, NORMAL RBCS				
Resulted by		[redacted]				

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client	Patient	DVM
86803 - UMC BLOOD DONOR SA	7537924 - Mike Feline Domestic Shorthair (DSH) Male Castrated 5.40 kg Nov 01, 2018	

[redacted]

Episode: 1098080
Date/Time: 5/9/24, 12:19 PM

Reason for Visit: Blood Donor

Lab - VHC:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood under a ketamine 0.4ml and Midazolam 0.4ml sedation. 60 mls of blood were obtained via the right jugular. Mike was recovered in ICU and returned to the ward. the next donation is no sooner than December 5th
PCV= 34%
TP= 7.2
Brief PE prior to sedation unrearkable.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew 60 mls of blood out of the right jugular under Ketamine 0.4ml and midazolam 0.4ml both IV Recovered uneventfully under observation and returned to OAR PCV=43 TP= 7.3
Brief PE prior to sedation unremarkable.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood under a ketamine and Midazolam sedation. 60 mls of blood were obtained via the left jugular after an attempt at the right. Mike was recovered in ICU and returned to the ward. the next donation is no sooner than september 23rd
PCV= 34%
TP= 7.3

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Uncovered by a White Coat Waste investigation

Drew blood under Ketamine and Midazolam sedation0.4 ml each. 60 mls of blood was obtained via the right jugular and recovered in ICU
PCV= 38%
TP=6.8

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood under a ketamine and Midazolam sedation. 60 mls of blood were obtained via the left jugular after an attempt at the right. Mike was recovered in ICU and returned to the ward. the next donation is no sooner than May 27th 25
PCV= 32%
TP= 7.2

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under Ketamine 0.4 ml and Midazolam 0.4 ml. 60 ml was removed for immediate use for ICU patient (8292325). he was recovered in ICU. His PCV=55% and TP= 7.5 his next donation will be no sooner than April 22, 25

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under a ketamine / Midazolam sedation 0.4 ml of each were given IV. eyes were lubed and he was recovered in endo in his carrier and returned to OAR A CBC, Maxi and UA were done for his annual wellness. PCV= 30% TP= 7.4

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donation on Jan. 29th disregard

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Sedated with ketamine and Midazolam .04ml each Iv Removed 60 mls blood via right jug and recovered in ICU. PCV= 40% and TP= 7.0
TPR was good

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donation on Sept. 26, 2024 drew 60 via the right jug under ketamine and midazolam 0.4 ml each
TPR=normal and recovered in ICU PCV=40% and TP=7.0

Comments:

Uncovered by a White Coat Waste investigation

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood under a Ketamine / midazolam 0.4 ml each IV. 60 mls of blood was removed via the right jugular a little pressure was applied for a few seconds and he was recovered in the ICU, to be returned to the ward the following AM. His TPR is recorded in TPR section.

Comments:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES Fe / BREED DSH

COLOR Tabby / White SEX F/N AGE 3

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC Blood donor

PRE-OP. DIAGNOSIS Presumed healthy Donor

PROPOSED PROCEDURE Blood donation.

PRIMARY CLINICIAN(S) COAN. ANESTHESIOLOGIST

PRIMARY STUDENT STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
9/26/24			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.9 kg	102.3	180	24	Pk /				
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine	0.4ml		IV.	
Midazolam	0.4mg		IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P Syst v
Diast ^
Mean —
Pulse ·
Resp ○
Ventil o—V—o
Assist o—A—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT

Temp.

$ET CO_2$

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E	T P	P.C.V

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

7.5 ml. midazolam wasted

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE [redacted]

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE [redacted]

Uncovered by a White Coat Waste Investigation

ANESTHESIA MEDICAL RECORD

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924
SPECIES feline BREED DSH
COLOR Tabby & White Sex Male Age PLACE LABEL HERE
NAME OR IDENTIFICATION Mike.
OWNER'S NAME UMC

PRE-OP. DIAGNOSIS Presumed healthy.
PROPOSED PROCEDURE Blood donation.
PRIMARY CLINICIAN(S) COAN. ANESTHESIOLOGIST
PRIMARY STUDENT STUDENT ANESTHETIST MARGARET

DATE 8-1-24 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.81 kg.	102.8	160	28	PK	<1			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
	Normal.							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 mL.	IV.	
Midazolam		0.4 mL	I.V.	

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____ mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P Syst v
Diast ^
Mean —
Pulse •
Resp ○
Ventil o—v—o
Assist o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

0.61 mL.
Midazolam waste
X [signature]

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical

Time	Temp	MM
HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P.C.V

TOTAL FLUIDS (mls)
Est Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE [redacted]
ANESTHESIOLOGIST SIGNATURE [redacted]

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste Investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924
SPECIES Fe BREED DSH
COLOR tabby/wht Sex M/N Age 5
NAME OR IDENTIFICATION Mike.
OWNER'S NAME UMC

PRE-OP. DIAGNOSIS Presumed healthy
PROPOSED PROCEDURE Bloodcconation.
PRIMARY CLINICIAN(S) CORN
ANESTHESIOLOGIST
PRIMARY STUDENT
STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
12/2/24			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.55	101.5	210	62	PA	<2			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE
						PHYSICAL STATUS 1 2 3 4 5 E		

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4ml.	IV.	9:55 AM
Ketamine		0.4ml.	IV	

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P Syst v
Diast ^
Mean
Pulse
Resp O
Ventil. o—v—o
Assist o—s—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

Wasted Midazolam 1.00 ml.
x Janett Stphs

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P C V

TOTAL FLUIDS (mls)
Est Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE [redacted]

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste Investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES feline BREED DSH

COLOR Brown tabby Sex Age

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS

PROPOSED PROCEDURE Blood donation

PRIMARY CLINICIAN(S) [redacted] ANESTHESIOLOGIST

PRIMARY STUDENT STUDENT ANESTHETIST

DATE 1/22/25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.5 kg	101.5	220	72					
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE
						PHYSICAL STATUS 1 2 3 4 5 E		

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml	IV	6:20p
Midazolam		0.4ml	IV	6:20p

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P. Syst v
Diast ^
Mean
Pulse
Resp O
Ventil
Assist

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp.
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

Wasted
1/22 @ 7:15pm
0.4 ml Midazolam
0.2 ml Midolam + Ketamine
0.6 ml Midazolam + Ketamine

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P.C.V

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE [redacted]

ANESTHESIOLOGIST SIGNATURE

Uncovered by a White Coat Waste Investigation

ANESTHESIA MEDICAL RECORD

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES Fe BREED DSH

COLOR Blk/wht Sex M/N Age 3

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC Blood donor.

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Good donation.

PRIMARY CLINICIAN(S): Colin

ANESTHESIOLOGIST: [redacted]

PRIMARY STUDENT

STUDENT ANESTHETIST

DATE 4-29-25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.95kg	101.1	200	64					
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam	0.4mL		IV.	
Ketamine	0.4mL		IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean =

Pulse ·

Resp. ○

Ventil. o—v—o

Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

0.75mL.

X [redacted]

Wasted Midazolam.

CONTROLLED DRUG WASTE:

RECOVERY: [redacted]

Post. Anesth. Physical | HR | RR | CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES fel. BREED DSH

COLOR tabby Sex M/N Age _____

NAME OR IDENTIFICATION Mike

OWNER'S NAME umc blood donor

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
Colin	
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
1-29-25			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.44	101.7	200	60	Pk	<2			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	END ANESTHESIA	END PROCEDURE
30%	7.4							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Bcf				
Ketamine	0.4	mL	IV	
Midazolam	0.4	mL	IV	

NC CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ ○
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean ~
Pulse •
Resp. ○
Ventil. o—v—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES Fel. BREED DSH

COLOR Tabby/wht. Sex M/N Age 3

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC Blood Donor

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood Donation.

PRIMARY CLINICIAN(S) | ANESTHESIOLOGIST

PRIMAR[redacted] | STUDENT ANESTHETIST

DATE 3/24/25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.24 kg.	102.1	204	52	Pk	2>			
PCV 55%	TP 7.5	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine	0.4mg.		IV	WDH
Midazolam	0.4mg.		IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse

Resp. ○

Ventil. ○—v—○

Assist. ○—a—○

COMMENTS/EVENTS:

MDOW

0.4 ml

0.7 ml. IV Wasted

Midazolam

[redacted]

CONTROLLED DRUG WASTE:

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

RECOVERY:

Post. Anesth. Physical | Time | Temp. | MM | HR | RR

ANESTHETIST SIGN [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537924

SPECIES Feline BREED DSH

COLOR Tabby / Wht Sex MN Age 3

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC.

PRE-OP. DIAGNOSIS: Presumed Healthy

PROPOSED PROCEDURE: Blood donation.

PRIMARY CLINICIAN(S): COHN.

ANESTHESIOLOGIST

PRIMARY STUDENT

STUDENT ANESTHETIST

DATE 6-27-25

CAGE/STALL#

FASTED YES NO

PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.75kg	102.1	144	60	PP / M	<2s			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	END ANESTHESIA	END PROCEDURE
						PHYSICAL STATUS 1 2 3 4 5 E		

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine	0.4	mL	IV	
Midazolam	0.4	mL	IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse ·

Resp. ○

Ventil. v

Assist. a

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

COMMENTS/EVENTS:

Midazolam Waste
0.1 mL

Hannah Sisco

CONTROLLED DRUG WASTE:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

RECOVERY:

Post. Anesth. Physical

Time	Temp.	MM
HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE:

[redacted]

ANESTHESIOLOGIST SIGNATURE:

[redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 753792A

SPECIES Fel. BREED DSH

COLOR Brindle & Wht Sex MN Age 3

NAME OR IDENTIFICATION Miko

OWNER'S NAME UMC Blood Donor

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S) HN | ANESTHESIOLOGIST

PRIMARY STUDENT [redacted] | STUDENT ANESTHETIST

DATE 11/5/25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.4kg	100.9	192	92	PR	<2			
PCV 34%	TP 7.2	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE | PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4ml	IV	11:07 AM
Ketamine		0.4ml	IV	11:10 AM

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ·
Resp. ○
Ventil. ○—Y—○
Assist. ○—B—○

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT
Temp.
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE: | EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

wasted
0.13 ml.
midazolam
x 1

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical | Time | Temp. | MM | HR | RR | CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

ANESTHESIA MEDICAL RECORD

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER _____

SPECIES Fel. BREED DSH

COLOR Tabby & Wht Sex _____ Age _____

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC Blood donor

PRE-OP. DIAGNOSIS: Presumed Healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
CDHN	
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
Oct. 6, 25			

WT	TEMP	HR	RR	MM	CR	SG	START ANESTHESIA	START PROCEDURE
5.54kg	101.6	180	48	< 2	Pr.			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
43	7.3							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam	0.4 mL		IV	
Ketamine	0.4 mL		IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: _____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: _____ O
O_2 flow Δ
L/min _____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ·
Resp. ○
Ventil. o—v—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

[redacted]

0.8mls midaz mel

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE:

MEM COLOR/CRT

Temp.

$ET CO_2$

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Eql BREED DSH

COLOR tabby/wht Sex MN Age

NAME OR IDENTIFICATION Mike

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS:

PROPOSED PROCEDURE:

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED	PERMIT
8-26-25		YES NO	YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.6 kg	101.0	140	60	pink	<2			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE
34	7.3							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4 mL	IV	8:50
Ketamine		0.4 mL	IV	8:54

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean

Pulse •

Resp. ○

Ventil. o—x—o

Assist. o—a—o

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

0.75 mL
wasted midazolam
x 1 vial PIV

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE: [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Patient Name: Mike (Mike) Case#: 7537924 7531967 Drug: Ketamine

Controlled Substance Charge Merge Request

If a controlled substance multi-dose vial is short and does not accurately contain enough volume in Omnicell, follow the below steps to obtain the remaining volume. The complete dose can be drawn up by draining the vial, zeroing out the bin, and logging a second transaction from a new vial.

1. Withdraw any remaining liquid from the vial. Leave the bin open.
2. Target (original) volume requested (in mL): 0.4 mL. (Target Volume)
3. Actual volume withdrawn from vial (in mL): 0.22 mL. (Bin Volume)
4. Tap the "Change Bin Level" button in the top right corner of the Omnicell display.
5. Enter "0" in the "quantity remaining" field. Omnicell will now incorrectly believe you have withdrawn your total needed volume, but the bin will be zeroed out when closed.
6. Note what remaining volume of drug you need for your targeted dose:
   Target volume 0.4 (Line 2) – actual amount in vial 0.22 (Line 3) = ~~0.18~~ 0.18 (Amount Short)
7. Create a second transaction for the volume calculated in line 6. Omnicell may alert you that this item has already been dispensed but should direct you to a new bin.

This will result in two charges being placed on the patient's bill. Submit completed form to pharmacy so that the charges can be corrected. This form will then be uploaded to the patient's chart for tracking and auditing purposes.

User Name (Print): [redacted] Date: 6-27-25

Signature: [redacted]

Below for Pharmacy Use Only

Pharmacy approver [redacted] Episode ID: 1094082-9

12/14/2023 MRP

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Immunization

Client	Patient	
UMC BLOOD DONOR SA [redacted]	Mike Domestic Shorthair (DSH) Male Castrated Feline	Case# 7537924 5.40 kg

Uncovered by a White Coat Waste investigation

Prescriptions

Client	Patient	
UMC BLOOD DONOR SA	Mike	Case# 7537924
[redacted]	Domestic Shorthair (DSH)	
	Male Castrated	5.40 kg
	Feline	

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Visit Date:5/9/24
Owner: UMC BLOOD DONOR SA
[redacted]

Client #:86803

Patient: Mike
Species: Feline
Breed: American Domestic Shorthair
Color: Brown Tabby/White

Patient #:7537924
DOB:11/01/2018
Sex:Male Castrated

RDVM:

RDVM #:

I have reviewed and verified the referring veterinarian information above. _____ Please initial

Is this your primary veterinarian? YES NO (circle one)

Did your veterinarian recommend that you come to the VHC for this appointment? YES NO (circle

AUTHORIZATION RELEASE
It is our policy to send information about your visit to your veterinarian. As owner or agent of the above named animal, I authorize release of information concerning this visit to the veterinarian(s) of my choice. Do we have your permission to send this information? YES NO (circle one) _____ Please initial

STATEMENT OF OWNERSHIP AND CONSENT
I am the owner/agent of the above-described animal and have authority to consent to its treatment. I assume full financial responsibility for all charges rendered for diagnostic, therapeutic, surgical, preventive procedures and infectious disease surveillance and control.

Owner's Signature _____

OUR PAYMENT POLICY
The Veterinary Health Center operates on a fee-for-service basis. Payment in full is expected at the time of discharge or dismissal. For animals admitted to the hospital, we require a deposit equal to one-half of the high end of the estimate range. The remainder of the balance should be paid upon discharge. All other payment arrangements must be made in advance. Please initial the expected method of payment.
Cash____ Check____ Credit Card____ Debit Card_____

I have read and agree to comply with the payment policy as stated above. _____ Please

Please indicate if your animal has any of the following conditions. (circle one)

Medication reaction Yes No Do not know
List drug and reaction if possible_____
Up to date on rabies vaccination Yes No Do not know

Does your pet have Pet Health Insurance?_____If yes, name of insurance co_____
By indicating yes to your pet having Pet Health Insurance, you have authorized release of your pet's VHC medical record to a PHI Company if requested.

Uncovered by a White Coat Waste investigation

No. 44521

Jefferson City Animal Shelter
2308 Hyde Park Rd.
Jefferson City, MO
573-634-6429

Adoption/Redemption Form

Owner Information

Name University of Missouri. VHC

Address [redacted]

City Columbia State MO Zip 65211

Telephone: Cell [redacted] Home

Email [redacted]

Animal Information

Animal unique ID 26628 NNN Microchip 608367358

Fees Due

Adoption $ placement Boarding $ Rabies $ Donation $ Total $ placement

Payment Method: ☐ Cash ☐ Credit/Debit Card ☐ Check # ____ Intent

Rabies

☒ Vaccination Current ☐ Vaccination Not Current ☐ Too Young for Vaccination

If my animal is not current on its rabies vaccination, I do hereby agree to obtain a rabies vaccine from a licensed veterinarian prior to the expiration date listed below, to be in compliance with Sections 5-22 & 5-45 of the Code of the City of Jefferson. Yearly re-vaccination for rabies is required by City Ordinance.

Voucher Expiration Date N/A *Pink copy, if provided to the owner is good for.*

☐ $ N/A *towards the cost of a rabies vaccination by a licensed veterinarian at a participating clinic for owner redeemed pets*

☐ *A no cost rabies vaccination by the Jefferson City Animal Shelter for an adopted pet that was too young at the time of adoption for its initial rabies vaccination included in the adoption fee*

Acknowledgment

I understand that the shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the time of adoption, which the adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

[redacted] 5/9/24

Signature of Owner Adoption/Redemption Date

Signature of Shelter Staff

Distribution: WHITE – Shelter copy YELLOW – Owner copy PINK – Rabies voucher

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Animal Profile

Pet Name	**MIKE**
Species	**CAT ID#: 26628**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Age	**5-6 YEARS**
Gender	**M/N/STERILIZED**
Color	**BB TABBY / WHITE**
Size	**LARGE**



Case Information

Case ID	**04-24-2024 4**	Status	**ADOPTION AVAILABLE**
Shelter ID/Kennel	**NNN**		
Arrival Date	**04-24-2024**	Collar Type	**NO COLLAR**
Arrival Reason	**UNWANTED**	Collar Color	
Transport	**OWNER TURN-IN**	License	
ACO		Vacc ID	**15476**
Precinct	**PRECINCT 1**	Microchip	**608367358**

Notes

Name: Mike
Age: 5-6 Years
Breed: Domestic Short Hair
Gender: Neutered Male
Good with Dogs: Friendly No Fighting
Good with Cats: Did not really care avoided each other
Good with Children: Has not been around kids
Current weight:
FeLv/FIV test: Neg

Adoption Fee: $50

Misc. Information: Mike was surrendered to the shelter due to him urinating outside of the litter box. It's possible that Mike's behavior could be influenced by the fact that there were three other cats in the home sharing only two litter boxes. This could have led to discomfort, causing him to seek alternative places to urinate. Otherwise, Mike is described as a very sweet and loving cat. However, he does not like to be picked up, so it's essential to keep this in mind when interacting with him. Mike is also quite shy and scared in the shelter environment, as his previous owner did mention that he is a shy cat. He is fearful and not a fan of loud noises such as vacuums and thunder, so it may be best for him to go to a calmer household with older children rather than younger ones if any at all.

The shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the of adoption, which adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

ALL adoptions are final! Animals can be returned to the shelter for any reason, however, there are NO refunds

Animals altered here are tattooed with a green abdominal line.

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Animal Profile
Case Information
Behavioral Assessment
Notes
or exchanges.

Animals altered here are tattooed with a green abdominal line.

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

ANIMAL MEDICAL HISTORY

University Of Mo Uhc
[redacted]

Species	**CAT ID#: 26628**
Pet Name	**MIKE**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Gender	**M/N/STERILIZED**
License	
Vacc ID	**15476**
Microchip	**608367358**
Age	**5-6 YEARS**
Size	**LARGE**

04-24-2024 - Medical Visit - these items were performed

Date	Category	Detailed Description	Status/Result
04-24-2024	Medical visit	Date:04-24-2024	Closed:05-09-2024
Note:	14lbs 4/25		
04-24-2024	Vitals	Weight=14.0,Temp=?	
04-24-2024	Vaccination	FVRCP 3W	
04-25-2024	Tested	FELV/FIV	NEGATIVE
04-25-2024	Vaccination	RABIES	
04-25-2024	Medication	STRONGID3W	
04-25-2024	Vaccination	REVOLUTION	
04-25-2024	Service	VETERINARIAN EXAM	
04-25-2024	Service	MICROCHIP IMPLANT	

Follow-ups - these items were scheduled and should be completed on the due date

Due Date	Category	Detailed Description
05-15-2024	Vaccination	FVRCP 3W
05-25-2024	Vaccination	REVOLUTION
04-25-2025	Vaccination	RABIES

End of items for this medical visit.

Animals altered here are tattooed with a green abdominal line.

Printed using Shelter Pro

Uncovered by a White Coat Waste Investigation

Jefferson City Animal Shelter

2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Vaccination Certificate

University Of Mo Uhc
[redacted]

Vaccination Information

ID	**15476**
Type	**RABIES**
Effective	**04-25-2024 TO 04-24-2025**
Precinct	**PRECINCT 1**
Issued by	**SCHALLER, TRAVIS**
Manufacturer	**ZOETIS DEFENSOR 1**
Lot / Serial	**690759**

About Mike

Species	**CAT ID#: 26628**
Breed	**DOMESTIC SHORT HAIR**
Gender	**M/N / STERILIZED**
Color	**BB TABBY / WHITE**
Microchip	**608367358**

Rabies vaccine admnistered by:
Travis Schaller, DVM License No. 2021018110
Greg Popp, DVM. License No. 03247

T J Schaller DVM

Animals altered here are tattooed with a green abdominal line.
Printed using ShelterPro

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Microchip Certificate

University Of Mo Uhc
[redacted]

Microchip Information

ID	**608367358**
Type	**AVID**
Effective	**04-25-2024**

About Mike

Species	**CAT ID#: 26628**
Breed	**DOMESTIC SHORT HAIR**
Gender	**M/N / STERILIZED**
Color	**BB TABBY / WHITE**

Microchip **608367358**

Animals altered here are tattooed with a green abdominal line.
Printed using ShelterPro

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA
[redacted]

Patient 7537724 - Milo
Feline
Domestic Shorthair (DSH)
Male 5.40 kg
Nov 01, 2021

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:14 PM

Local History

History

This young adult cat was acquired from the Jefferson City Animal shelter. His records from the shelter are attached to his medical record as a separate document. He appears healthy and passed preliminary health assessment at acquisition. He will undergo thorough health screening before use as a blood donor.

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA [redacted]

Patient 7537724 - Milo
Feline
Domestic Shorthair (DSH)
Male 5.40 kg
Nov 01, 2021

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:14 PM

Default Physical

Hydration	Normal	**Comments:**
Mm Color	Normal	**Comments:**
Capillary Refill Time	Normal	**Comments:**
Body Condition	Normal	**Comments:**
General Attitude	Normal	**Comments:**
Ocular (Pupillary Reflexes, Adnexa, Globe, Fundus)	Normal	**Comments:**
Head And Neck	Normal	**Comments:**
Ears (Pinna, Canal)	Normal	**Comments:**
Nose (Nares, Discharge)	Normal	**Comments:**
Teeth	Normal	**Comments:**
Tonsils	Not Evaluated	**Comments:**
Oral Mucosa, Tongue	Normal	**Comments:**
Skin & Subcu	Normal	**Comments:**
Musculoskeletal (Lameness/Limbs/Joints)	Normal	**Comments:**

Uncovered by a White Coat Waste investigation

Cardiovascular (Pulses/Veins/Membranes;Rhythm/Sound	Normal	no murmur
	Comments:	
Respiratory(Thorax/Sounds/Trachea/Nares/Pattern)	Normal	
	Comments:	
Abdomen (Spleen, Liver, Intestine)	Normal	
	Comments:	
Rectal Exam (Rectum, Stool, Anal Sacs)	Not Evaluated	
	Comments:	
Urinary (Kidneys, Bladder, Genitalia)	Normal	
	Comments:	
Reproduction (Mammary Glands, Prostate)	Normal	
	Comments:	
Nervous (Cranial Nerves, Gait, Posture, Reflexes)	Normal	
	Comments:	normal mentation, gait, CN
Mandibular Lymph Nodes	Normal	
	Comments:	
Prescapular Lymph Nodes	Normal	
	Comments:	
Axillary Lymph Nodes	Normal	
	Comments:	not palpable
Popliteal Lymph Nodes	Normal	
	Comments:	
Inguinal Lymph Nodes	Normal	
	Comments:	not palpable
Pain Assessment	Normal	
	Comments:	
Where Is Pain Located?	Normal	
	Comments:	no pain

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-003216
Reference Number: 2786000
Received: Feb 13, 2025
Finalized: Feb 13, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Milo
Case Ref #: 7537724
Specimen: Urine
Sampled: Sep 30, 2024

Clinical Pathology

Complete Urinalysis

Animal Id	Milo
Specimen Description	Urine
Collection	Cystocentesis
Color	Orange
Clarity	Cloudy
Urine Volume	4.0 mL
Specific Gravity	1.064
Glucose	Negative
Bilirubin	Negative
Ketones	Trace
Heme	3+
pH	8.5
Protein - Dipstick	3+
Urobilinogen	0.2 E.U./dL
WBC/hpf	Rare
RBC/hpf	> 40
Bacteria/hpf	None Seen
Transitional Cells/lpf	None Seen
Squamous Cells/lpf	None Seen
Casts/lpf	None Seen
Crystals/lpf	Few Struvite
Testing Completed On	February 13, 2025
Resulted by	[redacted]

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-003217
Reference Number: 2785999
Received: Feb 13, 2025
Finalized: Feb 13, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Milo
Case Ref #: 7537724
Specimen: Lithium Heparin - Whole Blood
Sampled: Sep 30, 2024

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood				
Sample Condition	CLEAR				
Glucose	106		mg/dL	80-155	02/13/2025 03:41 PM
Urea Nitrogen	22		mg/dL	19-35	02/13/2025 03:41 PM
Creatinine	1.6		mg/dL	0.9-2.0	02/13/2025 03:41 PM
Sodium	150		mEq/L	148-154	02/13/2025 03:41 PM
Potassium	3.2		mEq/L	3.1-4.6	02/13/2025 03:41 PM
Chloride	117		mEq/L	112-123	02/13/2025 03:41 PM
Bicarbonate	18		mEq/L	13-23	02/13/2025 03:41 PM
Anion Gap	18		mEq/L	13-26	02/13/2025 03:41 PM
Albumin	3.1		g/dL	2.8-3.7	02/13/2025 03:41 PM
Total Protein	6.6		g/dL	6.1-8.1	02/13/2025 03:41 PM
Globulin	3.5		g/dL	2.5-5.2	02/13/2025 03:41 PM
Calcium	10.2		mg/dL	8.6-10.4	02/13/2025 03:41 PM
Phosphorus	4.0		mg/dL	2.3-5.0	02/13/2025 03:41 PM
Magnesium	1.9		mg/dL	1.7-2.6	02/13/2025 03:41 PM
Cholesterol	177		mg/dL	80-258	02/13/2025 03:41 PM
Total Bilirubin	0.1		mg/dL	0.1 - 0.2	02/13/2025 03:41 PM
ALT	38		U/L	28 - 84	02/13/2025 03:41 PM
ALP	21		U/L	13 - 50	02/13/2025 03:41 PM
GGT	<3		U/L	0-3	02/13/2025 03:41 PM
CK	63	L	U/L	65 - 574	02/13/2025 03:41 PM
Resulted by	[redacted]				

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-003218
Reference Number: 2785998
Received: Feb 13, 2025
Finalized: Feb 13, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Milo
Case Ref #: 7537724
Specimen: Edta - Whole Blood
Sampled: Sep 30, 2024

Clinical Pathology

CBC, Small Animal

Specimen Description	Edta - Whole Blood				
WBC		10.16	x10^3/uL	4.25 - 18.32	02/13/2025 03:46 PM
RBC		8.69	x10^6/uL	5.91 - 10.69	02/13/2025 03:46 PM
HGB		13.4	g/dL	9.2 - 16.1	02/13/2025 03:46 PM
HCT		40.5	%	28.9 - 44.8	02/13/2025 03:46 PM
MCV		46.6	fL	36.3 - 49.1	02/13/2025 03:46 PM
MCH		15.4	pg	12.8 - 17.3	02/13/2025 03:46 PM
MCHC		33.0 L	g/dL	34.1 - 38.8	02/13/2025 03:46 PM
RDW		15.3	%	13.7 - 17.3	02/13/2025 03:46 PM
Abs Reticulocyte		43.0	x10^3 /uL	6.4 - 58.9	02/13/2025 03:46 PM
Minimum Platelet Count		203	x10^3/uL	127 - 450	02/13/2025 03:46 PM
Platelet Comment	FEW SMALL CLUMPS SEEN				
MPV		13.0	fL	10.1 - 24.7	02/13/2025 03:46 PM
PCT		0.26	%	0.10 - 0.52	02/13/2025 03:46 PM
Segmented Neutrophils	(45%)	4.57	x10^3 /uL	2.50 - 13.92	02/13/2025 03:46 PM
Band Neutrophils	(0%)	0.00	x10^3 /uL	0.00 - 0.07	02/13/2025 03:46 PM
Lymphocytes	(49%)	4.98	x10^3/uL	1.07 - 6.02	02/13/2025 03:46 PM
Monocytes	(2%)	0.20	x10^3 /uL	0.00 - 0.67	02/13/2025 03:46 PM
Eosinophils	(4%)	0.41	x10^3/uL	0.10 - 1.36	02/13/2025 03:46 PM
Basophils	(0%)	0.00	x10^3/uL	0.00 - 0.08	02/13/2025 03:46 PM
Other	(0%)	0.00	x10^3 /uL	No Ref Interval	02/13/2025 03:46 PM
NRBC/100 WBC		0.0		0.0 - 1.0	02/13/2025 03:46 PM
Absolute nRBC		0.00	x10^3/uL	No Ref Interval	02/13/2025 03:46 PM
Morphology	NORMAL RBCS				
Resulted by	[redacted]				

Uncovered by a White Coat Waste investigation

IDEXX Reference Laboratories
Division of IDEXX Laboratories
www.idexx.com

One IDEXX Drive
Westbrook, Maine 04092
United States

IDEXX Reference Laboratories
Customer Support
888 433 9987

IDEXX REFERENCE LABORATORY TEST REPORT

MILO UMC BLOOD DONOR SA

PET OWNER:	UMC BLOOD DONOR SA
PATIENT ID:	7537724
SPECIES:	FELINE
BREED:	SHORTHAIR, DOMESTIC
GENDER:	MALE NEUTERED
AGE:	2Y6M15D
MICROCHIP #:	

UMC VET CLIN PATH LAB
[redacted]

ACCOUNT #:	76576
ORDERED BY:	LEAH COHN

ACCESSION #:	4611694600
REQUISITION #:	235879781
DATE OF COLLECTION:	05/10/2024
DATE OF RECEIPT:	05/14/2024
DATE OF REPORT:	05/16/2024

IDEXX SERVICES: **26354 FELV QUANT REALPCR™ TEST**

MOLECULAR DIAGNOSTICS

TEST	RESULT	UNITS
FeLV Quant RealPCR [a]	NEGATIVE	

NOTES

MOLECULAR DIAGNOSTICS

a A NEGATIVE FeLV PCR result indicates that FeLV proviral DNA was not detected in the sample submitted. If FeLV antigen by ELISA test result is also negative, FeLV infection is very unlikely. Occasionally, the FeLV antigen test by ELISA may be positive in a cat with a negative FeLV PCR result. This combination is most consistent with a regressive or focal FeLV infection. Less commonly, the proviral DNA in the sample may be below the limit of detection, the sample may contain an uncommon strain that is not detected by PCR or the antigen test was falsely positive. Learn more at www.idexx.com/FeLVQuant.

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011532
Reference Number: 2735280
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 2 Y
Animal ID: Milo
Case Ref #: 7537724
Specimen: Lithium Heparin - Whole Blood

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood			
Sample Condition	CLEAR			
Glucose	128	mg/dL	80-155	05/09/2024 02:22 PM
Urea Nitrogen	22	mg/dL	19-35	05/09/2024 02:22 PM
Creatinine	1.3	mg/dL	0.9-2.0	05/09/2024 02:22 PM
Sodium	152	mEq/L	148-154	05/09/2024 02:22 PM
Potassium	3.7	mEq/L	3.1-4.6	05/09/2024 02:22 PM
Chloride	119	mEq/L	112-123	05/09/2024 02:22 PM
Bicarbonate	17	mEq/L	13-23	05/09/2024 02:22 PM
Anion Gap	20	mEq/L	13-26	05/09/2024 02:22 PM
Albumin	3.6	g/dL	2.8-3.7	05/09/2024 02:22 PM
Total Protein	7.1	g/dL	6.1-8.1	05/09/2024 02:22 PM
Globulin	3.5	g/dL	2.5-5.2	05/09/2024 02:22 PM
Calcium	9.8	mg/dL	8.6-10.4	05/09/2024 02:22 PM
Phosphorus	3.9	mg/dL	2.3-5.0	05/09/2024 02:22 PM
Magnesium	1.9	mg/dL	1.7-2.6	05/09/2024 02:22 PM
Cholesterol	186	mg/dL	80-258	05/09/2024 02:22 PM
Total Bilirubin	0.1	mg/dL	0.1 - 0.2	05/09/2024 02:22 PM
ALT	33	U/L	28 - 84	05/09/2024 02:22 PM
ALP	23	U/L	13 - 50	05/09/2024 02:22 PM
GGT	<3	U/L	0-3	05/09/2024 02:22 PM
CK	191	U/L	65 - 574	05/09/2024 02:22 PM
Resulted by	[redacted]			

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011533
Reference Number: 2735279
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 2 Y
Animal ID: Milo
Case Ref #: 7537724
Specimen: Edta - Whole Blood

Clinical Pathology

CBC, Small Animal

Specimen Description		Edta - Whole Blood				
WBC		10.29		x10^3/uL	4.25 - 18.32	05/09/2024 02:45 PM
RBC		10.21		x10^6/uL	5.91 - 10.69	05/09/2024 02:45 PM
HGB		15.3		g/dL	9.2 - 16.1	05/09/2024 02:45 PM
HCT		46.0	H	%	28.9 - 44.8	05/09/2024 02:45 PM
MCV		45.1		fL	36.3 - 49.1	05/09/2024 02:45 PM
MCH		15.0		pg	12.8 - 17.3	05/09/2024 02:45 PM
MCHC		33.3	L	g/dL	34.1 - 38.8	05/09/2024 02:45 PM
RDW		16.4		%	13.7 - 17.3	05/09/2024 02:45 PM
Abs Reticulocyte		56.0		x10^3 /uL	6.4 - 58.9	05/09/2024 02:45 PM
Minimum Platelet Count		144		x10^3/uL	127 - 450	05/09/2024 02:45 PM
Platelet Comment		MANY CLUMPS SEEN				
MPV		15.9		fL	10.1 - 24.7	05/09/2024 02:45 PM
PCT		0.23		%	0.10 - 0.52	05/09/2024 02:45 PM
Segmented Neutrophils	(34%)	3.50		x10^3 /uL	2.50 - 13.92	05/09/2024 02:45 PM
Band Neutrophils	(2%)	0.21	H	x10^3 /uL	0.00 - 0.07	05/09/2024 02:45 PM
Lymphocytes	(48%)	4.94		x10^3/uL	1.07 - 6.02	05/09/2024 02:45 PM
Monocytes	(7%)	0.72	H	x10^3 /uL	0.00 - 0.67	05/09/2024 02:45 PM
Eosinophils	(9%)	0.93		x10^3/uL	0.10 - 1.36	05/09/2024 02:45 PM
Basophils	(0%)	0.00		x10^3/uL	0.00 - 0.08	05/09/2024 02:45 PM
Other	(0%)	0.00		x10^3 /uL	No Ref Interval	05/09/2024 02:45 PM
NRBC/100 WBC		0.0			0.0 - 1.0	05/09/2024 02:45 PM
Absolute nRBC		0.00		x10^3/uL	No Ref Interval	05/09/2024 02:45 PM
Morphology		NORMAL RBCS				
Resulted by		[redacted]				

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client	Patient	DVM
86803 - UMC BLOOD DONOR SA	7537724 - Milo Feline Domestic Shorthair (DSH) Male Castrated 5.40 kg Nov 01, 2021	

[redacted]

Episode: 1098077
Date/Time: 5/9/24, 12:14 PM

Reason for Visit: Blood Donor

Lab - VHC:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Did a blood donation under Ketamine and Midazolam 0.4 ml each IV for sedation. 50 mls of blood was removed and stored for later use. His PCV=36% and TP=6.6. He will be available for another donation until December 14th. He was recovered in ICU uneventfully.
Brief PE prior to donation unremarkable.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

did a blood donation under ketamine and Midazolam 0.4 ml each Iv for sedation.60 mls of blood was removed and stored for later use His PCV=38% and TP=6.9 He will be available for another donation until October 25th. He was recovered in ICU uneventfully.
Brief PE prior to sedation unremarkable.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donated blood under a Ketamine and Midazolam sedation, 0.4 ml each were administered IV. His eyes were lubed and the right jugular prepped for the donation. A 19 gauge butterfly needle was used to obtain 62 mls of blood for storage for later use. His PCV= 39%
He will not be available for donation until after August 28th 2025.
Brief PE unremarkable. Recovery uneventful.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew 60 ml of blood via the right jugular under ketamine 0.4 ml and Midazolam 0.4 mg IV he was recovered

Uncovered by a White Coat Waste investigation

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under Ketamine 0.4 ml and Midazolam 0.4 ml. 60 ml was removed from the right jugular he was recovered in ICU. His PCV= 42 % and TP= 7.0 his next donation will be no sooner than May 30th, 2025

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under Ketamine 0.4 ml and Midazolam 0.4 ml. 60 ml was removed from the right jugular he was recovered in ICU. His PCV=37% and TP=6.8 his next donation will be no sooner than April 22, 25

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Blood donation 60mls via right jugular under ketamine and midazolam combo sedation 0.4mls each
PCV= 39% TP= 7.0

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Milo donated 60 mls via the left jugular after an attempt on the right. he was sedated with Ketamine 0.4 ml and Midazolam 0.4 ml IV and remaining Midazolam was wasted. His PCV= 35% TP= 6.7 he was recovered in Endo and returned to the ward same afternoon.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

attempted a blood donation under ketamine 0.4 ml and Midazolam 0.4 ml Iv and he flipped our after about 0.2 ml of the combination so the remainder was given IM. He did not become sedate enough after 15-20 minutes so he was given Dexmedetomidine IM at 4:45PM by anesthesia milo never became sedate enough to draw blood so I aborted mission and decided to try again Wednesday October 2 under Ketamine 0.4 ml and Midazolam 0.4ml IV this time it went better and I was able to get the 60 mls of blood via the right jugular and return him to ICU to recover and go to his ward the following day

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donated blood for patient usage under Ketamine 0.4 ml and midazolam 0.4 ml IV. 60 ml of blood was obtained and Milo was recovered in ICU. His PCV=31% and will be available for another donation on Sept. 26th.

Comments:

Uncovered by a White Coat Waste investigation

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

did first blood donation via the right jugular under Ketamine and Midazolam 0.4 ml each IV. 60 mls of blood was obtained and he was recovered in ICU overnight. His next donation is August 8th 2024. crit tubes broke in centrafuge

Comments:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Fe BREED DSH

COLOR Orange Tabby/White Sex M Age 1.5 yr

NAME OR IDENTIFICATION Miles

OWNER'S NAME UMC

PRE-OP DIAGNOSIS Presumed Healthy

PROPOSED PROCEDURE Blood donation.

PRIMARY CLINICIAN Coan

ANESTHESIOLOGIST

PRIMARY STUDENT

STUDENT ANESTHETIST Coan 1647

DATE 8-10-24 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
7.14kg.			48	PR				
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4ml.	IV.	3:00 pm
Ketamine		0.4ml	IV.	3:00 pm

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P Syst v
Diast ^
Mean –
Pulse .
Resp O
Ventil o–v–o
Assist o–a–o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
$ET CO_2$
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

Midazolam wasted 0.63 ml.

X BY

B. Lippert

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P C V.

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE

ANESTHESIOLOGIST SIGNATURE

Uncovered by a White Coat Waste Investigation

ANESTHESIA MEDICAL RECORD

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Fe BREED DSH

COLOR Tabby/wht Sex Age PLACE LABEL HERE

NAME OR IDENTIFICATION Milo

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS Presumed healthy

PROPOSED PROCEDURE Blood donation

PRIMARY CLINICIAN(S) COHN ANESTHESIOLOGIST HAIGHT

PRIMARY STUDENT STUDENT ANESTHETIST

DATE 10/1/24 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP.	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
7.5 kg		180	56	Pk	1			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 ml	IM	3:15pm
Midazolam		0.4 ml	IM	3:15pm
				4:30 0.4mL Midazolam

NO CODE ☐ CLOSED CHEST CODE ☐ OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE _____ mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ○ O_2 flow Δ L/min _____

SpO_2 Δ
B P. Syst v
Diast ^
Mean –
Pulse ·
Resp ○
Ventil v
Assist a

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS
0.1mL (0.05mg) dexmedetomidine IM @ 4:45pm

0. mL Midazolam wasted

CONTROLLED DRUG WASTE.

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE [redacted]

ANESTHESIOLOGIST SIGNATURE [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P C V

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724
SPECIES Fel. BREED _____
COLOR _____ Sex _____ Age _____
PLACE LABEL HERE
NAME OR IDENTIFICATION Milo
OWNER'S NAME UMC

PRE-OP DIAGNOSIS: Presumed Healthy
PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
8/27/24			

WT	TEMP 101.9	HR 162	RR 72	MM >1	CRT Pink	SG	START ANESTHESIA	START PROCEDURE
PCV 31%	TP 6.8	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Relamune		0.4ml	IV	5:30
M. Zolam		0.4ml	IV	5:30

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____ mls/hr

TIME 5:30 pm

AGENT ____ ○
O_2 flow Δ
L/min ____

SpO_2 Δ
B P. Syst v
Diast ^
Mean –
Pulse •
Resp ○
Ventil
Assist

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

0.62ml. Midazolam wasted

X [redacted]

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	TP	PCV

TOTAL FLUIDS (mls)
Est Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE [redacted]
ANESTHESIOLOGIST SIGNATURE [redacted]

ANESTHESIA MEDICATIONS REVIEWED

Uncovered by a White Coat Waste Investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Fe BREED DSH

COLOR Red tab Sex F/N Age 3

NAME OR IDENTIFICATION Milo

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed Healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
CeHN	
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
4-30-25			

WT	TEMP.	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
6.16 kg.	99.8	100	66	Pk	<2			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml	IV	
Midazolam		0.4ml	IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: _____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min _____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse •
Resp. ○
Ventil. v
Assist. a

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

CONTROLLED DRUG WASTE:

RECOVERY:

	Time	Temp.	MM
Post. Anesth. Physical	HR	RR	CRT

MEM COLOR/CRT									
Temp.									
ET CO_2									
FLUIDS									

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL/TRACHEAL

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 1537724
SPECIES Feline BREED _____
COLOR Calico & Tabby Sex m/n Age 3
NAME OR IDENTIFICATION Milo
OWNER'S NAME UMC.

PRE-OP. DIAGNOSIS: Presumed Healthy.
PROPOSED PROCEDURE: Blood donation
PRIMARY CLINICIAN(S): CHN
ANESTHESIOLOGIST
PRIMARY STUDENT
STUDENT ANESTHETIST

DATE 6-23-25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP. 100.7	HR. 160	RR 66	MM Pink	CRT <2	SG	START ANESTHESIA	START PROCEDURE
PCV 40%	TP 7.6	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4mL	IV.	2:10 PM.
Midazolam		0.4mL	IV.	

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse .
Resp. ○
Ventil. o–v–o
Assist. o–a–o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT															
Temp.															
ET CO_2															
FLUIDS															

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

Midazolam wasted x
X 0.7 / mL
x [redacted]

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)
Est. Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]
ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Fel. BREED DSH

COLOR Orange tabby Age 3

NAME OR IDENTIFICATION MILO

OWNER'S NAME UMC Blood Donor.

PRE-OP. DIAGNOSIS: Presumed healthy Donor.

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S): CHN	ANESTHESIOLOGIST
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE 3/24/23	CAGE/STALL#	FASTED YES NO	PERMIT YES NO

WT 6.3 kg	TEMP. 101.6	HR. 160	RR 48	MM Pk	CRT <2	SG	START ANESTHESIA	START PROCEDURE
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml.	IV.	4:40PM
Midazolam		0.4ml.	IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse •

Resp. ○

Ventil. v

Assist. a

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

COMMENTS/EVENTS:

0.75 ml. Midazolam wasted

[redacted]

CONTROLLED DRUG WASTE:

[redacted]

MEM COLOR/CRT										
Temp.										
ET CO_2										
FLUIDS										

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE:

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Feline BREED DSH

COLOR Orange Sex M/N Age 3.2

NAME OR IDENTIFICATION Milo

OWNER'S NAME UMC.

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S): COHN. | ANESTHESIOLOGIST

PRIMARY STUDENT | STUDENT ANESTHETIST

DATE 1-16-25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
7 kg.	100.9	200	60	PR E	1.5			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 ml.	IV.	10:30
Midazolam		0.4 ml.	IV.	prior

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2, Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse •

Resp. ○

Ventil. o—v—o

Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

$ET CO_2$

FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE: | EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

Wasted Midazolam 0.63 ml.

x [redacted]

CONTROLLED DRUG WASTE: /

RECOVERY:

Post. Anesth. Physical | Time | Temp. | MM | HR | RR | CRT

ANESTHETIST SIGNATURE: [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

[redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Feline BREED _____

COLOR Orange tabby Cat Age 3

NAME OR IDENTIFICATION Milo

OWNER'S NAME UMC Blood donor

PREOP. DIAGNOSIS Healthy Retained Blood donor.

PROPOSED PROCEDURE Blood donation.

PRIMARY CLINICIAN(S) Colin.	ANESTHESIOLOGIST
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE 2/13/25	CAGE/STALL#	FASTED YES NO	PERMIT YES NO

WT	TEMP 101.2	HR. 200	RR 36	MM PR	CRT 1.5	SG	START ANESTHESIA	START PROCEDURE
PCV 39%	TP 7.0	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Butorphanol		0.4ml.	IV.	2:30pm
Midazolam		0.4ml.	IV.	2:30pm

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse ·

Resp. ○

Ventil. v

Assist. a

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

COMMENTS/EVENTS:

Midazolam wasted 0.75 ml.

[redacted]

CONTROLLED DRUG WASTE:

RECOVERY:

MEM COLOR/CRT									
Temp.									
ET CO_2									
FLUIDS									

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

[redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES fel. BREED DSH

COLOR Orange Sex M/N Age 3

NAME OR IDENTIFICATION Milo

OWNER'S NAME UMC Blood donor

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation.

PRIMARY CLINICIAN(S) CORR. | ANESTHESIOLOGIST

PRIMARY STUDENT | STUDENT ANESTHETIST

DATE 7/31/25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.7 kg.	101.1	120	60	Pink	≤ 1			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
39 %								

SPECIAL ANESTHETIC PROCEDURE | PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml.	IV.	2:30
Midazolam		0.4ml		

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: | EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse ·

Resp. ○

Ventil. o—v—o

Assist. o—a—o

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

COMMENTS/EVENTS:

Midazolam .64ml. Wasted

X [redacted]

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp	MM
	RR	RR	CRT

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537724

SPECIES Feline BREED DSH

COLOR orange/white Sex MN Age 4

NAME OR IDENTIFICATION M.10

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed Healthy Donor

PROPOSED PROCEDURE: Blood Donation

PRIMARY CLINICIAN(S) Cohn | ANESTHESIOLOGIST

PRIMARY STUDENT [redacted] ANESTHETIST

DATE 11/14/25 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.4	100.2	200	72	PK	<2			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
36%	6.6							

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 ml	IV	9:55pm
Midazolam		0.4 ml	IV	9:55pm

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: | EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ·
Resp. O
Ventil. v
Assist. a

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

COMMENTS/EVENTS:

wasted 0.68 ml midazolam

X [redacted]

CONTROLLED DRUG WASTE:

MEM COLOR/CRT

Temp.

$ET CO_2$

FLUIDS

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 753772

SPECIES FE BREED DSH

COLOR Orange/wht Sex M/N Age 4

NAME OR IDENTIFICATION Milo.

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed healthy Donor

PROPOSED PROCEDURE: Blood donation.

PRIMARY CLINICIANS: Cottrell

ANESTHESIOLOGIST

STUDENT ANESTHETIST

[redacted]

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
9/25/25			

WT	TEMP	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.35kg	101.6	180	56	Pr	1.0			

PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE
38%	6.9							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml	IV	
Midazolam		0.4ml	IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse •

Resp. ○

Ventil. o—v—o

Assist. o—a—o

220 200 180 160 140 120 100 80 60 40 30 20 10 0

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

0.82mls wasted midaz mcl / [illegible]

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL STETH

[redacted]

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ANESTHESIA MEDICAL RECORD

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Immunization

Client	Patient	
UMC BLOOD DONOR SA	Milo	Case# 7537724
[redacted]	Domestic Shorthair (DSH)	
	Male Castrated	5.40 kg
	Feline	

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Prescriptions

Client	Patient	
UMC BLOOD DONOR SA	Milo	Case# 7537724
[redacted]	Domestic Shorthair (DSH)	
	Male Castrated	5.40 kg
	Feline	

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Visit Date:5/9/24
Owner: UMC BLOOD DONOR SA [redacted]

Client #:86803

Patient: Milo
Species: Feline
Breed: American Domestic Shorthair
Color: Orange

Patient #:7537724
DOB:11/01/2021
Sex:Male Castrated

RDVM:

RDVM #:

I have reviewed and verified the referring veterinarian information above. _____ Please initial

Is this your primary veterinarian? YES NO (circle one)

Did your veterinarian recommend that you come to the VHC for this appointment? YES NO (circle

AUTHORIZATION RELEASE
It is our policy to send information about your visit to your veterinarian. As owner or agent of the above named animal, I authorize release of information concerning this visit to the veterinarian(s) of my choice. Do we have your permission to send this information? YES NO (circle one) _____ Please initial

STATEMENT OF OWNERSHIP AND CONSENT
I am the owner/agent of the above-described animal and have authority to consent to its treatment. I assume full financial responsibility for all charges rendered for diagnostic, therapeutic, surgical, preventive procedures and infectious disease surveillance and control.

Owner's Signature _____

OUR PAYMENT POLICY
The Veterinary Health Center operates on a fee-for-service basis. Payment in full is expected at the time of discharge or dismissal. For animals admitted to the hospital, we require a deposit equal to one-half of the high end of the estimate range. The remainder of the balance should be paid upon discharge. All other payment arrangements must be made in advance. Please initial the expected method of payment.
Cash____ Check____ Credit Card____ Debit Card_____

I have read and agree to comply with the payment policy as stated above. _____ Please

Please indicate if your animal has any of the following conditions. (circle one)

Medication reaction Yes No Do not know
List drug and reaction if possible_____
Up to date on rabies vaccination Yes No Do not know

Does your pet have Pet Health Insurance?_____If yes, name of insurance co_____
By indicating yes to your pet having Pet Health Insurance, you have authorized release of your pet's VHC medical record to a PHI Company if requested.

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No. 44519

Jefferson City Animal Shelter
2308 Hyde Park Rd.
Jefferson City, MO
573-634-6429

Adoption/Redemption Form

Owner Information

Name University of Missouri VHC
Address [redacted]
City Columbia State Mo Zip 65211
Telephone: Cell Wo[redacted]me
Email [redacted]

Animal Information

Animal unique ID 71556 MS Microchip 900228297

Fees Due

Adoption $ Placement Boarding $ Rabies $ Donation $ Total $ Placem

Payment Method: ☐ Cash ☐ Credit/Debit Card ☐ Check # ____
Intent

Rabies

☐ Vaccination Current ☐ Vaccination Not Current ☐ Too Young for Vaccination

If my animal is not current on its rabies vaccination, I do hereby agree to obtain a rabies vaccine from a licensed veterinarian prior to the expiration date listed below, to be in compliance with Sections 5-22 & 5-45 of the Code of the City of Jefferson. Yearly re-vaccination for rabies is required by City Ordinance.

Voucher Expiration Date NP

Pink copy, if provided to the owner is good for

☐ $ NP *towards the cost of a rabies vaccination by a licensed veterinarian at a participating clinic for owner redeemed pets*

☐ *A no cost rabies vaccination by the Jefferson City Animal Shelter for an adopted pet that was too young at the time of adoption for its initial rabies vaccination included in the adoption fee.*

Acknowledgment

I understand that the shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the time of adoption, which the adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

Matthew S. Haight — 5/9/21
Signature of Owner — Adoption/Redemption Date

Signature of Shelter Staff

Distribution: WHITE – Shelter copy YELLOW – Owner copy PINK – Rabies voucher

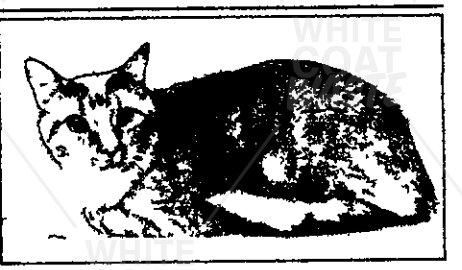
Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter

2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Animal Profile



Pet Name	**MILO**
Species	**CAT ID#: 21556**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Age	**2-3 YEAR**
Gender	**MALE/STERILIZED**
Color	**ORANGE / WHITE**
Size	**LARGE**

Case Information

Case ID	**04-23-2024 4**	Status	**ADOPTION AVAILABLE**
Shelter ID/Kennel	**MS**		
Arrival Date	**04-23-2024**	Collar Type	**NO COLLAR**
Arrival Reason	**UNWANTED**	Collar Color	
Transport	**OWNER TURN-IN**	License	
ACO		Vacc ID	**15470**
Precinct	**PRECINCT 1**	Microchip	**606278297**

Notes

Name: Milo
Age: 2-3 Years
Breed: Domestic Short Hair
Gender: Neutered Male
Good with Dogs: Friendly no fighting
Good with Cats: Didn't get along with other cat in home
Good with Children: Never been around them
Current weight: 13.7 lbs
FeLv/FIV test: Neg

Adoption Fee: $50

Misc. Information: Milo was surrendered to the shelter because he did not get along with the other cat in the home. He has been a very sweet boy while here at the shelter just a little shy and nervous. He was adopted as a kitten and has been an INDOOR ONLY cat. Milo would NOT do well outside! He loves to play with cat toys and feathers and scratching on his scratching post.

The shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the of adoption, which adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

ALL adoptions are final! Animals can be returned to the shelter for any reason, however, there are NO refunds or exchanges.

Animals altered here are tattooed with a green abdominal line.

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Animal Profile
Case Information
Behavioral Assessment
Notes

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Uncovered by a White Coat Waste investigation

Cat History Form

Jefferson City Animal Shelter
Jefferson City, MO
573-634-6429

For internal use only
Cage: MS
ShelterPro#: _____

In order for us to place your pet in the best suited environment, we ask that you complete this questionnaire as fully and truthfully as possible. All information will be provided to potential adopters.

Why are you surrendering your pet today? ***Please be more specific than "can no longer care for"***
Does not get along w/ other cats

Cat's Name: Milo **Age:** 2 **How old was this cat when you acquired him/her?** 0
Sex: X Male ____ Female **Sterilized (spayed or neutered):** X Yes ____ No
Has the cat had previous homes? ____Yes X No **If Yes, how many?** 0
Has this cat been declawed? X No ____Yes, front ____ All four declawed
If yes, when was this cat declawed _____ As a kitten _____ As an adult _____ Unsure

How was the cat obtained?
X Jefferson City Animal Shelter _____Free-to-good-home ad _____Pet shop _____Gift _____ Found as a stray
____Another Shelter: _____
____Friend/ Relative ____Stray ____ Breeder ____Born in my home
___ Other: _____

Has this cat ever been aggressive towards people or animals? (check all that apply)
____ Yes, has bit people ____Yes, has bit other cats ____ Yes has bit other animals X Yes, has scratched people
____ No, has shown no aggression towards animals or people
____ Other: _____

Where is the cat kept at the home (where does the cat spend its time)?
X Inside Only _____ Outdoor Only _____ Outbuilding (garage, shed, barn)
_____ Inside **and** Outside:
When is this cat inside? _____
When is this cat outside? _____

Medical History

Did this pet receive annual exam by a Veterinarian? X Yes ____ No ____ Unsure
Name of Veterinarian: Quail Valley
Is this cat current on his/her rabies vaccination? X Yes ____ No ____ Unsure

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Does this animal have any medical problems or previous injuries that require special attention or a special diet?

Explain:

_____

Please check all conditions that your cat has been diagnosed with or has been treated for:

____ Allergies ____ Urinary Tract Infection ____ Upper Respiratory Infection ____ Epilepsy/Seizures

____ Thyroid Disease ____ Skin Problems ____ Ringworm ____ Diabetes ____ Organ Failure

____ Heart murmur ____FIV ____ Feline Leukemia (FeLV) ____Obesity

____ Other: _____

_____

_____

Diet

Which of the following does your cat eat?

__X__ Dry food only ____Canned food only ____Combination of dry/canned ____ Table scraps

____Home cooked diet ____Other: _____

Do you feed your cat treats? ____ No ____ Yes What kind? _____

How often was this cat fed? ____ Once daily ____ Twice daily ____ Food always available

Exercise, Play and Behavior Information (Check all that apply)

What types of items does your cat play with?

____ **Cat toys** ____ **String** ____ **Feathers** **____ Balls** **____Bugs, birds, mice, etc.**

____ Other: _____

Does your cat use a scratching post? __X__ **YES** **____ NO**

What type of surfaces does your cat prefer to scratch on?

____ Cat tree **____ Scratching post** **____ Cardboard scratcher** **____ Carpet** __X__ **Upholstery** **____ Wood**

____ Drapes/Curtains **____ Other:** _____

Is this cat's activity level: **____ Low energy** **____ Medium energy** __X__ **High energy**

Is this cat most active: **____ Daytime** **____ Nighttime** __X__ **Both**

What makes this cat nervous, or causes it to behave in a different manner than usual?

____ Men **____ Women** **____ Children** __X__ **Strangers** **____ Going to the vet** **____ Going in the car**

__X__ **Cat carriers** **____ Bathing** **____ Brushing** **____ Nail clipping** **____ Other cats**

_____ Other animals: _____ **_____ Other:** _____

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Personality Profile

Where does the cat sleep? _X_ Wherever it wants _____ Cat bed _____in bed w/ adults _____in bed w/ children

_____ Other_____

Does the cat have any fears? (thunder, vacuum, etc?) _____

List any favorite toys: _____

Does the cat have any of the following behaviors?

_X_ Claws furniture _X_ Uses a scratching post _X_ Roams _____ Claws carpet _____Digs in plants

_____ Chews plants _____ Kills rodents _____ Kills birds _____Dumps trash _____Does not like to be picked up

How would you describe this cat?

_X_ Shy _____ Friendly _____ Demanding _____ Easygoing _____ Quiet _____ Fickle _____ Finicky

_____ Outgoing _____ One-person cat _____ Loving _____ Other: _____

Has this cat regularly been around children? _____ Yes _X_ No _____ Not Sure

If yes, please indicate the age range of children: ____ 0-2 yrs old ____ 2-5 yrs old ____ 6-10 yrs old ____11-18 yrs old

How does this cat behave around children? (check all that apply)

____ Friendly _____ Playful _____ Calm _____ Avoids certain children in family ____ Likes kids

____ Doesn't like kids _____ Easy for play to get out of control _____ Tolerates rough play from kids

____ Does not tolerate rough play from kids _X_ Other: hides from strangers

How does this cat behave around adults? (check all that apply)

____ Friendly ____ Playful _____ Calm _X_ Avoids certain adults _____ Easy for play to get out of control

____ Other: _____

Describe how this cat behaves when people come to visit: (check all that apply)

_____ Quiet _____ Friendly _____ Jumps up on them _X_ Hides and stays hidden

_____ Hisses or growls _____ Hides but then comes out after a while

_____ Starts out unsure, then gradually warms up and is friendly

Were there other animals in your home? _____ No _X_ Cat(s) _X_ Dog(s) _____ Small Mammals

_____ Birds Other: _____

If other cats, how many shared a litter box? _____ One _X_ Two or more _____ Many cats shared

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How does this cat behave around other cats? (check all that apply)

X Never been around other cats _____ Friendly, no fighting _____ Fight over certain situations or possessions

____ Didn't really care, avoided other cats _____ Did not get along at all, constantly fought _____This cat picked on other cats _____Other cats picked on this cat

How does this cat behave around dogs? (check all that apply)

____ Never been around dogs ___X__ Friendly, no fighting _____ Fight over certain situations or possessions

____ Didn't really care, avoided one another _____ Did not get along at all, constantly fought

____ This cat picked on dogs _____ Dogs picked on this cat

Additional Comments: _____

_____

_____

_____

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

ANIMAL MEDICAL HISTORY

University Of Mo Uhc
[redacted]

Species	**CAT ID#: 21556**
Pet Name	**MILO**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Gender	**MALE/STERILIZED**
License	
Vacc ID	**15470**
Microchip	**606278297**
Age	**2-3 YEAR**
Size	**LARGE**

04-24-2024 - Medical Visit - these items were performed

Date	Category	Detailed Description	Status/Result
04-24-2024	Medical visit	Date:04-24-2024	Closed:05-09-2024
04-24-2024	Vitals	Weight=?,Temp=?	
04-24-2024	Tested	FELV/FIV	NEGATIVE
04-24-2024	Vaccination	REVOLUTION	
04-24-2024	Medication	STRONGID3W	
04-24-2024	Vaccination	FVRCP 3W	
04-24-2024	Vaccination	RABIES	
04-24-2024	Service	VETERINARIAN EXAM	

Follow-ups - these items were scheduled and should be completed on the due date

Due Date	Category	Detailed Description
05-15-2024	Vaccination	FVRCP 3W
05-24-2024	Vaccination	REVOLUTION
04-24-2025	Vaccination	RABIES

End of items for this medical visit.

Animals altered here are tattooed with a green abdominal line.
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Jefferson City Animal Shelter

2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Vaccination Certificate

University Of Mo Uhc

[redacted]

Vaccination Information

ID	**15470**
Type	**RABIES**
Effective	**04-24-2024 TO 04-23-2025**
Precinct	**PRECINCT 1**
Issued by	**SCHALLER, TRAVIS**
Manufacturer	**ZOETIS DEFENSOR 1**
Lot / Serial	**690759**

About Milo

Species	**CAT ID#: 21556**
Breed	**DOMESTIC SHORT HAIR**
Gender	**MALE / STERILIZED**
Color	**ORANGE / WHITE**

Microchip **606278297**

Rabies vaccine admnistered by:
Travis Schaller, DVM License No. 2021018110
Greg Popp, DVM. License No. 03247

T J Schaller DVM

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Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Microchip Certificate

University Of Mo Uhc
[redacted]

Microchip Information

ID	**606278297**
Type	**AVID**
Effective	**11-23-2021**

About Milo

Species	**CAT ID#: 21556**
Breed	**DOMESTIC SHORT HAIR**
Gender	**MALE / STERILIZED**
Color	**ORANGE / WHITE**

Microchip **606278297**

Animals altered here are tattooed with a green abdominal line.
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Uncovered by a White Coat Waste Investigation

Transfusion Medicine in Small Animals

Beth Davidow, DVM, DACVECC[a,b,*]

KEYWORDS

• Transfusion medicine • Red blood cells • Plasma • Platelets • Albumin • TRALI • TACO • Transfusion reaction

KEY POINTS

- Transfusion medicine can be life saving and can be performed in veterinary clinics.
- Blood donors should be appropriately screened to minimize disease transmission.
- Blood typing is strongly recommended before transfusion in both dogs and cats.
- Crossmatching can be performed using gel technology.
- Red blood cell transfusions are the treatment of choice for anemia. Use of plasma is indicated for the treatment of active bleeding caused by coagulopathy. Other uses of plasma are controversial.
- Platelet transfusions can be used in small animal patients with active bleeding secondary to thrombocytopenia or thrombocytopathia.
- The use of human serum albumin is associated with a high risk of reactions.
- Reactions, both non–immune mediated and immune mediated, are a risk of transfusions.

Transfusions have been used as a life-saving modality for hundreds of years. The first documented transfusion in any species occurred in 1665 when Richard Lower withdrew blood from one dog and replaced it with blood from another dog. However, it was not until the 1950s that the use of transfusion medicine became more prominent in veterinary medicine because of the availability of equipment and techniques.[1] In the past 60 years, veterinary transfusion medicine has made remarkable advances. However, as knowledge has grown and the availability of different blood products has increased, transfusion therapy has become more complex. Availability of expanded donor screening, typing modalities, and crossmatching techniques make choosing the right donor unit for each patient potentially more complicated. Increased

Disclosures: The author has no funding sources or conflicts of interest to disclose.
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0195-5616/13/$ – see front matter © 2013 Elsevier Inc. All rights reserved.

availability of different blood components gives the clinician more options to tailor therapy more appropriately while avoiding transfusion-related reactions. This article summarizes recent advances in veterinary transfusion medicine and provides the clinician with evidence to guide transfusion decision making.

BLOOD DONOR SCREENING

In human medicine, individual blood units are screened for infectious diseases. A limited number of diseases are screened because of test availability and cost. Careful interview of donors is used to minimize risk of other diseases. In the veterinary field, it is usually cost prohibitive to test individual units. Therefore, a combination of careful interview and blood screening of the donor is used to minimize the risk of infectious disease transmission.

In 2005, the American College of Veterinary Internal Medicine (ACVIM) Consensus Statement on infectious disease testing for blood donors was published.[2] Since publication, polymerase chain reaction (PCR) assays have become more readily available for many diseases of concern.[3] When positive, PCR assays indicate that the organism has been identified in the blood stream and active infection is present. False-negatives are possible if the organism is present only in small amounts, because only a small sample of blood is examined. Several veterinary diagnostic laboratories offer donor-screening PCR panels, which typically include at least *Ehrlichia* spp, *Babesia* spp, *Anaplasma* spp, and *Mycoplasma hemocanis* or *Mycoplasma haemofelis*. When assessing which screening tests to perform, it is important to evaluate whether the diseases included are transmitted in blood, are present in asymptomatic donors, are geographically appropriate, and whether the test specificity and sensitivity for pertinent infectious diseases is acceptable. **Table 1** gives general recommendations of clinicopathologic screening for dogs and cats.

ADVANCES IN BLOOD TYPING

Dogs

Different blood antigens have been known to exist in dogs since 1910 and antigen groups have been described since the 1940s.[4] Although approximately 20 antigen specificities in 13 groups were originally identified by researchers, only 6 of these

Table 1
Recommended testing for canine and feline blood donors

Canine Donors	Feline Donors
Blood type	Blood type
Complete blood count	Complete blood count
Chemistry panel	Chemistry panel
Fecal analysis	Fecal analysis
Heartworm antigen	FeLV
Babesia spp	FIV
Ehrlichia spp	*M haemofelis*
Neorickettsia spp	*Bartonella* spp
Bartonella spp	—
M hemocanis	*Ehrlichia, Anaplasma, Neorickettsia* spp (geographic)
Leishmania spp (geographic)	*Cytauxzoon felis* (geographic)
Trypanosoma cruzi (geographic)	
Brucella canis (breeding animals)	

Abbreviations: FeLV, feline leukemia; FIV, feline immunodeficiency virus.

antigens, dog erythrocyte antigen (DEA) 1.1, DEA 1.2, DEA 3, DEA 4, DEA 5, and DEA 7, can be routinely identified by typing.[5–7] A newer antigen, Dal, exists at high frequency in most dogs but is missing in some Dalmatians.[8] Antibodies against Dal in vitro seem to induce a strong agglutination reaction and could cause a severe transfusion reaction. Dal has been shown to be independent from known DEA antigens.[9] Dogs do not have natural antibodies to the DEA 1 system. It has been shown that natural antibodies are present in some dogs for DEA 3, DEA 5, and DEA 7, and in occasional dogs for less common antigens.[7,9,10] At present, a universal donor in dogs is considered to be negative for DEA 1.1, DEA 1.2, DEA 3, DEA 5, and DEA 7, and positive for DEA 4.

The DEA 1 system, with its allelic subtypes (DEA 1.1, DEA 1.2, and possibly DEA 1.3), is most important because of its high degree of antigenicity. Dogs that are DEA 1.1 negative do not react to DEA 1.1–positive blood on initial transfusion because of lack of natural antibodies to this allele. However, the second time a DEA 1.1–negative dog is given DEA 1.1–positive blood, it may develop a severe hemolytic reaction.[11] Likewise, natural antibodies to DEA 3, DEA 5, and DEA 7 can result in transfusion reactions that are usually mild or delayed; also, a significantly shorter survival time of the transfused red blood cells (RBCs) may be seen.[12]

In order to identify dogs that are true universal donors, typing must be done through a commercial laboratory (Animal Blood Resources International) rather than through point-of-care blood typing tests. Specific blood typing is done with polyclonal antibodies using a tube agglutination method. A gel column test using a monoclonal antibody against DEA 1.1 was developed; however, this is no longer commercially available. Research is ongoing to develop gel methodology for other DEA antigens. Availability of testing for DEA 3 and DEA 5 has been variable in recent years.

In-house typing kits are available; however, these only test for the most antigenic type system, DEA 1.1. Although this is typically beneficial for the emergent patient, it should not be used as a sole method for blood screening prospective donors. The most common in-house typing kits are the DMS card test (CARD; DMS Laboratories, NJ) and the Quick test DEA 1.1 (CHROM; Alvedia, France), both of which use different monoclonal anti–DEA 1.1 antibodies. The benefits of the CHROM testing system is that it is not affected by the presence of autoagglutination (ie, the agglutinates do not move across the test strip and therefore do not interfere with typing).[13] With the tube agglutination method or the CARD, autoagglutination can prevent accurate typing.

A recent study compared the gel column test, CARD, and CHROM tests in both healthy dogs and those who were sick, and found identical results in 69 of 88 samples (78%). In 6 cases, the CHROM result was DEA 1.1 negative, whereas the gel and CARD both were positive. In 18 cases, the CARD result was different from both the gel and CHROM, reading positive in 15 and negative in 3. Because of the disparity of results in these systems, it is strongly recommended that blood typing for donors be confirmed by an outside laboratory.[13] For recipients, it is better to use a test that has false-negative results, because this leads to a true DEA 1.1–positive dog receiving a negative blood unit, which is safe, rather than a test with a high false-positive rate, which could lead to true DEA 1.1–negative dog receiving a positive transfusion (after which a transfusion reaction is more apt to occur).

Cats

Cats are either type A, type B, or type AB. Recently, the MiK antigen has also been identified.[14] Note that there is no universal blood type in cats, and all cats must be tested before receiving any type of transfusion. Blood type is determined by 3 alleles,

with A being dominant over the rare ab, which is dominant over b. Cats with a genotype A/A, A/ab, or A/b are type A, whereas only cats with b/b are type B. Rare AB cats are genotypically ab/ab or ab/b.

The distribution of these blood types varies geographically, with the highest incidence of B reported in Australia (36% in Sydney).[15] It was originally reported that 97% of cats in the United States were type A and that only 0.3% of cats in the northeast part of the United States were type B.[16] However, a 2005 study from the Animal Medical Center of New York City revealed a 6% incidence of the B blood type.[17] The B blood type is more common in certain exotic cat breeds, including Devon rex (41%), British shorthair (36%), Cornish rex (31%), exotic shorthair (27%), and Scottish fold (19%), but is also seen in domestic breed cats.[18] Type AB has been reported to occur in less than 1% of the general cat population. However, this incidence may be higher because new methodologies seem better at identifying these cats.[19]

Type A cats may have weak anti-B alloantibodies that can cause shortened RBC survival if a B donor is used. However, type B cats have strong anti-A antibodies and can have a fatal reaction from as little as 1 mL of transfused type A blood.[20] Type AB cats have no alloantibodies against either type A or B blood in their sera; they should receive either AB or A blood products if they need a transfusion because of the strong anti-A antibodies present in B serum. Most cats have MiK antigen but, in cats that do not, naturally occurring antibodies can lead to a hemolytic transfusion reaction with a first-time transfusion.

Original typing for cats was done using serum from type B cats with strong anti-A antibodies to identify type A cats. Anti-B antibodies are weaker in A serum but lectin from *Triticum vulgaris* reacts strongly with type B antigen.[21] At present, there are 2 commercially available in-house typing kits for cats (Rapid®-VetH Feline, DMS Laboratories, Flemington, NJ [CARD] and Quick test A+B, Alvedia, France [CHROM]). Both kits use monoclonal antibodies against type A and type B. In a recent study comparing typing methodologies in cats, the gold standard tube agglutination test, CARD, and CHROM gave identical results in 52 of 58 cats (89.7%). Overall, the CARD had 91.4% accuracy and CHROM had an overall accuracy of 94.8%. In 2 of the 6 cats with discordant resultants, feline leukemia (FeLV) infection was present.[22] It is recommended that B and AB cats be confirmed by a second method or through an outside laboratory.[19,22] At present, there is no in-house typing system available for the MiK antigen.

Blood typing is recommended before all transfusions in both dogs and cats. In an emergent situation in which time for blood typing is precluded, DEA 1.1–negative blood can be given to a dog of unknown type safely. However, typing and use of DEA 1.1–specific blood is recommended whenever possible to maximize the efficient use of canine blood donors that can be used. In cats, there are no universal donors and cats must always be blood typed before transfusion to prevent a life-threatening reaction.

CROSSMATCHING

In-house crossmatching can be used when blood typing is not available. It can also be used in conjunction with blood typing results. In dogs, crossmatching is recommended if transfusion history is unknown, if a hemolytic reaction is noted during a first transfusion, if more than 7 days has lapsed between administration of transfusions, or if the donor DEA 7 type is unknown.[7,23] In the past, crossmatching was recommended in dogs that had previously been pregnant. However, a recent study showed that pregnancy does not seem to sensitize dogs to antigens on RBCs.[8] In cats, both typing and

crossmatching should be strongly considered before an initial transfusion, because of the identification of antigens such as MiK with naturally occurring antibodies and the potential for a potential fatal transfusion reaction (eg, when type A blood is given to a type B cat). In addition, because in-house typing methodologies are not perfect, both typing and crossmatching on the first transfusion helps to identify any missed type incompatibilities in cats.[7]

Crossmatching has historically been done using a manual procedure that includes both a wash and an incubation step. **Box 1** gives a description of the crossmatching technique.[24] The procedure is time consuming and interpretation has been shown to be operator dependent. Gel crossmatch technology has recently been developed. One benefit is that the gels can be saved to show to others for verification (vs blood typing cards, which dry up). Gel technology also requires less blood than a standard crossmatch (0.5 mL vs 2–3 mL). In addition, the gel crossmatch can be used even if the patient is autoagglutinating.[25] The gel allows agglutinates to be trapped in a matrix but free cells to sink to the bottom, allowing easier interpretation of compatibility. A gel system for companion animals is available for both major and minor crossmatches (RapidVet®-H, DMS Laboratories Inc., NJ) **Fig. 1**.[9]

TRANSFUSION COLLECTION AND ADMINISTRATION

Whole blood can be collected for transfusion in many hospitals when proper bags and preservatives are available. Dogs and cats should be appropriately screened and a full physical examination should be performed before to each donation to ensure general health and lack of ectoparasites. If sedatives are necessary (typically required for cats), acepromazine should be avoided because of potential effects on platelet function. Blood is usually drawn from the jugular vein once the area has been aseptically prepped (to avoid contamination of the blood product). Dogs can safely donate 15 to 20 mL/kg, whereas cats can donate 10 to 15 mL/kg (lean body weight).

Blood should ideally be collected with the use of an anticoagulant preservative (eg, citrate-phosphate-dextrose-adenine [CPDA-1], Adsol) at a ratio of 1 mL of anticoagulant per 9 mL of blood.[24] Note that blood cannot be stored in syringes long-term,



Fig. 1. Gel crossmatch kit for use in both dogs and cats. (*Courtesy of* RapidVet®-H Feline/ DMS Laboratories, Inc., Flemington, NJ; with permission.)

Box 1
Crossmatching technique

1. Obtain blood in both ethylenediamine tetraacetic acid (EDTA) tube (purple top) and in a nonadditive tube (red top) from both donor and recipient. If using a stored RBC unit, use one of the provided tags and empty the sample into a red-top tube to spin.
2. Centrifuge and separate plasma and serum from the RBCs. Save the removed serum in a separate tube. Discard the plasma from the EDTA tube.
3. Wash the RBCs from the EDTA tube (purple top).
   a. Place the RBC in a second tube 12 × 75 mm and fill three-quarters full with saline.
   b. Centrifuge for 1 minute, decant the saline, and repeat 3 times, removing the supernatant the last time.
4. Resuspend the cells to make a 2% to 4% solution (0.2 mL of blood in 4.8 mL of saline gives a 4% solution).
5. Label tubes to make the following mixtures:
   a. Major crossmatch: 2 drops patient serum with 1 drop donor RBC suspension
   b. Minor crossmatch: 1 drop patient RBC suspension with 2 drops donor serum
   c. Control: 1 drop patient RBC suspension with 1 drop patient serum
6. Incubate for 15 to 30 minutes at 37°C.
7. Centrifuge for 15 seconds.
8. Interpretation of results:
   a. If either hemolysis or hemagglutination is seen macroscopically, or if agglutination is seen microscopically, the donor is not a good match

Modified from Lanevschi A, Wardrop KJ. Principles of transfusion medicine in small animals. Can Vet J 2001;42:447–54.

and should only be stored if collected aseptically into sealed, gas-diffusible blood bags.

The process of transfusion administration can potentially affect the survival of the RBCs, and technique, use of delivery system, and filter use must be correct when transfusing a patient. All blood products should be warmed to body temperature before administration. Plasma should be thawed by double bagging the product and placing in a warm water bath. Microwaves should not be used to thaw or warm blood products because of potential RBC or protein damage. RBCs should ideally be administered through a large-bore peripheral catheter (ideally >22 gauge). Initial administration of transfusions should be done slowly (eg, 0.5–1 mL/kg/h for the first 15 minutes) to monitor for an acute reaction, time permitting. Vital signs should be monitored every 15 minutes during the first hour and every 30 to 60 minutes thereafter. All transfusions should be administered within 4 hours, to prevent increased risk of bacterial contamination of the unit.[7]

In 2011, a study showed a significant decrease in the short-term probability of RBC survival in those canine blood transfusions administered with either a volumetric or syringe pump.[26] However, a similar study in cats showed no loss of RBCs with the use of a syringe pump and HemoNate filter.[27] As a result of these studies, the author recommends that canine packed RBCs (pRBCs) be given through an in-line blood filter but not through a fluid pump. Feline pRBCs can be given using a syringe and HemoNate filter.

If available, leukoreduction (the process by which white blood cells [WBCs] are removed from an RBC unit) can be considered. Leukocytes in stored pRBCs can lead to release of inflammatory compounds such as histamine and plasminogen activator inhibitor-1, potentially predisposing to transfusion reaction.[28] The benefit of leukoreduction is that it can decrease the incidence of nonhemolytic transfusion reactions and the immunomodulation that has been seen after transfusion in some human patients.[28] Leukoreduction can be performed either before storage or at the time of administration, and is also available for purchase through commercial blood banks as leukoreduced pRBC (Hemosolutions, Colorado Springs, CO).

The process of leukoreduction is now more readily available for canine blood, and has been shown to be associated with smaller increases in WBCs, fibrinogen, and C-reactive protein levels in recipients than with than nonleukoreduced transfusions.[28] A pilot study was recently done with feline RBC; however, the use of the neonatal leukoreduction filters resulted in loss of 8 mL of blood and may not be justified at this time.[29]

BLOOD COMPONENTS

The appropriate use of blood components allows more specific treatment while avoiding the risks of giving the unnecessary parts of the blood.[30] Component therapy is a more efficient way of using blood resources, allowing 1 donated unit to be split into multiple products (which may then benefit more than 1 patient). For example, a chronically anemic FeLV-positive cat may only require RBCs rather than whole blood (WB), provided the patient is not coagulopathic or thrombocytopenic. Likewise, a coagulopathic patient secondary to long-acting anticoagulant toxicosis may not be clinically anemic, and may only require fresh frozen plasma (FFP) or frozen plasma (FP) rather than WB, thereby saving the RBCs for another patient.

Appropriate storage of blood components is also necessary to allow efficient and maximal use of blood products. For example, the storage of component therapy (eg, FFP and pRBCs) allows longer storage time (compared with WB alone).[31,32] Available blood products, storage times, and indications for use are summarized in **Table 2**.[33–35]

RBC PRODUCTS

RBCs are indicated for the treatment of anemia. Anemia is defined as a deficiency of RBCs or hemoglobin (Hb). Anemic animals are usually pale, exercise intolerant, weak, tachypneic, and potentially tachycardiac and hypotensive (consistent with hemorrhagic shock). These clinical signs of anemia are related to decreased oxygen delivery (Do_2), because Hb plays a significant role in delivering oxygen to cells. This is explained in the formula for Do_2:

$$Do_2 = \text{cardiac output} \times \text{oxygen content of blood } (Cao_2)$$

$$Cao_2 = [(Pao_2 \times 0.003) + (Sao_2 \times Hb \times 1.34)]$$

$$CO = \text{stroke volume} \times \text{heart rate}$$

Sao_2 is arterial oxygen concentration.

The transfusion trigger is the Hb level at which an animal's Do_2 has decreased enough that anaerobic metabolism occurs. In critically ill humans, transfusions are recommended at a Hb level of 7 g/dL (approximate hematocrit [HCT] of 21%) unless

Table 2
Summary of Blood Components

Blood Product	Definition	Composition	Stability	Indications for Use	Other Information	Dose
WB	Blood pulled from a donor with no processing	RBCs, WBCs, platelets, all components of plasma	In 1–6°C refrigerator, 28 d in CPDA-1, 30 d in ACD	Massive blood loss, need for multiple components	Platelets lose activity after refrigeration, must be used within 4–6 h if not refrigerated	Transfusion amount (mL) = ($PCV_{desired}$ − $PCV_{current}$)/ PCV_{donor} × blood volume (mL/kg) × wt (kg) Blood volume dog = 88 mL/kg, Blood volume cat = 66 mL/kg
Packed RBCs	RBCs centrifuged and most of the plasma removed	RBCs, may have some WBCs	In 1–6°C refrigerator: 20 d in CPDA-1, 35 d in CPDA-1 with Optisol or Nutricel, 37 d in CPDA-1 with Adsol	Anemia	—	
Leukoreduced RBCs	Packed RBCs where WBC have been removed before storage	RBCs	37 d in CPDA in 1–6°C refrigerator	Anemia; leukoreduction may reduce immunomodulation and nonhemolytic febrile reactions	—	
PRP	Plasma and platelets separated from RBCs using a soft spin	Platelets, plasma	5 d in gas-diffusible bags with constant agitation at 22°C	Severe thrombocytopenia or thrombocytopathia with active hemorrhage or need for an invasive procedure	—	1 unit per 10 kg
Platelet concentrate	Further centrifugation of PRP to have a smaller volume or platelets obtained via plateletpheresis	Platelets, plasma	5 d in gas-diffusible bags with constant agitation at 22°C	Severe thrombocytopenia or thrombocytopathia with active hemorrhage or need for an invasive procedure	—	1 unit per 10 kg

Frozen platelets	Platelet concentrate created by plateletpheresis and then frozen using DMSO for platelet stability	Platelets	6 mo at −20 to −30°C	Severe thrombocytopenia or thrombocytopathia with active hemorrhage or need for an invasive procedure	—	1 unit per 10 kg
Lyophilized platelets	Platelet concentrate created by plateletpheresis, aldehyde cross-linked for platelet stability and then lyophilized	Platelets	2 y in refrigerator	Severe thrombocytopenia or thrombocytopathia with active hemorrhage or need for an invasive procedure	—	Research product in development
FFP	Plasma separated from WB and frozen within 8 h; <1 y of age	Coagulation factors, anticoagulation factors such at antithrombin, alpha-macroglobulin, albumin	1 y in freezer maintained at −20 to −30°C	Coagulation disorders resulting in active hemorrhage or as prophylaxis before invasive surgery in an animal with a known significant clotting factor deficiency	Potential use for treatment of severe necrotizing pancreatitis, DIC	10–30 mL/kg, higher dose for vWF
FP	Plasma separated from WB but not frozen completely within 8 h or plasma that has been frozen >1 y but <4 y	Contains stable coagulation factors II, VII, IX, X, albumin	5 y in freezer maintained at −20 to −30°C	Coagulation deficiencies, specifically of II, VII, IX, or X resulting in active hemorrhage	—	10–15 mL/kg
Cryoprecipitate-poor plasma	Supernatant that remains after preparation of cryoprecipitate	Contains stable coagulation factors II, VII, IX, X, anticoagulant, and fibrinolytic factors, albumin	1 y from original collection date in freezer maintained at −20 to −30°C	Coagulation deficiencies of II, VII, IX, or X resulting in active hemorrhage that does not require vWF	—	—

(*continued on next page*)

Table 2
(*continued*)

Blood Product	Definition	Composition	Stability	Indications for Use	Other Information	Dose
Cryoprecipitate	The precipitate containing cold insoluble proteins, formed when FFP is slowly thawed and spun	Contains concentrated vWF, VIII, XIII, fibrinogen, and fibronectin	10 mo when stored at −20°C or colder	When active hemorrhage from specific deficiency in vWF and VIII, or as prophylaxis when invasive procedure needed for animal with known deficiency of one of these factors	—	1 unit per 10 kg
Albumin	Protein is extracted from pooled plasma	Albumin	3 y at 20–24°C	Treatment of hypotension or hypovolemia in the septic patient with severe hypoalbuminemia	Both canine and HSA available; only canine albumin should be used ideally in canine patients because of risk of reaction from human product	Albumin deficit = 0.3 × BW (kg) × 10 × (albumin desired − current albumin)
IVIG	Pooled IgG extracted from multiple human B9 donors	IgG	36 mo in refrigerator at 1–6°C	Treatment of immune disease	Monitor carefully for type I, III reactions	0.5 g/kg

Abbreviation: ACD, Acid-citrate-dextrose.

the patient has clinically significant heart disease, in which case a level of 10 g/dL (approximate HCT of 30%) is recommended.[36] Although the appropriate veterinary transfusion trigger has not been well studied or identified, animal data suggest that an HCT of 15% to 18% may be a reasonable guideline.[7,37] However, animals with low volume or poor contractility may require a higher Hb to maintain D_{O_2}.

Fresh WB (FWB) has been the most common blood product traditionally used in veterinary medicine, because it is readily available to clinicians (often from an in-house donor or pet). WB is indicated when the patient is anemic, has a blood volume loss of more than 50%, or when the patient requires multiple components of blood (eg, RBC, clotting factors, platelets).[24] If WB is used before refrigeration, it is a good source of functional platelets.[38] One 500-mL unit of FWB obtained from a canine donor is estimated to contain 7×10^{10} platelets. Once refrigerated, platelet function decreases dramatically (discussed later). Recent work in human trauma has shown that maintaining the ratio of plasma, platelets, and RBCs close to 1:1:1 significantly improves the outcome in cases with large-volume blood loss.[39]

The amount of WB to administer can be calculated using the following formula[7]:

$$\text{Transfusion amount (mL)} = (PCV_{desired} - PCV_{current})/PCV_{donor} \times \text{blood volume (mL/kg)} \times \text{wt (kg)}$$

where PCV is packed cell volume.

pRBCs are indicated when blood volume is normal (eg, as shown clinically by a normal total protein) but the animal requires oxygen carrying capacity (eg, Hb). This is typically seen clinically when the anemia is caused by lack of production (eg, lack of erythropoietin, aplastic anemia), or a destructive process (eg, hemolysis).[24] Component therapy (eg, use of pRBC and FFP together) can be used to replace significant blood loss if the patient's platelet count is adequate and a WB donor is not available. The dose of pRBC can be calculated as listed for WB. When using pRBC with a PCV of approximately 60%, the formula: 1.5 mL $\times$ desired % increase in PCV $\times$ BW (kg), where *BW* is body weight, provides an accurate volume to administer.[40]

PLASMA PRODUCTS

Plasma contains albumin, globulins, clotting proteins, and anticoagulants. It can be stored and separated into many products. Indications for plasma use are debated in both human and veterinary medicine.[30,41] Plasma has been proved to be effective in actively bleeding patients with documented coagulation factor defects.[42] When dosing plasma products, a general guideline is 10 to 20 mL/kg. For example, a dose of 10 to 20 mL/kg of FFP is recommended for coagulopathic patients suffering from factor deficiency from vitamin K antagonist rodenticide toxicity,[35] whereas slightly higher doses (15–30 mL/kg) are needed to provide adequate factors to reverse coagulopathy from von Willebrand disease (vWD) or hemophilia A in dogs.[43]

The use of adequate dosing of plasma has also been proved, in humans, to be critical in preventing the dilutional coagulopathy that occurs with massive RBC transfusions.[39] As previously mentioned, studies in both civilian and military human patients with trauma have shown that 30-day survival is increased when maintaining a 1:1 ratio of plasma to RBC transfusion in massively transfused patients.[39,44–46]

Plasma is often administered to patients with increased coagulation parameters (but no active bleeding) before invasive procedures. However, there are no studies in human or veterinary medicine proving plasma's efficacy in prevention of hemorrhage.[42,47] Most human studies evaluating liver biopsies have found no association between coagulation times and the risk of bleeding.[48,49] One human study showed a mild increase in risk of hemorrhage with hyperbilirubinemia and a severely increased international

normalized ratio (INR).[50] A single veterinary study showed that the risk of hemorrhage after liver biopsy was more associated with platelet count than either prothrombin (PT) or activated partial thromboplastin time (PTT). The author recommends that the use of prophylactic plasma only be reserved for those patients with significant coagulopathy or those with documented severe factor deficiency undergoing a significant procedure (eg, splenectomy).

The use of FFP for the treatment of both hypercoagulability and hypocoagulability associated with disseminated intravascular coagulation (DIC) is controversial.[30] A study of dogs with severe illness and decreased antithrombin (AT) levels showed maintenance of AT with administered FFP; however, AT levels were not maintained when FFP was given with concurrent heparin.[51] There have been no prospective studies of dose or timing of FFP administration with DIC.

Plasma has also been used in patients with severe pancreatitis to help replace alpha-macroglobulins and maintain albumin levels, because survival in humans is known to be decreased in patients with low alpha-macroglobulin.[52] However, no benefit was seen compared with the control group when plasma was given to human patients with pancreatitis, even though an increase in alpha-macroglobulin levels was seen.[53] However, the control group comprised patients receiving colloid support in the form of albumin; so it is unknown whether colloid support from the albumin may still be useful for patients with pancreatitis. In veterinary medicine, a recent canine retrospective study evaluated the use of FFP with pancreatitis[54]; no improvement in mortality was seen with plasma administration. However, albumin levels were not reported before or after treatment of either group.

Hypoalbuminemia is also a controversial indication for plasma transfusion. Low albumin levels have been correlated with increased mortality in both humans and animals.[55,56] Albumin plays a key role in the body, because it makes up 70% to 80% of colloid osmotic pressure (COP), acts as a primary carrier molecule (eg, for medications), is important for the removal of bacterial toxins and free radicals, acts as a buffer, helps maintain microvascular integrity, and reduces platelet aggregation.[55] Despite the numerous theoretic benefits, exogenous albumin supplementation has not been proved to improve survival in human or veterinary medicine. Several large meta-analyses have examined human trials of albumin administration and found no overall benefit.[57,58] In a large trial of 6997 people resuscitated with either crystalloids or 5% albumin, there was a trend toward worse outcome with the use of albumin in patients with trauma[59] and no benefit in patients with burns.[57] However, a current meta-analysis found evidence for benefit of resuscitation with an albumin-containing fluid in human patients with sepsis.[60]

If plasma is used to increase serum albumin, 22.5 mL/kg is needed to raise the serum albumin by 0.5 g/dL if there is no ongoing loss. However, in one canine study evaluating FFP transfusions, no increase in albumin level was seen with average administration rates of 15 to 18 mL/kg.[41] Concentrated albumin may be more effective in animals with severely low albumin and concurrent peripheral edema. Synthetic colloids may be as or more effective in raising the COP in hypoalbuminemic animals.

Available plasma products include FFP, FP, cryoprecipitate, cryoprecipitate-poor plasma (CPP), human serum albumin (HSA), canine albumin, and intravenous immunoglobulin (IVIG). Each is discussed later in further detail; readers are also referred to the article "Fluid Therapy for the Emergent Small Animal Patient" by Elisa elsewhere in this issue for additional information.

FFP is plasma that has been separated from RBCs and frozen within 8 hours of collection of the blood. It contains all the coagulation factors, along with anticoagulants, fibrinogen, fibronectin, albumin, and alpha-macroglobulin. FFP is considered fresh when frozen at −40°C for 1 year.[35] Coagulation factors are defined as either

labile or nonlabile, based on their activity with storage. Labile clotting factors include factors V and VIII, whereas nonlabile factors generally include the vitamin K-dependent factors II, VII, IX, and X.

FP is plasma that has been separated from RBC and frozen greater than 8 hours after blood collection or when FFP is more than 12 months old but less than 5 years of age.[7] The labile coagulation factors and anticoagulants are not consistently active but the nonlabile coagulation factors (eg, vitamin K–dependent factors) are readily available, making it the transfusion treatment of choice for a coagulopathic patient secondary to long-acting anticoagulant rodenticide toxicosis. Likewise, it can potentially be used as source of albumin.

Cryoprecipitate is made by slow thawing and centrifugation of FFP. The supernatant is removed and the remaining slush/sediment, which contains cold insoluble proteins, is a concentrated form of factor VIII, von Willebrand factor (vWF), and fibrinogen. The cryoprecipitate can then be refrozen and is good for 10 months from the date of the initial blood draw.[61] Cryoprecipitate is the preferred treatment of prophylactic or active bleeding in dogs with hemophilia A or vWD.[43,62] Recommendations for dosing are 1 unit of cryoprecipitate for every 10 kg of body weight. Although FFP can also be used, the administration of cryoprecipitate allows a more concentrated amount of desirable factors versus the administration of much larger amounts of FFP to accomplish the same goal. Lyophilized (freeze-dried) canine cryoprecipitate is now commercially available (ABRI, Dixon, CA).

CPP is the supernatant removed in the process of making cryoprecipitate.[7] This plasma can be refrozen for 12 months and is a source of the nonlabile clotting factors II, VII, IX, and X; thus, CPP can be used to treat anticoagulant rodenticide toxicosis. It is also a source of albumin, although large amounts are required to increase the albumin. Dosing is similar to that for FFP or FP for coagulopathy.

Albumin can be extracted from plasma to make a concentrated product. Concentrated HSA has been used in both canine and feline patients with extremely low albumin levels (typically secondary to septic peritonitis, and so forth).[63–65] Doses for canine administration have been extrapolated from human medicine, and recommendations vary. A simple formula is 1.5 g/kg or the albumin deficit can be calculated as grams = 10 $\times$ (albumin desired – albumin current) $\times$ kg $\times$ 0.3.[66] This volume should be started slowly to monitor for reactions and then slowly titrated based on the stability of the patient. Although several articles have shown safety with HSA in sick patients, 2 healthy dogs died when given HSA in a research trial; this was caused by severe type III hypersensitivity reactions and development of profound vasculitis and membranoproliferative glomerulonephritis.[67] In addition, a follow-up study showed that even sick, potentially immunocompromised canine patients developed significant antibodies to HSA after administration.[68] With the current availability of canine albumin, the use of a potentially antigenic human product is not recommended.

Canine albumin is available (ABRI, Dixon, CA) as a lyophilized product. Each bottle contains 5 g of albumin, and typically ranges from $125 to $175. Canine albumin dosing can be calculated as listed for human albumin. Canine albumin is generally considered to be safer than HSA because of less immunogenicity. Initial safety studies have shown no signs of reactions in 6 dogs who received the product weekly for 4 weeks.[69]

Human immunoglobulin (IVIG) is polyvalent immunoglobulin G (IgG) that has been extracted and pooled from the plasma from multiple donors. It has been used in veterinary medicine to treat multiple immune diseases, including immune-mediated thrombocytopenia (ITP), immune-mediated hemolytic anemia, nonregenerative anemias, cutaneous diseases, polyradiculoneuritis, myasthenia gravis, and sudden

acquired retinal degeneration.[70–73] As with HSA, it is derived from humans, so antibody formation and type III hypersensitivity reactions are risks with its use in veterinary patients.[70] Type I hypersensitivity reactions and hypercoagulability are additional risks. Dosing for IVIG is extrapolated from human medicine, and is currently recommended at 0.5 g/kg.[70,72]

Specific clotting factors and anticoagulants are also extracted from human plasma and made into concentrates but these products are not yet used or produced in veterinary medicine. Examples include concentrates of factor VII, VIII, IX, and antithrombin.[74]

PLATELET PRODUCTS

Until recently, platelet transfusions were not viable, readily available options in veterinary medicine. Recent advances in platelet storage may make platelet transfusion more readily accessible to veterinarians, including the availability of lyophilized platelets. However, there are limited publications in veterinary medicine about triggers for prophylaxis or therapeutic efficacy of platelet products in actively bleeding patients.[75–78]

In humans, platelets are recommended for prophylaxis in any patient with a count less than 10,000/μL[74,79] and in patients who require an invasive procedure with counts less than 50,000/μL. Platelets are also recommended in patients with drug or hereditary impairments of platelet function that require an invasive procedure. In actively bleeding patients, the use of therapeutic platelet transfusions is also warranted with platelet counts less than 20,000/μL.[74,79] With ITP, the use of platelet transfusions is controversial because of the rapid destruction of any administered platelet unless the patient has evidence of life-threatening bleeding (eg, spinal cord bleed, bleeding into the brain).[74]

The risk of bleeding with severe thrombocytopenia is affected by the degree of anemia. RBCs scavenge nitric oxide, which leads to increases in platelet activity. In addition, high hematocrit values push platelets toward the endothelium and reduce sheer stress.[80] In cases of moderate thrombocytopenia and concurrent anemia, the risk of bleeding is potentially lessened with pRBC transfusion alone.

As previously discussed, FWB is the product most veterinarians have available to them to supply a limited number of platelets. A dose of 10 mL/kg of FWB is expected to raise the platelet count about 10,000/μLl, which is generally considered to be minimal; however, this may be life saving with severe thrombocytopenia.[75]

The advantage of FWB is that no platelets are lost, compared with component therapy production. In addition, the platelets are less activated than platelets obtained via centrifugation for concentrate.[76] FWB at room temperature is considered safe for use for 4 to 8 hours. Refrigeration of human platelets, either in WB or in platelet concentrate, rapidly leads to platelet aggregation and activation. In addition, refrigerated platelet survival is half that of platelets held at room temperature.[81] This effect is caused by clustering of the vWF receptors in response to temperature, leading to increased clearance by hepatic macrophages.[82] No studies have been done of canine refrigerated platelets to confirm whether a similar response occurs.[76] However, refrigeration of FWB should be avoided if used primarily for platelet transfusion.

Fresh platelet concentrate has traditionally been made by using a soft spin of WB, which separates the platelets into the plasma component. This technique allows appropriate allocation of component therapy and minimizes the volume or component of transfusion products that the patient may be receiving unnecessarily. After a soft spin, the plasma is expressed into a separate bag, and this plasma is then known as platelet-rich plasma (PRP). The PRP is spun again to create a platelet concentrate and the plasma is removed and stored as FFP.[7,74] One unit of platelet concentrate is

the amount made from 1 unit (500 mL) of WB but contains fewer platelets. Studies have shown average in vivo platelet recovery of 80%.[83,84] The dose is normally calculated as 1 unit/10 kg.

Fresh platelet concentrate can also be made through plateletpheresis. In this process, blood is taken from the donor and split into components (using an apheresis machine). During plateletpheresis, platelets are retained and the remaining volume is returned to the donor. The process is time consuming for the donor; however, large amount of platelets can be collected in this manner (eg, typically $1–4 \times 10^{11}$ platelets). This amount is typically 4 to 6 times the amount collected in a centrifuged platelet concentrate. Plateletpheresis also allows maximum sterility of the product. In addition, during plateletpheresis, there is negligible WBC and RBC contamination, minimizing potential transfusion reaction. Fresh platelets must be stored in gas-soluble bags at room temperature with constant agitation to remain active. Bacterial contamination is a concern at room temperature and storage is limited to 5 days.[76,85,86]

Frozen platelet concentrate is made by stabilizing apheresed platelets with 6% Dimethyl sulfoxide (DMSO) or with 2% DMSO and Thrombosol.[83] Canine platelet recovery (after freezing with 6% DMSO at −62°C [−80°F]) was shown to be 70% with a half-life of 2 days versus 3.5 days for fresh platelets.[87] The platelets were effective in halting active bleeding in thrombocytopenic dogs.[88] A more recent study comparing 6% DMSO with 2% DMSO and Thrombosol showed only 49% and 44% platelet recovery, respectively. Platelet half-life was confirmed to be about 2 days.[83] Frozen platelet concentrate with 6% DMSO is commercially available in veterinary medicine (ABRI, Dixon, CA). Dosing is recommended at 1 unit/10 kg of body weight given over 4 hours (see package insert for further information).

Lyophilized platelets (LYO) are an experimental product still undergoing research. Canine LYO are not yet commercially available but research is ongoing. Platelets are stabilized using a mild aldehyde cross-linking of membrane proteins and lipids, and then lyophilized and reconstituted with preservation of platelet structure and function. LYO can be stored for up to 24 months in the refrigerator, and can be reconstituted with saline immediately before use.[89] Research studies on LYO have shown that they bind to collagen, vWF and damaged endothelium. Receptors are activated normally and bind fibrinogen.[90] These platelets also retain the ability to increase procoagulant activity.[89] In a study of dogs on cardiac bypass, infusion of the LYO product led to improvement in venous bleeding time that was most pronounced at 20 to 30 minutes after infusion.[91]

A prospective, multicenter trial studied the use of fresh platelet concentrate (FRESH) and LYO in 37 dogs with active bleeding and thrombocytopenia. In this study, 22 dogs received LYO and 15 received FRESH. The incidence of transfusion reaction was low in both groups, and there was no difference in hospitalization time or mortality between the groups. The use of LYO seemed to be safe in dogs but larger studies are needed to study efficacy.[77] LYO products may also be used as hemostatic agents even if platelet numbers and function are normal. In a recent study, 20 swine were subjected to liver injury and then treated with either a placebo or LYO. Overall, 80% survived in the LYO group, whereas only 20% survived in the placebo group. One pig had evidence of thrombi in other locations on necropsy, indicating the need for further evaluation before these products are used in clinical patients.[92]

RISKS OF TRANSFUSION PRODUCTS

With administration of any type of blood product, the benefits and potential risks must be evaluated. Although potentially life saving, transfusions can result in acute or

delayed reactions. Reactions have been reported to occur in 8% to 13% of pRBC transfusions in dogs and cats.[17,31] **Table 3** shows a list of non–immune-mediated and immune-related transfusion reactions.

Transfusion-associated circulatory overload (TACO) and nonhemolytic febrile reactions are the most common reactions seen in dogs and cats.[93,94] Most blood products cause significant oncotic pull, which is more pronounced with those containing albumin (eg, WB, FFP), which can be useful in animals that have severe peripheral edema or in animals that are severely hypotensive; however, volume overload with resultant pulmonary edema is a potential risk in those animals that are already normovolemic. Blood pressure should be monitored while administering blood products and patients should be appropriately monitored for signs of volume overload (eg, tachypnea, weight gain, hemodilution, serous nasal discharge, development of pulmonary edema or pleural effusion).

Nonhemolytic febrile reactions are defined as a temperature increase of 1 to 2°C within 1 to 2 hours of a transfusion. Nonhemolytic reactions are usually caused by antibody reactions against donor leukocyte or platelet antigens and are greatly reduced when leukoreduction filters are used before storage of blood. It is important to monitor patients carefully, because fever may also be an early indicator of a more severe reaction (eg, hemolytic reaction, sepsis). If a nonhemolytic febrile reaction is suspected, slowing of the transfusion or drug administration may be warranted.

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related mortality in human medicine. TRALI manifests clinically as respiratory distress with severe noncardiogenic edema within 24 hours of transfusion and is seen mostly in patients receiving multiple plasma transfusions.[94] In human medicine, the development of TRALI is associated with use of plasma from female donors, particularly those who are parturient. The use of plasma from male donors, plasma from women who have never been pregnant, or from plasma that has been screened for the presence of WBC antibodies greatly decreases the incidence of TRALI.[94,95] In veterinary medicine, it is unclear whether TRALI occurs. RBC alloantibodies do not seem to be increased in dogs with repeated pregnancies but WBC alloantibodies have not been studied.[96] The incidence of parturient canine blood donors is typically rare in veterinary medicine, so TRALI may also be rare.

Type I hypersensitivity can also occur with any type of blood product and includes pruritus and angioedema, and can progress to bronchoconstriction and hypotension.

Table 3
Types of transfusion reactions

Immunologic Reactions	Nonimmunologic Reactions
Allergic reactions (type I hypersensitivity)	Sepsis
Hemolytic reactions	Citrate toxicity: hypocalcemia
Nonhemolytic febrile reactions	TACO
TRALI	Hyperammonemia
TRIM	Hypothermia
Decreased RBC survival	Hypophosphatemia
—	Hyperkalemia
—	Infectious disease transmission

Abbreviations: TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury; TRIM, transfusion-related immunomodulation.

It has recently been found that humans with preexisting allergic conditions such as atopy seem to be more at risk.[94]

SUMMARY

Transfusion medicine may be life saving in the emergent or critically ill veterinary patient. Blood products are becoming readily available, and transfusions can be performed in many clinic settings. The appropriate use of transfusion medicine should balance the potential risks, albeit rare, associated with transfusions. Patients should be appropriately screened with blood typing and crossmatching before therapy, and component therapy should be used when possible. Ongoing research may provide even better cage-side typing, longer storage times for components, and a larger variety of products that are more specific for each species.

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Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA
[redacted]

Patient 7537824 - Wesley
Feline
Domestic Shorthair (DSH)
Male 4.71 kg
Nov 01, 2020

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:17 PM

Local History

History

This young adult cat was acquired from the Jefferson city Animal Shelter. He is apparently healthy and will be used as a blood donor. All of the shelter information, including vaccinations administered there, are attached to his medical records as a separate document.

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client 86803 - UMC BLOOD DONOR SA
[redacted]

Patient 7537824 - Wesley
Feline
Domestic Shorthair (DSH)
Male 4.71 kg
Nov 01, 2020

DVM

Visit Date:

Admission Date/Time: 5/9/24, 12:17 PM

Local Physical

Physical Summary

The OAR team does all the physical exams and preventative maintenance. There were no concerns on examination.

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client	Patient	DVM
86803 - UMC BLOOD DONOR SA	7537824 - Wesley	
[redacted]	Feline	
	Domestic Shorthair (DSH)	
	Male Castrated 4.71 kg	
	Nov 01, 2020	

Episode: 1098079
Date/Time: 5/9/24, 12:17 PM

Reason for Visit: Blood donor

Temperature, Pulse, Respiration, and Other Vitals:

Date	Weight	Temperature	Pulse	Respiratory	Body Condition	Lameness
Nov 5 2025	4.71 kg 10 lb 6 oz	101.00 F /	140	72		
Appetite: PCV= 36%						
Urine:						
Bowl Movement: TP=6.3						
Comments:						
Sep 25 2025		101.30 F /	212	60		
Appetite: 35%						
Urine:						
Bowl Movement: 6.6						
Comments:						
Aug 26 2025	5.60 kg 12 lb 6 oz	101.00 F /	140	60		
Appetite: PCV= 34%						
Urine:						
Bowl Movement: TP= 7.3						
Comments:						
Aug 26 2025		101.30 F /	196	40		
Appetite: PCV= 33%						
Urine:						
Bowl Movement: TP= 6.4						
Comments:						

Uncovered by a White Coat Waste investigation

Date	Weight	Temperature	Pulse	Respiratory	Body Condition	Lameness
Jul 1 2025	4.55 kg 10 lb 1 oz	101.80 F /	186	66		
Appetite: PCV= 35%						
Urine:						
Bowl Movement: TP= 6.3						
Comments:						
Apr 24 2025	4.40 kg 9 lb 11 oz	101.00 F /	200	72		
Appetite: PCV= 35%						
Urine:						
Bowl Movement: TP= 6.5						
Comments:						
Mar 12 2025	4.97 kg 10 lb 15 oz	101.30 F /	168	72		
Appetite: pk<1 MM						
Urine:						
Bowl Movement:						
Comments:						
Oct 2 2024	3.20 kg 7 lb 1 oz	102.20 F /	175	40		
Appetite: PCV=34%						
Urine:						
Bowl Movement:						
Comments:						
Aug 28 2024	6.00 kg 13 lb 4 oz	101.50 F /	180	28		
Appetite: PCV= 38%						
Urine:						
Bowl Movement:						
Comments:						
Jun 27 2024	5.48 kg 12 lb 1 oz	101.90 F /	160	90		
Appetite: PK<2						
Urine: TP= 7.0 mg/dl						
Bowl Movement: PCV= 35%						
Comments:						

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001969
Reference Number: 2824143
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 4 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Urine

Clinical Pathology

Complete Urinalysis

Animal Id	Wesley
Specimen Description	Urine
Collection	Cystocentesis
Color	Yellow
Clarity	Cloudy
Urine Volume	5.0 mL
Specific Gravity	1.059
Glucose	Negative
Bilirubin	Negative
Ketones	Trace
Heme	2+
pH	7.0
Protein - Dipstick	2+
Urobilinogen	0.2 E.U./dL
WBC/hpf	Rare
RBC/hpf	21-40
Bacteria/hpf	None Seen
Transitional Cells/lpf	None Seen
Squamous Cells/lpf	None Seen
Casts/lpf	Rare Granular
Crystals/lpf	Few Struvite
Testing Completed On	January 29, 2025
Resulted by	[redacted]

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001970
Reference Number: 2824142
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 4 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Lithium Heparin - Whole Blood

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood			
Sample Condition	CLEAR			
Glucose	111	mg/dL	80-155	01/29/2025 11:07 AM
Urea Nitrogen	21	mg/dL	19-35	01/29/2025 11:07 AM
Creatinine	1.7	mg/dL	0.9-2.0	01/29/2025 11:07 AM
Sodium	153	mEq/L	148-154	01/29/2025 11:07 AM
Potassium	3.2	mEq/L	3.1-4.6	01/29/2025 11:07 AM
Chloride	118	mEq/L	112-123	01/29/2025 11:07 AM
Bicarbonate	21	mEq/L	13-23	01/29/2025 11:07 AM
Anion Gap	17	mEq/L	13-26	01/29/2025 11:07 AM
Albumin	3.0	g/dL	2.8-3.7	01/29/2025 11:07 AM
Total Protein	6.3	g/dL	6.1-8.1	01/29/2025 11:07 AM
Globulin	3.3	g/dL	2.5-5.2	01/29/2025 11:07 AM
Calcium	9.8	mg/dL	8.6-10.4	01/29/2025 11:07 AM
Phosphorus	4.6	mg/dL	2.3-5.0	01/29/2025 11:07 AM
Magnesium	1.9	mg/dL	1.7-2.6	01/29/2025 11:07 AM
Cholesterol	151	mg/dL	80-258	01/29/2025 11:07 AM
Total Bilirubin	0.1	mg/dL	0.1 - 0.2	01/29/2025 11:07 AM
ALT	56	U/L	28 - 84	01/29/2025 11:07 AM
ALP	18	U/L	13 - 50	01/29/2025 11:07 AM
GGT	<3	U/L	0-3	01/29/2025 11:07 AM
CK	88	U/L	65 - 574	01/29/2025 11:07 AM
Resulted by	[redacted]			

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H25-001971
Reference Number: 2824141
Received: Jan 29, 2025
Finalized: Jan 29, 2025
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 4 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Edta - Whole Blood

Clinical Pathology

CBC, Small Animal

Specimen Description	Edta - Whole Blood					
WBC		4.28		x10^3/uL	4.25 - 18.32	01/29/2025 10:55 AM
RBC		7.92		x10^6/uL	5.91 - 10.69	01/29/2025 10:55 AM
HGB		12.1		g/dL	9.2 - 16.1	01/29/2025 10:55 AM
HCT		34.8		%	28.9 - 44.8	01/29/2025 10:55 AM
MCV		44.0		fL	36.3 - 49.1	01/29/2025 10:55 AM
MCH		15.3		pg	12.8 - 17.3	01/29/2025 10:55 AM
MCHC		34.8		g/dL	34.1 - 38.8	01/29/2025 10:55 AM
RDW		15.9		%	13.7 - 17.3	01/29/2025 10:55 AM
Abs Reticulocyte		31.0		x10^3 /uL	6.4 - 58.9	01/29/2025 10:55 AM
Minimum Platelet Count		125	L	x10^3/uL	127 - 450	01/29/2025 10:55 AM
Platelet Comment	FEW SMALL CLUMPS SEEN					
MPV		16.5		fL	10.1 - 24.7	01/29/2025 10:55 AM
PCT		0.21		%	0.10 - 0.52	01/29/2025 10:55 AM
Segmented Neutrophils	(44%)	1.88	L	x10^3 /uL	2.50 - 13.92	01/29/2025 10:55 AM
Band Neutrophils	(0%)	0.00		x10^3 /uL	0.00 - 0.07	01/29/2025 10:55 AM
Lymphocytes	(47%)	2.01		x10^3/uL	1.07 - 6.02	01/29/2025 10:55 AM
Monocytes	(3%)	0.13		x10^3 /uL	0.00 - 0.67	01/29/2025 10:55 AM
Eosinophils	(5%)	0.21		x10^3/uL	0.10 - 1.36	01/29/2025 10:55 AM
Basophils	(1%)	0.04		x10^3/uL	0.00 - 0.08	01/29/2025 10:55 AM
Other	(0%)	0.00		x10^3 /uL	No Ref Interval	01/29/2025 10:55 AM
NRBC/100 WBC		0.0			0.0 - 1.0	01/29/2025 10:55 AM
Absolute nRBC		0.00		x10^3/uL	No Ref Interval	01/29/2025 10:55 AM
Morphology	NORMAL RBCS					
Resulted by	[redacted]					

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011534
Reference Number: 2735276
Received: May 09, 2024
Finalized: May 16, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Edta - Whole Blood

Clinical Pathology

FeIV PCR

MAIL OUT COMMENT

Testing run by IDEXX, 6100 E Shelby Dr, Memphis, TN 38121.
Results are available as an attachment to this document.

Resulted by [redacted]

Uncovered by a White Coat Waste investigation

This report supersedes any previous report issued for this case prior to 12/02/2025 at 12:22 PM

IDEXX Reference Laboratories
Division of IDEXX Laboratories
www.idexx.com

One IDEXX Drive
Westbrook, Maine 04092
United States

IDEXX [illegible]ence Laboratories
Customer Support
888 433 9987

IDEXX REFERENCE LABORATORY TEST REPORT

WESLEY UMC BLOOD DONOR SA

PET OWNER:	**UMC BLOOD DONOR SA**	**UMC VET CLIN PATH LAB**		ACCESSION #	4611694599
PATIENT ID:	7537824	[redacted]		REQUISITION #:	235880043
SPECIES:	FELINE			DATE OF COLLECTION:	**05/10/2024**
BREED:	SHORTHAIR, DOMESTIC			DATE OF RECEIPT:	**05/14/2024**
GENDER:	MALE NEUTERED	ACCOUNT #:	76576	DATE OF REPORT:	**05/16/2024**
AGE:	3Y6M15D	ORDERED BY:	LEAH COHN		
MICROCHIP #:					

IDEXX SERVICES: **26354 FELV QUANT REALPCR™ TEST**

MOLECULAR DIAGNOSTICS

TEST	RESULT	UNITS
FeLV Quant RealPCR [a]	NEGATIVE	

NOTES

MOLECULAR DIAGNOSTICS

a A NEGATIVE FeLV PCR result indicates that FeLV proviral DNA was not detected in the sample submitted. If FeLV antigen by ELISA test result is also negative, FeLV infection is very unlikely. Occasionally, the FeLV antigen test by ELISA may be positive in a cat with a negative FeLV PCR result. This combination is most consistent with a regressive or focal FeLV infection. Less commonly, the proviral DNA in the sample may be below the limit of detection, the sample may contain an uncommon strain that is not detected by PCR or the antigen test was falsely positive. Learn more at www.idexx.com/FeLVQuant.

Discover more: For complete access to this patient's diagnostic results, including historic values and images, login to www.vetconnectplus.com now!

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011535
Reference Number: 2735275
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Urine

Clinical Pathology

Complete Urinalysis

Animal Id	Wesley
Specimen Description	Urine
Collection	Cystocentesis
Color	Orange
Clarity	Cloudy
Urine Volume	5.0 mL
Specific Gravity	1.058
Glucose	Negative
Bilirubin	Negative
Ketones	Negative
Heme	3+
pH	5.5
Protein - Dipstick	1+
Urobilinogen	0.2 E.U./dL
WBC/hpf	Rare
RBC/hpf	> 40
Bacteria/hpf	None Seen
Transitional Cells/lpf	None Seen
Squamous Cells/lpf	Rare
Casts/lpf	None Seen
Crystals/lpf	None Seen
Testing Completed On	May 9, 2024
Resulted by	[redacted]
Reviewed by	

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location [redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - S A U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011536
Reference Number: 2735274
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Lithium Heparin - Whole Blood

Clinical Pathology

Small Animal Maxi Profile

Specimen Description	Lithium Heparin - Whole Blood				
Sample Condition	CLEAR				
Glucose	114		mg/dL	80-155	05/09/2024 02:33 PM
Urea Nitrogen	24		mg/dL	19-35	05/09/2024 02:33 PM
Creatinine	0.9		mg/dL	0.9-2.0	05/09/2024 02:33 PM
Sodium	149		mEq/L	148-154	05/09/2024 02:33 PM
Potassium	3.6		mEq/L	3.1-4.6	05/09/2024 02:33 PM
Chloride	116		mEq/L	112-123	05/09/2024 02:33 PM
Bicarbonate	19		mEq/L	13-23	05/09/2024 02:33 PM
Anion Gap	18		mEq/L	13-26	05/09/2024 02:33 PM
Albumin	3.6		g/dL	2.8-3.7	05/09/2024 02:33 PM
Total Protein	7.5		g/dL	6.1-8.1	05/09/2024 02:33 PM
Globulin	3.9		g/dL	2.5-5.2	05/09/2024 02:33 PM
Calcium	10.6	H	mg/dL	8.6-10.4	05/09/2024 02:33 PM
Phosphorus	5.8	H	mg/dL	2.3-5.0	05/09/2024 02:33 PM
Magnesium	1.6	L	mg/dL	1.7-2.6	05/09/2024 02:33 PM
Cholesterol	108		mg/dL	80-258	05/09/2024 02:33 PM
Total Bilirubin	0.1		mg/dL	0.1 - 0.2	05/09/2024 02:33 PM
ALT	43		U/L	28 - 84	05/09/2024 02:33 PM
ALP	23		U/L	13 - 50	05/09/2024 02:33 PM
GGT	<3		U/L	0-3	05/09/2024 02:33 PM
CK	178		U/L	65 - 574	05/09/2024 02:33 PM
Resulted by	[redacted]				

Uncovered by a White Coat Waste investigation

Veterinary Medical Diagnostic Laboratory Report

Mailing Address
PO Box 6023
Columbia, MO 65205

Location
[redacted]

Phone [redacted]
Web http://vmdl.missouri.edu

Owner:
BLOOD DONOR - SA U M C
[redacted]

RDVM:
Dr Leah Cohn

Clinic:
VETERINARY HEALTH CENTER
[redacted]

Accession Number: H24-011537
Reference Number: 2735273
Received: May 09, 2024
Finalized: May 09, 2024
Species: Feline
Breed: Domestic Shorthair (DSH)
Sex: Male Castrated
Age: 3 Y
Animal ID: Wesley
Case Ref #: 7537824
Specimen: Edta - Whole Blood

Clinical Pathology

CBC, Small Animal

Specimen Description		Edta - Whole Blood				
WBC		3.81	L	x10^3/uL	4.25 - 18.32	05/09/2024 02:35 PM
RBC		8.13		x10^6/uL	5.91 - 10.69	05/09/2024 02:35 PM
HGB		12.5		g/dL	9.2 - 16.1	05/09/2024 02:35 PM
HCT		39.5		%	28.9 - 44.8	05/09/2024 02:35 PM
MCV		48.6		fL	36.3 - 49.1	05/09/2024 02:35 PM
MCH		15.3		pg	12.8 - 17.3	05/09/2024 02:35 PM
MCHC		31.6	L	g/dL	34.1 - 38.8	05/09/2024 02:35 PM
RDW		15.8		%	13.7 - 17.3	05/09/2024 02:35 PM
Abs Reticulocyte		43.0		x10^3 /uL	6.4 - 58.9	05/09/2024 02:35 PM
Minimum Platelet Count		192		x10^3/uL	127 - 450	05/09/2024 02:35 PM
Platelet Comment		FEW SMALL CLUMPS SEEN				
MPV		10.0	L	fL	10.1 - 24.7	05/09/2024 02:35 PM
PCT		0.19		%	0.10 - 0.52	05/09/2024 02:35 PM
Segmented Neutrophils	(39%)	1.49	L	x10^3 /uL	2.50 - 13.92	05/09/2024 02:35 PM
Band Neutrophils	(0%)	0.00		x10^3 /uL	0.00 - 0.07	05/09/2024 02:35 PM
Lymphocytes	(26%)	0.99	L	x10^3/uL	1.07 - 6.02	05/09/2024 02:35 PM
Monocytes	(5%)	0.19		x10^3 /uL	0.00 - 0.67	05/09/2024 02:35 PM
Eosinophils	(30%)	1.14		x10^3/uL	0.10 - 1.36	05/09/2024 02:35 PM
Basophils	(0%)	0.00		x10^3/uL	0.00 - 0.08	05/09/2024 02:35 PM
Other	(0%)	0.00		x10^3 /uL	No Ref Interval	05/09/2024 02:35 PM
NRBC/100 WBC		0.0			0.0 - 1.0	05/09/2024 02:35 PM
Absolute nRBC		0.00		x10^3/uL	No Ref Interval	05/09/2024 02:35 PM
Morphology		FEW REACTIVE LYMPHOCYTES, NORMAL RBCS				
Resulted by		[redacted]				

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine: [redacted]
Food Animal: [redacted]

Client	Patient	DVM
86803 - UMC BLOOD DONOR SA	7537824 - Wesley	
[redacted]	Feline	
	Domestic Shorthair (DSH)	
	Male Castrated 4.71 kg	
	Nov 01, 2020	

Episode: 1098079
Date/Time: 5/9/24, 12:17 PM

Reason for Visit: Blood donor

Lab - VHC:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood under a ketamine 0.4ml and Midazolam 0.4ml sedation. 60 mls of blood were obtained via the right jugular. Mike was recovered in ICU and returned to the ward. the next donation is no sooner than December 5th
PCV= 36%
TP= 6.3
Brief PE was unremarkable and recovery uneventful.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

did a blood donation under ketamine and Midazolam 0.4 ml each Iv for sedation.60 mls of blood was removed and stored for later use His PCV=35% and TP=6.6 He will be available for another donation until October 25th. He was recovered in ICU.
Brief PE was unremarkable.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Wesley donated 60 mls for blood for stored use his TPR is listed in the TPR section and his PCV= 33% and TP= 6.4 he will not be available until sept. 25th for another blood donation. Sedated with 0.4 mls of ketamine and .4 mls of midazolam recovered in ICU Sedated with 0.4 mls of ketamine and .4 mls of midazolam recovered in ICU

Comments:

Uncovered by a White Coat Waste investigation

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Wesley donated 60 mls fo blood for stored use his TPR is listed in the TPR section and his PCV= 33% and TP= 6.4 he will not be available until sept. 25th for another blood donation.

this is a duplicate

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donated 60 mls of blood for stored use. sedated with Ketamine and midazolam 0.4 ml of each. blood was drawn via the right jugular and he was recovered in ICU.
PCV=36% TP=6.6

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Donated 60 mls of blood for stored use. sedated with Ketamine and midazolam 0.4 ml of each. blood was drawn via the right jugular and he was recovered in ICU. PCV= 34%00TP= 07.1 TPR within normal limits

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under Ketamine 0.4 ml and Midazolam 0.4 ml IV. 60 mls of blood were removed and he was recovered in ICU. His next donation is no sooner than May 22 2025.
PCV= 35%
TP= 6.5

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew 60 mls of blood under Ketamine 0.4 ml and Midazolam 0.4 ml sedation. blood was obtained from the right jugular and he was recovered in ICU overnight. the crit tubes broke in the centrifuge. TPR is in TPR section.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew blood via the right jugular under a ketamine / Midazolam sedation 0.4 ml of each were given IV. eyes were lubed and he was recovered in endo in his carrier and returned to OAR A CBC, Maxi and UA were done for his annual wellness. PCV= 38% TP= 9.9

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Blood was obtained under Ketamine and Midazolam sedation 0.4 ml each. 60 mls was obtained via the right jugular. He was recovered and returned to OAR.
PCV= 38%

Uncovered by a White Coat Waste investigation

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew 60 mls of blood under ketamine 0.4 ml and Midazolam 0.4 ml IV sedation. Drew blood (60 mls)via the right jugular and recovered in ICU.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Drew 60 ml of blood under ketamine 0.4 ml and midazolam 0.4 ml IV. the right jugular was used and he was recovered in ICU overnight. His PCV=38% and will available Sept. 26 for his next donation.

Comments:

BLOOD DONATION FOR THERAPEUTIC USE (430:1500)

Findings

Wesley was sedated with 0.4 ml ketamine and 0.4 ml Midazolam IV. 60ml of blood was obtained from the right jugular. a light wrap was applied and he was recovered in ICU for the day and returned to the Vivarium. This is his first donation and he will be available for donation on or after July 27th
His PCV= 34%
Tp= 7.0 mg/dl

Comments:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7937824
SPECIES Fe BREED DSH
COLOR Black Sex M/F Age 1
NAME OR IDENTIFICATION Wesley
OWNER'S NAME UMC

PLACE LABEL HERE

PRE-OP. DIAGNOSIS Presumed healthy
PROPOSED PROCEDURE: Blood donation.
PRIMARY CLINICIAN(S) COHN
ANESTHESIOLOGIST HAIGHT
PRIMARY STUDENT
STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
10/1/24			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
3.2kg	102.2	175	40	Pk	1.5			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml	IV.	3:20p
Midazolam		0.4ml	IV.	3:20p

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM. ☐ NR ☐ CIRCLE FLUID RATE ____mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst v
Diast ^
Mean –
Pulse ·
Resp ○
Ventil o–V–o
Assist. o–S–o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

0.66 mls.
Midazolam, wasted
X [redacted]

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T P.	P C.V.

TOTAL FLUIDS (mls) [redacted]
Est Blood Loss (mls)
ANESTHESIOLOGIST SIGNATURE [redacted]
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste Investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824
SPECIES Fel. BREED DSH
COLOR Black LABEL MISSING Sex M/N Age 3
NAME OR IDENTIFICATION Wesley
OWNER'S NAME Unc

PRE-OP. DIAGNOSIS Presumed healthy
PROPOSED PROCEDURE Blood donation.
PRIMARY CLINICIAN(S) COHN.
ANESTHESIOLOGIST
PRIMARY STUDENT
STUDENT ANESTHETIST Haight

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
8-7-24			

WT	TEMP 100.7	HR 140	RR 28	MM Pk	CRT <1	SG	START ANESTHESIA	START PROCEDURE
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine	0.4 mL		IV.	
Midazolam	0.4 mL		IV.	

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst v
Diast ^
Mean –
Pulse .
Resp ○
Ventil o–v–o
Assist o–s–o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

0.7 mL Midazolam waste
X [redacted]

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	TP	PCV

TOTAL FLUIDS (mls)
Est Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE [redacted]
ANESTHESIOLOGIST SIGNATURE [redacted]

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7531824

SPECIES _____ BREED _____

COLOR PLACE LABEL HERE Sex Age

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS

PROPOSED PROCEDURE Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE 8/27/24	CAGE/STALL#	FASTED YES NO	PERMIT YES NO

WT [redacted]	TEMP 101.5	HR 180	RR 28	MM PK	CRT <2s	SG	START ANESTHESIA	START PROCEDURE
PCV 38%	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.2 mL	IV	8:00 am
Midazolam		0.4 mL	IV	8:00 am

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

COMMENTS/EVENTS

Wasted midazolam 0.68 mL.

[redacted] CONTROLLED DRUG WASTE

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P Syst v
Diast ^
Mean –
Pulse .
Resp O
Ventil o–v–o
Assist o–a–o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

MEM COLOR/CRT
Temp
ET CO_2
FLUIDS

RECOVERY

Post Anesth Physical

Time	Temp	MM
HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B E	T P	P C V.

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE [redacted]

ANESTHESIOLOGIST SIGNATURE [redacted]

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste Investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fe BREED DSH

COLOR Blk/white Sex M/N Age PLACE LABEL HERE

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC Donor

PRE-OP. DIAGNOSIS Presumed healthy

PROPOSED PROCEDURE Blood donation.

PRIMARY CLINICIAN(S) COHN

ANESTHESIOLOGIST LAIGHT

PRIMARY STUDENT

STUDENT ANESTHETIST

DATE 6-27-24 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam	0.4mL		IV.	9:00 AM
Ketamine	0.4mL		IV.	9:00AM

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE	EPHEDRINE
EPINEPHRINE	

MASK ☐ INTUBATED ☐ SIZE SYSTEM ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME 0 15 30 45 0 15 30 45 0 15 30 45

AGENT ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B P Syst v
Diast ^
Mean –
Pulse .
Resp O
Ventil o—v—o
Assist o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

COMMENTS/EVENTS

Wasted Midazolam

X

CONTROLLED DRUG WASTE

RECOVERY

Post Anesth Physical	Time	Temp	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE [redacted]

ANESTHESIOLOGIST SIGNATURE [redacted]

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E	T P	P C V

TOTAL FLUIDS (mls)

Est Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste investigation

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7557824 Page ___ of ___

SPECIES Fel. BREED DSH

COLOR Black Sex N/N

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC blood donor.

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S): CHT

PRIMARY STUDENT

ANESTHESIOLOGIST

STUDENT ANESTHETIST

DATE 1-29-25

CAGE/STALL#

FASTED YES NO

PERMIT YES NO

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.51 kg	101.9	240	48	Pink	<2			

PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE
39%	9.9							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml.	IV.	
Midazolam		0.4ml.	IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ___ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ___ O

O_2 flow Δ

L/min ___

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse •

Resp. ○

Ventil. o—v—o

Assist. o—a—o

EMERGENCY DRUG CALCULATIONS

ATROPINE:

EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical — Time | Temp. | MM | HR | RR | CRT

V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

[redacted]

[redacted]

ANESTHESIA MEDICAL RECORD

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fe BREED DSH

COLOR Blk Sex M/N Age 3

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S)	ANESTHESIOLOGIST
CohN	
PRIMARY STUDENT	STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
4-24-25			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
4.4 kg	101.0	200	72	Pk	<2			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
35	6.5							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine	0.4	0.4	IV	
Midazolam	0.4	0.4	IV	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse •
Resp. ○
Ventil. o—v—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

Midazolam wasted 0.75 mls

[redacted]

CONTROLLED DRUG WASTE:

MEM COLOR/CRT													
Temp.													
ET CO_2													
FLUIDS													

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fe BREED _____

COLOR Blk/Wht Sex M/N Age 3

NAME OR IDENTIFICATION Wesley.

OWNER'S NAME U M C.

PRE-OP. DIAGNOSIS Presumed Healty.

PROPOSED PROCEDURE: Blood draw.

PRIMARY CLINICIAN(S) COHN. | ANESTHESIOLOGIST

PRIMARY STUDENT | STUDENT ANESTHETIST

DATE 3/12/24 | CAGE/STALL# | FASTED YES NO | PERMIT YES NO

WT 4.97	TEMP 101.3	HR. 168	RR 72	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4ml.	IV.	310pm
Ketamine		0.4ml.	IV.	310pm

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: | EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ♥
Resp. ○
Ventil. o—v—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER

COMMENTS/EVENTS:

0.7ml Midazolam wasted.

[redacted]

CONTROLLED DRUG WASTE:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

RECOVERY:

Post. Anesth. Physical | Time | Temp. | MM | HR | RR | CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE:

ANESTHESIOLOGIST SIGNATURE: [redacted]

Uncovered by a White Coat Waste investigation

Page ____ of ____

Admission Date ____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fel. BREED ____

COLOR Blk Sex MN Age 3

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation.

PRIMARY CLINICIAN(S) | ANESTHESIOLOGIST

PRIMARY STUDENT | STUDENT ANESTHETIST

DATE	CAGE/STALL#	FASTED YES NO	PERMIT YES NO
12-24-24			

WT	TEMP	HR	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
5.8 kg.	99.8	272	40	PK	< 1.5			
PCV 36%	TP 6.8	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 mL.	IV.	
Midazolam		0.4 mL.	IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse ·

Resp. ○

Ventil. v

Assist. ^

MONITORING

☐ ECG ☐ IBP

☐ ESOPH ☐ NIBP

☐ TEMP ☐ AIRWAY

☐ OXIMETRY

☐ OTHER:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

Combo ½ & ½ 0.45 mL

X [redacted]

Wasted Midazolam 0.69 mL. ATL

X [redacted] ATL

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical | Time | Temp | MM | HR | RR | CRT

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHETIST SIGNATURE: [redacted]

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824
SPECIES FE BREED
COLOR Black Sex Age
NAME OR IDENTIFICATION Wesley
OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed healthy
PROPOSED PROCEDURE: Blood donation
PRIMARY CLINICIAN(S) COHN
ANESTHESIOLOGIST
PRIMARY STUDENT
STUDENT ANESTHETIST

DATE 7-1-25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT 4.65 kg	TEMP. 101.8	HR. 186	RR 66	MM PR	CRT 1	SG	START ANESTHESIA	START PROCEDURE
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4 mL	IV	3:40 MDX
Ketamine		0.4 mL	IV	3:40 MDX

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse .
Resp. ○
Ventil. o—Y—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT
Temp.
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:
EPINEPHRINE:

COMMENTS/EVENTS:

Wasted 0.7 ml. Midazolam

[redacted]

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical Time Temp. MM HR RR CRT

ANESTHETIST SIGNATURE: [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)
Est. Blood Loss (mls)
EXTUBATION
ESOPHAGEAL REMOVAL

Uncovered by a White Coat Waste investigation

Admission Date _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

PRE-OP. DIAGNOSIS:	Presumed Healthy
PROPOSED PROCEDURE:	Blood donation.
PRIMARY CLINICIAN(S):	Conn
ANESTHESIOLOGIST	
PRIMARY STUDENT	
STUDENT ANESTHETIST	
DATE:	6/3/25
CAGE/STALL#	
FASTED YES NO	
PERMIT YES NO	

CA:
SPI
CO
NAI
OW

Wesley 7537824
Domestic Shorthair (DSH) DOB: 11/01/20 (Y: 4, M: 7)
Feline
Black/White
Male Castrated 86803
SA, UMC BLOOD DONOR
900 E CAMPUS
COLUMBIA, MO 65211
H 0
C (484) 354-9648 W (573) 882-7821

WT 4.65	TEMP.	HR 104	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
PCV 37%	TP 6.5	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4mL	IV.	
Ketamine		0.4mL	IV.	

NO CODE ☐
CLOSED CHEST CODE ☐
OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ·
Resp. ○
Ventil. o—x—o
Assist. o—a—o

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

MEM COLOR/CRT
Temp.
ET CO_2
FLUIDS

EMERGENCY DRUG CALCULATIONS

ATROPINE:	EPHEDRINE:
EPINEPHRINE:	

COMMENTS/EVENTS:

Midazolam waste
0.8 mL.
[redacted]
X
Murdock

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

ANESTHETIST SIGNATURE: [redacted]

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)
Est. Blood Loss (mls)
EXTUBATION
ESOPH. AGE/ANESTHESIA

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fe BREED DSH

COLOR Blk Sex M/N Age 4

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC

PRE-OP. DIAGNOSIS: Presumed healthy Donor

PROPOSED PROCEDURE: Blood donation.

PRIMARY CLINICIAN: Cerin

ANESTHESIOLOGIST

PRIMAR [redacted]

STUDENT ANESTHETIST

DATE 9-25-25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP.	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
101.3		212 Str	60	PK	2			
PCV	TP	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4 mL	IV.	
Midazolam		0.4 mL	IV.	

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse •

Resp. ○

Ventil. v

Assist. a

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

COMMENTS/EVENTS:

0.7 mls wasted of midaz mel/ [redacted]

CONTROLLED DRUG WASTE:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

RECOVERY:

Post. Anesth. Physical	Time	Temp.	MM
	HR	RR	CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

[redacted]

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fel. BREED _____

COLOR Black & White Sex M/N Age 3

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC Blood donor

PRE-OP. DIAGNOSIS: Presumed healthy

PROPOSED PROCEDURE: Blood donation

PRIMARY CLINICIAN(S) CHN

ANESTHESIOLOGIST

PRIMARY STUDENT [redacted]

STUDENT ANESTHETIST

DATE 11/5/25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP	HR.	RR	MM	CRT	SG	START ANESTHESIA	START PROCEDURE
4.71 kg	101.0	140	72	Ph	<2			
PCV	**TP**	**BUN**	**CREAT**	**GLU**	**ALB**	**K**	**END ANESTHESIA**	**END PROCEDURE**
36%	6.3							

SPECIAL ANESTHETIC PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Ketamine		0.4ml.	IV	10:50
Midazolam		0.4ml.	IV	WNL

NO CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O

O_2 flow Δ

L/min ____

SpO_2 Δ

B.P. Syst. v

Diast. ^

Mean –

Pulse ·

Resp. ○

Ventil. v

Assist. a

MONITORING

☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

COMMENTS/EVENTS:

Wasted
0.72 mL.
Midazolam

[redacted]

CONTROLLED DRUG WASTE:

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

RECOVERY:

Post. Anesth. Physical | Time | Temp. | MM | HR | RR | CRT

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

[redacted]

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Admission Date _____ Page _____ of _____

Veterinary Health Center
University of Missouri

ANESTHESIA RECORD

CASE NUMBER 7537824

SPECIES Fel. BREED DSH

COLOR Blk SEX MN AGE

NAME OR IDENTIFICATION Wesley

OWNER'S NAME UMC.

PRE-OP. DIAGNOSIS Planned Healthy.

PROPOSED PROCEDURE Blood donation

PRIMARY CLINICIAN(S) CORN

ANESTHESIOLOGIST

PRIMARY STUDENT

STUDENT ANESTHETIST

DATE 8/26/25 CAGE/STALL# FASTED YES NO PERMIT YES NO

WT	TEMP. 101.3	HR. 196	RR 40	MM Pk	CRT <1	SG	START ANESTHESIA	START PROCEDURE
PCV 33%	TP 6.4	BUN	CREAT	GLU	ALB	K	END ANESTHESIA	END PROCEDURE

PHYSICAL STATUS 1 2 3 4 5 E

SPECIAL ANESTHETIC PROCEDURE

PRE-ANESTHETIC DRUGS

DRUG	MG	ML	ROUTE	TIME
Midazolam		0.4 ml.	IV	4:10
Ketamine		0.4 ml.	IV.	

CODE ☐

CLOSED CHEST CODE ☐

OPEN CHEST CODE ☐

ANESTHETIC INDUCTION

DRUG	MG	ML	ROUTE	TIME

MASK ☐ INTUBATED ☐ SIZE: SYSTEM: ☐ NR ☐ CIRCLE FLUID RATE: ____ mls/hr

TIME: 0 15 30 45 0 15 30 45 0 15 30 45

AGENT: ____ O
O_2 flow Δ
L/min ____

SpO_2 Δ
B.P. Syst. v
Diast. ^
Mean –
Pulse ·
Resp. ○
Ventil. v
Assist. B

MONITORING
☐ ECG ☐ IBP
☐ ESOPH ☐ NIBP
☐ TEMP ☐ AIRWAY
☐ OXIMETRY
☐ OTHER:

EMERGENCY DRUG CALCULATIONS

ATROPINE: EPHEDRINE:

EPINEPHRINE:

COMMENTS/EVENTS:

ne.

wasted midazola

x 0.75

[redacted]

CONTROLLED DRUG WASTE:

RECOVERY:

Post. Anesth. Physical Time Temp. MM HR RR CRT

[redacted]

MEM COLOR/CRT

Temp.

ET CO_2

FLUIDS

A/V	Time	pH	PO_2	PCO_2	HCO_3	TCO_2	B.E.	T.P.	P.C.V.

TOTAL FLUIDS (mls)

Est. Blood Loss (mls)

EXTUBATION

ESOPHAGEAL REMOVAL

ANESTHESIOLOGIST SIGNATURE:

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Animal: [redacted]
Wentzville: [redacted]

Equine [redacted]
Food Animal: [redacted]

Immunization

Client	Patient	
UMC BLOOD DONOR SA	Wesley	Case# 7537824
[redacted]	Domestic Shorthair (DSH)	
	Male Castrated	4.71 kg
	Feline	

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Prescriptions

Client	Patient	
UMC BLOOD DONOR SA	Wesley	Case# 7537824
[redacted]	Domestic Shorthair (DSH)	
	Male Castrated	4.71 kg
	Feline	

Uncovered by a White Coat Waste investigation

Veterinary Health Center

[redacted]

Web: vhc.missouri.edu

Small Anim[redacted]
Wentzville: [redacted]

Equine[redacted]
Food Animal: [redacted]

Visit Date:5/9/24
Owner: UMC BLOOD DONOR SA
[redacted]

Client #:86803

Patient: Wesley
Species: Feline
Breed: American Domestic Shorthair
Color: Black/White

Patient #:7537824
DOB:11/01/2020
Sex: Male Castrated

RDVM:

RDVM #:

I have reviewed and verified the referring veterinarian information above. _____ Please initial

Is this your primary veterinarian? YES NO (circle one)

Did your veterinarian recommend that you come to the VHC for this appointment? YES NO (circle

AUTHORIZATION RELEASE
It is our policy to send information about your visit to your veterinarian. As owner or agent of the above named animal, I authorize release of information concerning this visit to the veterinarian(s) of my choice. Do we have your permission to send this information? YES NO (circle one) _____ Please initial

STATEMENT OF OWNERSHIP AND CONSENT
I am the owner/agent of the above-described animal and have authority to consent to its treatment. I assume full financial responsibility for all charges rendered for diagnostic, therapeutic, surgical, preventive procedures and infectious disease surveillance and control.

Owner's Signature _____

OUR PAYMENT POLICY
The Veterinary Health Center operates on a fee-for-service basis. Payment in full is expected at the time of discharge or dismissal. For animals admitted to the hospital, we require a deposit equal to one-half of the high end of the estimate range. The remainder of the balance should be paid upon discharge. All other payment arrangements must be made in advance. Please initial the expected method of payment.
Cash____ Check____ Credit Card____ Debit Card_____

I have read and agree to comply with the payment policy as stated above. _____ Please

Please indicate if your animal has any of the following conditions. (circle one)

Medication reaction Yes No Do not know
List drug and reaction if possible_____
Up to date on rabies vaccination Yes No Do not know

Does your pet have Pet Health Insurance?_____If yes, name of insurance co_____
By indicating yes to your pet having Pet Health Insurance, you have authorized release of your pet's VHC medical record to a PHI Company if requested.

Uncovered by a White Coat Waste investigation

No. 44520

Jefferson City Animal Shelter
2308 Hyde Park Rd.
Jefferson City, MO
573-634-6429

Adoption/Redemption Form

Owner Information

Name University of Missouri VHC
Address [redacted]
City Columbia State Mo Zip 65211
Telephone: Cell [redacted] Home
Email [redacted]

Animal Information

Animal unique ID 26591 BBB Microchip 982366292

Fees Due

Adoption $ Placement Boarding $ Rabies $ Donation $ Total $ Placement

Payment Method: ☐ Cash ☐ Credit/Debit Card ☐ Check # ____ Intent

Rabies

☑ Vaccination Current ☐ Vaccination Not Current ☐ Too Young for Vaccination

If my animal is not current on its rabies vaccination, I do hereby agree to obtain a rabies vaccine from a licensed veterinarian prior to the expiration date listed below, to be in compliance with Sections 5-22 & 5-45 of the Code of the City of Jefferson. Yearly re-vaccination for rabies is required by City Ordinance.

Voucher Expiration Date N/A *Pink copy, if provided to the owner is good for.*

☐ $ N/A *towards the cost of a rabies vaccination by a licensed veterinarian at a participating clinic for owner redeemed pets*

☐ *A no cost rabies vaccination by the Jefferson City Animal Shelter for an adopted pet that was too young at the time of adoption for its initial rabies vaccination included in the adoption fee*

Acknowledgment

I understand that the shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the time of adoption, which the adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

[redacted] 5/9/24
Signature of Owner Adoption/Redemption Date

Signature of Shelter Staff

Distribution: WHITE – Shelter copy YELLOW – Owner copy PINK – Rabies voucher

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter

2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Feed 1.5c dry SID



Animal Profile

Pet Name	**WESLEY**
Species	**CAT ID#: 26591**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Age	**3-4 YEARS DOB: 01-01-1900 0 DAYS**
Gender	**MALE/STERILIZED**
Color	**BLACK / WHITE**
Size	**MEDIUM**

Case Information

Case ID	**04-17-2024 3**	Status	**HOLDING TX URI**
Shelter ID/Kennel	**BBB**		
Arrival Date	**04-17-2024**	Collar Type	**NO COLLAR**
Arrival Reason	**STRAY**	Collar Color	
Transport	**CITIZEN TURN-IN**	License	
ACO		Vacc ID	**15467**
Precinct	**PRECINCT 2**	Microchip	**608366292**

Notes

Name: Wesley
Age: 3-4 Years
Breed: Domestic Short Hair
Gender: Neutered Male
Good with Dogs: unknown
Good with Cats: Unknown
Good with Children: Unknown
Current weight: 10.3 lbs
FeLv/FIV test: Neg

[Doxycycline 0.9mL BID x 5days]
24 25 26 27 28
29

Adoption Fee: $50

Misc. Information: Wesley as brought to the shelter as a stray, so there's limited information about his background. He has been a pretty sweet boy while he has been here at the shelter

The shelter provides a 5-day health guarantee for adopted animals. This includes health conditions not present or known about by the shelter at the of adoption, which adopter becomes aware of within 5 days. Injuries or accidents that occur at the adopter's home are not covered. The shelter is not responsible for incurred costs from local veterinarians due to non-compliance.

ALL adoptions are final! Animals can be returned to the shelter for any reason, however, there are NO refunds or exchanges.

Animals altered here are tattooed with a green abdominal line.

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Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Kennel Card

Animal Profile
Case Information
Behavioral Assessment
Notes

Uncovered by a White Coat Waste investigation

Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Microchip Certificate

University Of Mo Uhc
[redacted]

Microchip Information

ID	**608366292**
Type	**AVID**
Effective	**04-24-2024**

About Wesley

Species	**CAT ID#: 26591**
Breed	**DOMESTIC SHORT HAIR**
Gender	**MALE / STERILIZED**
Color	**BLACK / WHITE**

Microchip **608366292**

Animals altered here are tattooed with a green abdominal line.
Printed Using: Shelter Pro

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Jefferson City Animal Shelter

2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

Vaccination Certificate

University Of Mo Uhc
[redacted]

Vaccination Information

ID	**15467**
Type	**RABIES**
Effective	**04-24-2024 TO 04-23-2025**
Precinct	**PRECINCT 1**
Issued by	**SCHALLER, TRAVIS**
Manufacturer	**ZOETIS DEFENSOR 1**
Lot / Serial	**690759**

About Wesley

Species	**CAT ID#: 26591**
Breed	**DOMESTIC SHORT HAIR**
Gender	**MALE / STERILIZED**
Color	**BLACK / WHITE**

Microchip **608366292**

Rabies vaccine admnistered by:
Travis Schaller, DVM License No. 2021018110
Greg Popp, DVM. License No. 03247

Schaller DVM

Animals altered here are tattooed with a green abdominal line.

Printed using ShelterPro

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Jefferson City Animal Shelter
2308 HYDE PARK ROAD
JEFFERSON CITY, MO 65109
Phone: (573) 634-6429

ANIMAL MEDICAL HISTORY

University Of Mo Uhc
[redacted]

Species	**CAT ID#: 26591**
Pet Name	**WESLEY**
Primary Breed	**DOMESTIC SHORT HAIR**
Crossbreed	
Gender	**MALE/STERILIZED**
License	
Vacc ID	**15467**
Microchip	**608366292**
Age	**3-4 YEARS**
Size	**MEDIUM**

04-17-2024 - Medical Visit - these items were performed

Date	Category	Detailed Description	Status/Result
04-17-2024	Medical visit	Date:04-17-2024	Closed:05-09-2024
Note:	4/24: 10.3 lbs		
04-17-2024	Vitals	Weight=?,Temp=?	
04-17-2024	Vaccination	FVRCP 3W	
04-24-2024	Vaccination	RABIES	
04-24-2024	Medication	STRONGID3W	
04-24-2024	Vaccination	REVOLUTION	
04-24-2024	Service	VETERINARIAN EXAM	
04-24-2024	Service	SPAY/NEUTER	
04-24-2024	Service	MICROCHIP IMPLANT	

Follow-ups - these items were scheduled and should be completed on the due date

Due Date	Category	Detailed Description
05-08-2024	Vaccination	FVRCP 3W
05-24-2024	Vaccination	REVOLUTION
04-24-2025	Vaccination	RABIES

End of items for this medical visit.

Animals altered here are tattooed with a green abdominal line.

Printed using Shelter Pro

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Protocol 24580 Amendment 5.0

Approval date	02/02/2023
Expiration date	07/21/2024

1. Basic Information

1. Elements ID

   For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

   10200

2. eACUC Number (Automatically Assigned)

   24580

3. Principal Investigator

   Lyons, Leslie A

Job title	GILBREATH-MCLORN ENDOWED PROFESSOR OF COMPARATIVE MEDICINE ADJUNCT PROF. DIVISION OF ANIMAL SCIENCES
Department	Veterinary Medicine & Surgery
Division	Veterinary Medicine
Business unit	University of MO-Columbia

4. Protocol Title

   Dietary Management of Polycystic Kidney Disease 10200

5. Triennial Re-write

   Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

   ○ Yes ● No

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

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Species	Strain/Stock/ Breed	Age/Weight	Pain/ Distress Category	Authorized	Ordered	Received	Adjustment	Available
Cat	Domestic cats with and without DNA mutation for polycystic kidney disease.	Post-weaning ages and all weights for breeding; and feeding trial, kittens will be produced for this study but start trial post-weaning.	USDA Category C	30		9		21
Total Cats:				30	0	9	0	21

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

○ Yes ● No

3. Proposal Overview

1. Purpose

Purpose of the study:

Cats are a biomedical model for humans with polycystic kidney disease (PKD). We hypothesize the cells with autosomal dominant polycystic kidney disease (ADPKD) variants in PKD1 are highly dependent on glucose, which can be used as a therapeutic approach to the treatment of ADPKD in humans and cats via dietary interventions. The purpose of the study is to prove ketogenic diets, which limits glucose, reduce cystic volume, and thereby kidney volume, in cat patients with PKD. Reduction of cystic and kidney volume will prolong kidney function and support a longer and better quality of life of patients with PKD. Cats will be fed a ketogenic diet and kidney cyst and kidney volumes will be measured by radiographic imaging techniques to qualify changes during the time course of the feeding trials. A fasting trial in cats was previously conducted and was highly successful (see attached - published Torres et al.), suggesting the ketogenic diets have good promise for effective treatments for PKD.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

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Autosomal dominant (ADPKD) is a common, life-threatening genetic disease that causes immense human suffering and places a significant economic burden on the health care system. Most human patients will require dialysis or kidney
transplantation. Although PKD is a very common genetic disease, there is no treatment stopping the development of the cysts, in humans or cats. Recent studies have suggested diets low in sugar, glucose, may cause the cysts to become smaller and perhaps rupture and disappear (See attached published paper (Torres et al., and other literature). High protein, high fat, low carb diets (i.e., Atkins, interval or fasting diets) are know to induce ketosis, which is when the body burns fat to produce energy. The cats on this study will be provided a balanced diet for normal growth and maintenance. However, the diet will be modified to induce a keotgenic state in the cats, i.e., a diet high in fat, adequate in protein and low in carbohydrates and containing beta-hydroxybuturate (BHB). Should the ketogenic diet suggest cyst supression or regression, humans could also use diets and not drugs to prevent progression of their PKD and thereby avoid end-stage renal disease.

PKD negative cats will firstly be provided a formulated but non-ketogenic diet to establish palatability and time to transition to the formulated diet from their current commerical diet (Royal Canin Adult Persian) . Next, the PKD negative cats will be transistioned to a formulate ketogenic diet, again to establish palatability and transition time but also to establish the time to induction of ketosis. Weight, activity level, general health and fecal scores will be monitored during the transitions and feeding trials. Should the cats have continued diarrhea or weight loss, they will be removed from the study and transitioned back to the standard commercial diet. Once the formulated ketogenic diet is accepted by the PKD negative cats, the ketogenic diet will be similarly introduced to the PKD positive cats as part of the feeding trial. The cats will be individually or small grouped housed once they readily accept the new food. Periodically, blood and urine samples will be collected to monitor ketosis in the PKD postivie and negative cats. Cat cystic and kidney volumes will be monitored by ultrasound (US) (pre-trial, monthly, post-trial) and quantified by computed tomography (CT) imaging at the VHC (pre-trial, mid-trial and post-trial, 3 month intervals). Blood and urine samples (cystocentesis) will be collected prior to imaging. Mild to full sedation may be required for imaging. The attending anesthesiologist (Dr. John Dodam) will select and monitor the anesthesia and post-anesthesia recovery. Weekly monitoring of ketosis can be conducted by ear pick blood sampling and urine will be collected using specialized litter boxes. We do not expect pain beyond that experienced during placement of a catheter for CT imaging, blood collection by venipuncture or urine collection by cystocentesis. We expect the cats to have balanced nutrition for maintenance and growth. Once transitioned to the trial diets, we anticipate normal group housing for the cats. Cats will be transitioned to the trial diets after weaning but the ketogenic trials will begin at 8 months of age, once a majority of growth has been completed. Post-trial, cats will be euthanized to procure kidney tissues for further investigations or be maintained for future projects or adopted with ACUC approval.

3. Lay Term Description of Experimental Design

To put something in layman's terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This section should be written so that someone with a **10th grade science education can easily understand the project.**

Autosomal dominant (ADPKD) is a common, life-threatening genetic disease that causes immense human suffering and places a significant economic burden on the health care system. Most patients will require dialysis or kidney
transplantation. Polycystic kidney disease (PKD) is an inherited condition that causes the growth of fluid-filled cysts in the kidneys. The cysts become so large they eventually destroy the normal kidney tissue and cause kidney failure. Mutations in the gene called polycystin - 1 (PKD1) causes a majority of this disease in humans. Cats have the exact same disease in the exact same gene. Although PKD is a very common genetic disease, there is no treatment stopping the development of the cysts, in humans or cats. Recent studies have suggested diets low in sugar, glucose, may cause the cysts to become smaller and perhaps rupture and disappear (See attached paper (Torrres et al.) and other literature). These high protein, high fat, low carb diets

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(i.e., Atkins, interval or fasting diets) are know to induce ketosis, which is when the body burns fat, not sugar, to produce energy. Because we have cats with PKD, and we have good ways to measure the sizes of the cysts and the kidney sizes with ultrasound, MRI and or CT, this study will test if low glucose (ketosis) will shrink the cysts in the cats with PKD. To cause low glucose, cats will be fed a diet rich in fat and protein but the diet will still be balanced for proper nutrition and growth. Blood and urine samples will be collected to determine when ketosis occurrs and is maintained and also the health of the cats. Weight, body condition and fecal scores and or behavioral changes will also indicate the cats' health and acceptance of the formulated diets. Non-invasive imaging techniques, including ultrasound (US) and computed tomography (CT), will be used to monitor the size of the cysts in the cats' kidneys. The feeding trial will continue for a minimum of 6 months and a maximum of 12 months, with CT monitoring at pre-trial, mid-trial, post-trial, and 3 month intervals. Significantly smaller cyst volumes in PKD positive cats on the diet versus the cyst sizes of control PKD - positive cats not on diet will indicate success and dictate the end of the trial, between 6 - 12 months.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

Induction of the metabolic state of ketosis leads to strong inhibition of renal cyst growth, and even regression of existing cyst burden. Ketosis is the natural response of the body to periods of fasting that involves a shift in the utilization of fuels for energy. Whereas glucose is normally the primary fuel for most cells, fatty acids and ketone bodies become the primary fuel during ketosis. A mild reduction in food intake surprisingly strongly inhibits PKD progression in a Pkd1 mouse model, and our recent fasting study in cats indicated the same effects. The beneficial effect was actually not due to caloric restriction per se but rather to the effects of ketosis. Our pilot study on 72-hr fasted cats indicated over a 10% reduction in cystic volume in PKD affected cats. Therefore, this NIH funded study will formally investigate ketogenic diets for dietary management of PKD using the domestic cat model for ADPKD.

1. Cats from our breeding colony (Protocol 9642) will be mated to produce age and gender matched cohorts for the feeding trails. Six (6) PKD negative cats will be used on a feeding trial (see below). Cats on feeding trials = 24 (PKD +) + 6 (PKD -) = Category C.

2. All kittens will be genotyped for the PKD mutation by the Lyons laboratory using DNA isolated from buccal swabs of each kitten.

3. A balanced non - ketogenic diet will be formulated and 6 PKD NEGATIVE cats (after weaning, ages less than one year, ~5 - 7 months of age) will be transitioned to the diet to determine palatability. Changes in diet palatability, composition and transition time may be required to get the cats to accept the diet. (Diet is overseen by Dr. Backus (VHC nutritionist). Cats are expected to be on the diet for 2 - 3 weeks, long enough to determine palatability and get a baseline of any health changes. CBC, serum chemistries, urinalyses, and BHB quantification will be determined at baseline and after acceptance of the diet - expected within 2 - 3 weeks (5 ml anti-coagulant / serum sample collection, urine by cystocentesis). Weight, body condition and fecal scores and or behavioral changes will also indicate the cats' health and acceptance of the formulated diet.

4. After acceptance of the formulated, non-ketogenic diet, the same 6 PKD negative kittens will be transitioned to a balanced ketogenic diet to determine palatability and induction of ketosis (`8 months age). Changes in diet palatability, composition and transition time may be required to get the cats to accept the diet. (Diet is overseen by Dr. Backus (VHC nutritionist). Cats are expected to be on the diet for 2 - 3 weeks, long enough to determine palatability and get a baseline of any health changes and induction of ketosis. CBC, serum chemistries, urinalyses, and BHB quantification will be determined at baseline and after acceptance of the diet - expected within 2 - 3 weeks (5 ml anti-coagulant / serum sample collection, urine by cystocentesis).

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Cats will be monitored for ketosis by blood collection at day 5 and day 10 via blood collection (500 ul serum - likely ear prick). Weight, body condition and fecal scores and or behavioral changes will also indicate the cats' health and acceptance of the formulated diet. Kittens will be transitioned back to the normal diet after completion of the diet assessments. These cats can be maintained as new breeding stock, euthanized, transitioned to other protocols or adopted with ACUC permission.

5. PKD positive kittens will be imaged at ~ 6 - 8 months of age to determine rate of progression of the PKD disease (Attached publication - Cat PKD Imaging Progression).

6. Age / gender/ PKD progression matched cohorts will be established for cats with PKD. 50% of PKD positive cats will be on the formulated ketogenic diet and 50% will be on the formulated non-ketogenic diet. (3M:3F fast, 3M:3F slow - on diet; 3M:3F fast, 3M:3F slow - formulated, non-ketogenic diet = 24 cats). Slow and fast are relative to the cohort. Slow progression cats will be the kittens with the least number and lowest cystic volumes. Fast will imply the kittens with the most number of cysts and greatest cystic volume.

7. The cats of the feeding trial cohorts (PKD Positive cats) will be imaged by CT to establish baseline cystic and kidney volumes. CBC, serum chemistries, urinalyses, and BHB quantification will be determined at baseline (3 ml anti-coagulant / serum sample collection, urine by cystocentesis). Sedation or anesthesia and blood and urine sample collection will be conducted by Dr. Dodam (VHC radiologist). Imaging will be overseen by Dr. Karnia (VHC radiologist).

8. Feeding trial cohort PKD positive cats will be transitioned to firstly the formulated diet and then the formulated ketogenic diet using the time-frame established by the PKD negative cats. Monitoring of the cats will be the same as indicated for the PKD negative cats.

Addendum 03 June 2022 - Water consumption will be monitored via deuterium oxide dilution determination of body water mass and body water turnover to confirm cats are maintaining sufficient hydration, which could affect renal function and cystic volumes if compromised.

Food will be withheld overnight while water is continuously present. After body weight determination, each cat will receive a single subcutaneous injection of 0.7 g per kg body weight of deuterium oxide (D2O) containing dissolved sodium chloride at isosmotic strength (9.0 g/L). The "salinated" D2O solution will be made sterile by passage through a non-pyrogenic sterile disk filter with submicron pores (0.2 micron) into a sterile multidose vial. The injected D2O will be allowed to equilibrate in body water for 3 hours while food continues to be withheld. Thereafter, jugular venous blood (1 mL) with be collected by venipuncture for extraction of serum to be later analyzed by FTIR spectrometry for deuterium content. Cats will be then allowed access to food. The jugular venous blood collection will be repeated on days 3, 7 and 14 after the D2O injection so the rate of D2O washout and therefore water turnover can be estimated. Descriptions of the body water and water turnover estimation conducted by Co-I, RCB, are reported in the literature (Hooper et al 2017)."

Addendum 31 January 2023 - all 3 ml blood draws changed to 3 - 5 mls so that ketones can be evaluated with different methods. Allows maximum collection with one blood draw.

9. Once on the ketogenic diet, the cohort PKD positive cats cats will be monitored for ketosis at day 5 and day 10 via blood collection (500 ul serum - likely ear prick). Ketones will also be monitored by urine collection using inert litter in specialized urine collection litter boxes.

10. At 3 month intervals, cohort cats will be monitored for cyst and kidney volume by CT and or ultrasound imaging. Only mild sedation is expected for CT or US imaging. US imaging may not require any sedation. Full cbc, serum chemistries, urinalyses will be performed at time of imaging.

11. Feeding trial will continue for 6 - 12 months. Feeding trials could end at 6 or 9 months if significant cystic

Uncovered by a White Coat Waste investigation

volume reduction is observed.

12. Feeding trial cats may be euthanized for kidney sample collection at the end of the feeding trials. Otherwise, cats could be established as new breeding colony stock or adopted with ACUC permission.

4. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

Autosomal dominant (ADPKD) is a common, life-threatening genetic disease that causes immense human suffering and places a significant economic burden on the health care system. Most patients will require dialysis or kidney
transplantation. The vasopressin receptor antagonist tolvaptan has been FDA-approved as the first, and so far, only therapy for ADPKD in April 2018. However, tolvaptan is only moderately effective, problematic due to side effects including polyuria and potential liver toxicity, and its high cost has called the cost-effectiveness into question. Many other potential pharmacological approaches are being developed/tested and are promising in short-term preclinical studies using rodent models. However, translation of such approaches to the clinic has proved extremely challenging because ADPKD is a slowly progressing disease that likely requires pharmacological intervention for years and decades, starting long before patients may even exhibit significant symptoms of renal failure. Virtually all of the molecular drug targets implicated in ADPKD are not unique to polycystic kidneys. Therefore, potentially effective drugs also tend to cause extra-renal, long-term effects that would be prohibitive in clinical practice. Therapies for ADPKD need to demonstrate reduction in cystic and kidney volume to be considered effective. Tissue culture can not recapitulate the 3-dimensional structure and function of the kidney.

2. Justify Animal Species

Justify the choice of species for your study.

The cat has kidney and cysts sizes amenable to the use of imaging to monitor cyst enlargment or reduction. Also, the cat has a longer lifespan to demonstrate the efficacy of longterm therapies for ADPKD. Cats also have a mutation in PKD1 that causes similar protein loss of function to many humans with ADPKD.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total numbers should match the requested numbers in the species section.

- Animal Numbers Justification
- The Logical Determination of “N” in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

A previous pilot study used 4 cats on trial and 4 controls and successfully indicated reduction of cystic volume due to fasting. This study will induce ketosis at a slower rate, which needs to be maintained, therefore, the effects on the kidney cysts may be slower and must be demonstrated to be consistent over time. Cyst rate of progression in cats is highly variable, as in humans, thus the cohorts must be balanced with slow and fast progressors and cats of different genders. Ideally, a minimum of 3 fast, 3 slow progressors of each gender

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will be in a cohort (12 cats). Two cohorts - one on feeding trial, one not on feeding trial, implying 24 study cats - all PKD positive cats. The cohorts will also be aged matched. PKD negative cats (~6 cats) will be used to evaluate palatability of the diet, induction of ketosis, and time to transition required from the standard to the ketogenic diet. The same cats, all cats on the protocol, will be included in the water study.

5. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

	Facility
1	[redacted]

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

○ Yes ● No

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

● Yes ○ No

A. Description of Transportation

Describe how animals will be moved, including caging/transport carriers used (covered microisolator cages, dog/cat carrier, etc.), type of transport used (i.e. personal car or van), and projected transport time. (Note: Animals should be transported in a temperature controlled environment.)

Animals will be moved from [redacted] to [redacted] by hand carry using cat kennels. Transport would be across the parking lot to the next building, ~ 200 feet.

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

● Yes
○ No
○ Overnight only

i. Explain any food/fluid regulation and justify why it is needed

The cats will be provided a ketogenic diet and water, ad lib, they can eat as much as they chose. The D2O study is to monitor water intake to determine if weight change (if there is weight change) is fat or muscle loss.

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ii. Length of time the animal will undergo food/fluid regulation

The cats will be provided a ketogenic diet and water, ad lib, they can eat as much as they chose. The D2O study is to monitor water intake to determine if weight change (if there is weight change) is fat or muscle loss. The D20 study will be conducted once the cats have transitioned to the ketogenic diet and repeated at 6 months and end of trial, which may be 6 months. Food (but not water) will be withheld overnight (15 to 17 hours) prior to administration of D2O and for 3 hours thereafter to allow the D2O to equilibrate in the body water before blood sampling.

iii. Methods to Ensure Nutritional Intake and Hydration

Check all the methods that will be used to ensure adequate nutritional intake and hydration.

- ☐ Body weight
- ☐ Urine output
- ☐ Fecal output
- ☐ BUN
- ☐ Hct
- ☐ Food intake
- ☐ Fluid intake
- ☑ Other

i. Explain "other" method:

Time of diet presentation will be decreased for less than 24 hours on on each of two days that D2O is administered. When food is represented, the cats will be monitored to determine whether food intake resumed.

ii. Frequency of Checks

Initially after food is represented and the next day to assess the amount consumed.

iv. Restriction Baseline

Restriction protocols typically base the restriction amount relative to a baseline, (free-choice consumption) parameter (body weight, intake amount). What will this restriction amount use as the baseline?

The time of diet restriction will be less than 24 hours so body weight loss is not anticipated.

v. Maximum % Restriction

What is the maximum % restriction for any animal?

The time of diet restriction will be less than 24 hours so the percentage restriction will be be negligible.

vi. Provision to Assure Nutritional Needs are Met

Growing animals must be frequently re-assessed to ensure normal growth patterns. What provisions will be made for these animals to assure that their nutritional needs are maintained?

The time of diet restriction will be less than 24 hours so the nutritional impact will be be negligible.

vii. Water/Fluid Availability

The Animal Welfare Act states that if “water is not continually available, it must be offered to them as often as necessary to ensure their health and well-being, but not less than twice daily for at least

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1 hour each time, unless otherwise required by the attending veterinarian". Will animals be provided access to water/fluids twice daily for at least 1 hour each time (continuously for dogs)?

● Yes ○ No

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

- ☐ Single housing of social species
- ☐ Wire-bottom cages
- ☑ Special diet/water
- ☐ Extended time to weaning
- ☐ Extended time between cage changes
- ☐ Alternative light cycles
- ☐ Out of range temperatures
- ☐ Cage-size exceptions
- ☐ Other

i. Explain non-standard husbandry and list the length of time the animal will undergo non-standard husbandry.

Two semi-purified diets formulated and prepared by the Co-I (Backus) are to be used (one ketogenic, one standard). The standard will be nutritionally complete and balanced for feline growth. This diet will not be formulated to promote ketogenesis and will be fed to post-weaned cats (> 5 - 7 months) to habituate them to acceptance of a purified diet. Ingredients and nutrient composition of the diet will be similar to those used in previous research (Backus et al 2007 - attached). Protein, fat, and carbohydrate contents of the diet will be similar to those commonly in commercially available feline growth diets. A second diet will be intended to promote ketogenesis. This diet will be complete and balanced for feline maintenance and will be high in fat, adequate in protein, low in carbohydrate, and containing a magnesium salt of beta-hydroxybutyrate. This diet will be given to cats greater than 8 months in age, an age when cats are near mature body weight and high nutrients demands of growth have waned. The beta-hydroxybutyrate will be added in an amount found tolerated and effective in a rat model of PCKD (Torres et al 2019 - attached). The cats will be daily presented with diet for ad libitum consumption unless undesired weight gain is observed, when as needed amount of diet presented may be limited for maintaining a healthy body condition. Acceptance and tolerance of the diet will be assessed from monitoring of amount of diet daily consumed and change in body weight every two weeks. Full trial 6 - 12 months.; overnight restriction only for CT imaging. Food (but not water) will be withheld overnight (15 to 17 hours) prior to administration of D2O and for 3 hours thereafter to allow the D2O to equilibrate in the body water before blood sampling. After the blood sampling, food will be represented.

6. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

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● Yes ○ No

A. Sample Type

Type of sample(s):

Blood sample; urine sample

B. Sample Volume

Volume of sample(s):

Blood - 500 ul, 5 ml; urine 5 - 10 ml

C. Sampling Frequency and Duration

Frequency of collection and for how long:

Blood 500 ul at 5 & 10 days to monitor ketones; 5 ml - pre-trial at CT, every 3 months, post-trial at CT; urine - free catch litter box - weekly to monitor ketones; pre-trial at CT, every 3 months, post-trial at CT. Urine collection is via cystocentesis during CT and or US monitoring. 5 ml at time 0, day 3, day 7 and day 14 for deuterium oxide wash out.

D. Sampling Method

Method of collection:

500 ul ear prick or venipuncture jugular; 5 ml - venipuncture jugular; urine - free catch litter box weekly; cystocentesis pre-trial at CT, every 3 months, post-trial at CT. venipuncture jugular for deuterium oxide wash out.

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

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	Procedure	Description of procedure	Building name	Room number or area
1	Overall health monitoring	Health parameters will be monitored on a regular basis, including weight, body condition score (BCS), fecal scores, activity level for all cats on the protocl. Weights and BCS will be examined weekly, fecal scores and activity level daily. Any suggestion of detrioration in health, consistent loss of weight, decreasing BCS or reduced activity, poor fecal scores, will suggest the cat is not adapting to the diets and the cats will be transitioned back to normal diets. We anticipate that some cats may not transistion to the formulated diets, jsut as any cats may have more particularly dietary preferences.	[redacted]	[redacted]
2	CT Imaging	CT scans will be performed using a third generation 64 slice instrument (Aquillion 64, Canon Medical Systems, Tustin, CA, USA) using mild sedation at the VHC.. Each cat will be placed in dorsal recumbency and pre- and post-contrast images will be obtained from the diaphragm through the anus (slice thickness 1–2 mm, kvp 80–100, mAs 230–500). Intravenous non-ionic, iodinated contrast media (Omnipaque 350 Iohexol injection, GE Healthcare, Marlboro, MA, USA) will be administered at a dose of 0.5 mg/kg and post-contrast images will be obtained after a three-minute delay.	[redacted]	[redacted]
3	US Imaging	The US examinations will be performed using an 8 MHz micro-convex transducer on a dedicated ultrasound unit (Logiq 9, GE Healthcare, Wauwatosa, WI, USA). Each kidney will be scanned in sagittal and transverse planes. Dr. Karnia wil perform US imaging. The length will be measured, using internal digital calipers, as the longest point between the cranial and caudal poles in the sagittal plane. Width and height will be measured in the transverse plane. Total kidney volume will be calculated using the prolate ellipsoid formula	[redacted]	[redacted]
4	Water intake monitoring via deuterium wash out	Food will be withheld overnight while water is continuously present. After body weight determination, each cat will receive a single subcutaneous injection of 0.7 g per kg body weight of deuterium oxide (D2O) containing dissolved sodium chloride at isosmotic strength (9.0 g/L). The “salinated” D2O solution will be made sterile by passage through a non-pyrogenic sterile disk filter with submicron pores (0.2 micron) into a sterile multidose vial. The injected D2O will be allowed to equilibrate in body water for 3 hours while food continues to be withheld. Thereafter, jugular venous blood (5 mL) with be collected by venipuncture for extraction of serum to be later analyzed later by FTIR spectrometry for deuterium content. Cats will be then allowed access to food. The jugular venous blood collection will be repeated on days 3, 7 and 14 after the D2O injection so the rate of D2O washout and therefore water turnover can be estimated. Descriptions of the body water and water turnover estimation conducted by Co-I, RCB, are reported in the literature (Hooper et al 2017).	[redacted]	[redacted]

7. Substances Used in Animals

1. Substances Used in Animals

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List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

	Substance	Amount/ Dose/ Volume	Route	Frequency/ Duration	Hazard	Pharmaceutical Grade
1	Formulated Ketogenic diet	ad lib	by mouth	daily / 6 - 8 months	No	No
2	Formulared growth diet	ad lib	by mouth	daily / ongoing	No	No
3	Omnipaque (Iohexol)	0.5 mg/kg	I.V.	twice / pre and post trial CT	No	Yes
4	Deuterium oxide (D2O) (99.8% Sigma-Aldrich, St. Louis, USA) containing dissolved sodium chloride (MSDS attached)at isosmotic strength (9.0 g/L)	0.7 g per kg body weight	sub-cutaneous	3 times / 10 min	No	No

2. Non-Pharmaceutical Grade Substances

For those substances that are marked “no” as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

Deuterium oxide (D2O) is a non-radioactive, stable-isotopic form of water that occurs naturally in water at a concentration of about 150 parts per million. D2O behaves chemically the same as water (protium oxide, H2O) and is not hazardous so PPE is not needed when it is handled. Feeding studies on mice show that as much as 40% of body water can be in the form of D2O without ill effect. An isotonic, sterile solution at the D2O enrichment to be used is not commercially available. The solution will be prepared by dissolving pharmaceutical grade 0.9 g of sodium chloride in 100 g of water that is 998 parts per million of D2O. The solution will be passed though a sterile, nonpyrogenic, 0.2 micron, syringe filter into a sterile glass vial fitted with a crimp-top, rubber stopper for later aseptic insertions of a hypodermic needle for withdrawal of the contents.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., “All substances” or “non-hazardous substances”) if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

	Substance	Gloves	Eye Protection	Lab Coat	Face Mask	Fume hood	Biosafety cabinet	Double-Gloves	Other	Other PPE
1	deuterium oxide	☑	☐	☑	☐	☐	☐	☐	☐	

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Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

8. Hazardous Materials

1. Will you use any Biological Hazards?

○ Yes ● No

2. Will you use any Chemical Hazards?

○ Yes ● No

3. Will you use any Radiation Hazards?

○ Yes ● No

9. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

● Yes ○ No

2. Preanesthetic Agent(s)

List preanesthetic agents here

No Preanesthetic Agents...

3. Anesthetic Agent(s)

List anesthetic agents here. **Do not list isoflurane here, it will be listed later in the form.**

	Species	Agent	Controlled Substance	Dose/Volume	Route	Frequency of Admin.
1	Cat	formulary table attached		formulary table attached	formulary table attached	formulatory table atached

4. Isoflurane Use

A. Will you use isoflurane?

● Yes ○ No

B. What species?

domestic cat

C. How will it be administered?

☑ Vaporizer with nose cone

☐ Vaporizer with endotracheal tube

☐ Induction box connected to vaporizer

☐ Open drop method (bell jar, etc.)

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☐ Other

5. Non-Pharmaceutical Grade Anesthesia

If non-pharmaceutical grade anesthesia must be used, strong scientific justification must be provided. In addition, describe how the solution will be made and stored. Also, describe how pH, osmolarity, and sterility will be kept in acceptable physiological ranges. Please see ACUC policies for Anesthesia and Non-pharmaceutical Anesthesia and ACUC Guidelines for pentobarbital, avertin, inactin, and urethane/chloralose.

n/a

6. Monitoring and Life Support

Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:

Monitoring and life support: For animals that have undergone sedation, monitoring and life support will follow standard veterinary practices. Heart rate and respiration will be monitored along with mucous membrane color and body temperature when indicated. Intravenous crystalloids or other supportive therapy will be administered when needed. Depth of anesthesia will be monitored by assessing heart and respiratory rates, palpebral reflex, and withdrawal reflex. Life support parameters evaluated every 5 minutes and recorded every 15 minutes until sternal. Imaging procedures from time of sedation to sternal recovery is expected to be less than one hour.

7. Post Anesthetic Recovery

Complete description of post-anesthetic recovery monitoring and care:

The animals will be recovered from sedation in the room adjacent to the CT imaging room in the VHC. The animals will be directly observed until completely recovered by one of the veterinarians or veterinary technicians Hourly, serial neurologic examinations will be performed by assessing mentation, body position, mobility and cranial nerve evaluation, and rectal temperature, pulse and respiration will be monitored hourly until the cats are sternal, bright, alert and responsive. If rectal temperature falls below 100 F a thermal heating blanket will be placed beneath the animal..

8. Pharmaceutical Analgesia

● Yes ○ No

9. Pharmaceutical Analgesia

	Species	Agent/Non-pharm.	Dose/Volume	Route	Frequency of Admin.
1	Cat	Formulary attached	formulary attached	formulary attached	formulary attached

10. Non-pharmacologic control of pain

○ Yes ● No

11. Paralytic Agents

○ Yes ● No

12. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

● Yes ○ No

13. Antibiotics and Other Agents

List other agents such as antibiotics and other emergency drugs

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	Species	Agent	Dose/Volume	Route	Frequency of Admin.
1	Cat	formulary attached	formulary attached	formulary attached	formulary attached

10. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

○ Yes ● No

11. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

Diarrhea or other gastric distress from diet; hematoma from blood draws; infection from cystocentesis, normal risks of anesthesia. The D20 solution injections will present no greater risk for adverse effects than sterile isotonic saline injections. As with any injection, an unanticipated result could be development of a hematoma or an inflammatory response secondary to trauma or sepsis.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

The protocol is not intended to cause pain or distress. Momentary pain for catheter placement, cystocentesis, restraint for ultrasound and blood sampling. Cats will be handled often for behavioral enrichment and to minimize stress during restraint for sample collection and imaging. Trained personnel will collect samples, cats will be sedated if more than mild restraint is required. Cats with diarrhea or gastrointestinal distress will be transistioned to normal diet and treated according by the veterinarians for fluid loss and electrolyte balance. After blood collections and subcutaneous injections, activity and appetite will be assessed and hypodermic needle insertion sites will be inspected for development of swelling, pain, and/or discharge on the day of injection and during the week thereafter for determination of need for treatment.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

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○ Yes ● No

12. Disposition of Animals

1. Animal Disposition

Check all that apply

- ☑ Adoption (See MU adoption policy)
- ☐ Market
- ☑ Euthanasia
- ☑ Transfer to different project, PI, or another institution
- ☑ Returns to breeding colony, herd, source, owner, or wildlife site
- ☐ Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, "Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue."

A. Method of Euthanasia

Select the method of euthanasia

- ☐ Inhalant agent
- ☐ Physical Method without Anesthesia
- ☐ Physical Method with Anesthesia
- ☑ Noninhalent Pharmaceutical Agent

B. Euthanasia Descriptions

	Species	Agent/Method	Dose/Volume	Route
1	Cat	Barbituate overdose	> 86 mg/Kg	i.v.

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

Cats will be sedated prior to IV administration of barbituate overdose for euthanasia.

D. Scientific Justification for Use

- ☑ AVMA Approved Method
- ☐ Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

- ☐ Bilateral pneumothorax
- ☐

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Cervical dislocation

- [ ] Decapitation
- [ ] Exsanguination
- [ ] Removal of vital organs
- [x] Other

i. Please specify:

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Auscultation of heart and lungs; reflex responses

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13. Project Information

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1.

Associate	Role	Responsibilities	OHSP Training	Animal Care & Use	Survival Surgery	P&D Training
Lyons, Leslie A [redacted]	Principal Investigator Authorized to order animals Editor		☑ Jul 24, 2023	☑ May 24, 2024	⊖	⊖
[redacted]	Co-Investigator Access to view cages Editor		☑ Sep 25, 2022	⊖	⊖	⊖
[redacted]	Key Personnel Access to view cages		⊖	☑ Jan 26, 2022	⊖	⊖
[redacted]	Key Personnel Access to view cages		☑ Dec 11, 2021	☑ Oct 21, 2021	☑ Oct 21, 2021	⊖
[redacted]	Key Personnel Access to view cages	P&D assessment	☑ Jan 30, 2023	☑ Nov 11, 2022	⊖	⊖
[redacted]	Key Personnel Access to view cages	P&D assessment	☑ Jan 26, 2023	☑ Nov 20, 2022	⊖	⊖
[redacted]	Key Personnel		⊖	⊖	☑ Oct 14, 2014	⊖
[redacted]	Key Personnel Access to view cages		☑ Dec 13, 2023	☑ May 29, 2024	⊖	⊖
[redacted]	Key Personnel		☑ Mar 24, 2023	☑ Mar 24, 2023	☑ May 12, 2020	⊖
[redacted]	Key Personnel Access to view cages		☑ Aug 27, 2023	☑ Aug 31, 2023	⊖	⊖
[redacted]	Key Personnel Access to view cages	P&D assessment	☑ Jun 14, 2022	☑ Oct 20, 2021	⊖	⊖
[redacted]	Key Personnel Access to view cages	P&D assessment	☑ Jun 14, 2022	☑ Oct 18, 2021	⊖	⊖
[redacted]	Key Personnel Access to view cages		☑ Sep 15, 2021	☑ Sep 15, 2021	⊖	⊖

2. Training and Qualifications

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Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	[redacted]	Veterinary nutritionist	Develop food for feeding trial, advice on trial, blood sample collection	years	Full-time employee
2	[redacted]	Veterinary anesthesiologist	Support for radiological imaging	years	Full-time employee
3	[redacted]	>4 years	Colony manager	>4 years	Full-time employee
4	[redacted]	Veterinary radiologist	Support ultrasound and CT imaging	years	Part-time employee
5	[redacted]	Veterinary student	Environmental enrichment, support with breeding and cat transports, blood sample collection, weights.	Veterinary student	Grad student/ Professional student
6	Lyons, Leslie A	29 years	Principal investigator of the project	All aspects	Full-time employee
7	[redacted]	Novice	Enrichment	Novice	Undergraduate student
8	[redacted]	Adjunct Instructor in the CVM's Department of Veterinary Medicine and Surgery employed to assist in the training second and third year veterinary professional students in surgery. Received a DVM degree from the University of California-Davis in 1989.	Assistance with restraint and blood collection	Experiences include one year in small animal practice in [redacted], ten years as a veterinary specialty technician supervisor at the teaching hospital of the [redacted], and seven years as an ICU technician at the [redacted].	Full-time employee

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	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
9	[redacted]	Veterinary resident [redacted]. Prior experience includes a 1 year rotating small animal internship at the [redacted]. Additional training includes veterinary medical education from [redacted].	Restraint for blood collection, animal feeding	Veterinary resident [redacted]. Prior experience includes a 1 year rotating small animal internship at the [redacted]. Additional training includes veterinary medical education from [redacted].	Grad student/ Professional student
10	[redacted]	Veterinary technician	Assist with blood draws, feeding, enrichment	Veterinary technician	Undergraduate student
11	[redacted]	Novice	enrichment, feeding	Training from Lyons laboratory	Undergraduate student
12	[redacted]	Novice	enrichment, feeding	Training from Lyons laboratory	Undergraduate student
13	[redacted]	Novice	enrichment, feeding	Training from Lyons laboratory	Undergraduate student

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- [x] PHS (NIH, CDC, FDA, NSF, NASA)
- [ ] DoD
- [ ] VA
- [ ] AHA
- [ ] USDA
- [ ] Foundation/Industry

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- ☐ Internal
- ☐ Other

14. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

○ Yes ● No

15. Investigator Assurances

1. ABSL-2 Assurance

I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

- ● Yes, I will meet the requirements of this statement.
- ○ No, I will not meet the requirements of this statement.
- ○ Not Applicable

2. Investigator Assurances

- ☑ 1. The information provided herein is accurate to the best of my knowledge.
- ☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
- ☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.
- ☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.
- ☑ 5. I assure that I am not unnecessarily duplicating previous experiments.
- ☑ 6. I have considered alternative methods to using animals.
- ☑ 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

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Recipient Information

1. Recipient Name
UNIVERSITY OF CALIFORNIA, SANTA BARBARA
3227 CHEADLE HALL
SANTA BARBARA, CA 93106

2. Congressional District of Recipient
24

3. Payment System Identifier (ID)
1956006145A1

4. Employer Identification Number (EIN)
956006145

5. Data Universal Numbering System (DUNS)
094878394

6. Recipient's Unique Entity Identifier
G9QBQDH39DF4

7. Project Director or Principal Investigator
Thomas Weimbs, PHD
Professor
weimbs@lifesci.ucsb.edu
805-893-4144

8. Authorized Official
Sarah Payne
proposals@research.ucsb.edu
805-893-4584

Federal Agency Information

9. Awarding Agency Contact Information
Tommy Gunter

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
gunterto@niddk.nih.gov
301-451-3447

10. Program Official Contact Information
Christine Maric-Bilkan
Program Officer
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
christine.maric-bilkan@nih.gov
301-435-0486

Federal Award Information

11. Award Number
5R01DK124895-05

12. Unique Federal Award Identification Number (FAIN)
R01DK124895

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
Ketosis as a therapy for polycystic kidney disease

15. Assistance Listing Number
93.847

16. Assistance Listing Program Title
Diabetes, Digestive, and Kidney Diseases Extramural Research

17. Award Action Type
Non-Competing Continuation (REVISED)

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information	
19. Budget Period Start Date 02/01/2024 **– End Date** 01/31/2025	
20. Total Amount of Federal Funds Obligated by this Action	$28,051
20 a. Direct Cost Amount	$19,625
20 b. Indirect Cost Amount	$8,426
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$448,839
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$448,839
26. Project Period Start Date 04/01/2020 **– End Date** 01/31/2025	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$2,183,834

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Norma G DeGuzman

30. Remarks

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

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RESEARCH
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION I – AWARD DATA – 5R01DK124895-05 REVISED

Principal Investigator(s):
Thomas Weimbs, PHD

Award e-mailed to: awards@research.ucsb.edu

Dear Authorized Official:

The National Institutes of Health hereby revises this award to reflect an increase in the amount of $28,051 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF CALIFORNIA SANTA BARBARA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Diabetes And Digestive And Kidney Diseases of the National Institutes of Health under Award Number R01DK124895. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please direct questions to the Federal Agency contacts.

Sincerely yours,

Norma G DeGuzman
Grants Management Officer
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Uncovered by a White Coat Waste investigation

Salaries and Wages	$138,180
Fringe Benefits	$26,184
Personnel Costs (Subtotal)	$164,364
Materials & Supplies	$35,904
Travel	$3,379
Other	$37,171
Subawards/Consortium/Contractual Costs	$71,083
Publication Costs	$2,112
Federal Direct Costs	$314,013
Federal F&A Costs	$134,826
Approved Budget	$448,839
Total Amount of Federal Funds Authorized (Federal Share)	$448,839
TOTAL FEDERAL AWARD AMOUNT	$448,839
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$28,051

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
5	$448,839	$448,839

Fiscal Information:
Payment System Identifier: 1956006145A1
Document Number: RDK124895A
PMS Account Type: P (Subaccount)
Fiscal Year: 2024

IC	CAN	2024
DK	8472278	$448,839

NIH Administrative Data:
PCC: KMB KPKD / **OC**: 41025 / **Released**: 05/13/2024
Award Processed: 05/14/2024 12:11:05 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R01DK124895-05 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – STANDARD TERMS AND CONDITIONS – 5R01DK124895-05 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor

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should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VII Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to obtain a unique entity identifier (UEI) and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a UEI requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01DK124895. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the Payment Management System (PMS) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the real-time cash drawdown data in PMS. NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.*

NIH requires electronic submission of the final invention statement through the Closeout feature in the Commons.

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NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and must be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

Recipients must administer the project in compliance with federal civil rights laws that prohibit discrimination on the basis of race, color, national origin, disability, age, and comply with applicable conscience protections. The recipient will comply with applicable laws that prohibit discrimination on the basis of sex, which includes discrimination on the basis of gender identity, sexual orientation, and pregnancy. Compliance with these laws requires taking reasonable steps to provide meaningful access to persons with limited English proficiency and providing programs that are accessible to and usable by persons with disabilities. The HHS Office for Civil Rights provides guidance on complying with civil rights laws enforced by HHS. See https://www.hhs.gov/civil-rights/for-providers/provider-obligations/index.html and https://www.hhs.gov/.

- Recipients of FFA must ensure that their programs are accessible to persons with limited English proficiency. For guidance on meeting the legal obligation to take reasonable steps to ensure meaningful access to programs or activities by limited English proficient individuals, see https://www.hhs.gov/civil-rights/for-individuals/special-topics/limited-english-proficiency/fact-sheet-guidance/index.html and https://www.lep.gov.
- For information on an institution's specific legal obligations for serving qualified individuals with disabilities, including providing program access, reasonable modifications, and to provide effective communication, see http://www.hhs.gov/ocr/civilrights/understanding/disability/index.html.
- HHS funded health and education programs must be administered in an environment free of sexual harassment; see https://www.hhs.gov/civil-rights/for-individuals/sex-discrimination/index.html. For information about NIH's commitment to supporting a safe and respectful work environment, who to contact with questions or concerns, and what NIH's expectations are for institutions and the individuals supported on NIH-funded awards, please see https://grants.nih.gov/grants/policy/harassment.htm.
- For guidance on administering programs in compliance with applicable federal religious nondiscrimination laws and applicable federal conscience protection and associated anti-discrimination laws, see https://www.hhs.gov/conscience/conscience-protections/index.html and https://www.hhs.gov/conscience/religious-freedom/index.html.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – DK SPECIFIC AWARD CONDITIONS – 5R01DK124895-05 REVISED

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

REVISION # 1:
In accordance with NOT-OD-24-109, and the availability of FY2024 appropriated funds, this award provides funding at the 96% committed level.

THE FOLLOWING TERMS FROM THE PREVIOUS NOTICE OF AWARD ALSO APPLY TO THIS AWARD:

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In accordance with the NIH Grants Policy Statement (NIH GPS) Section 2.3.7.9 Graduate Student Compensation, total direct costs (salary, fringe benefits and tuition remission) for graduate students must not exceed the Ruth L. Kirschstein National Research Service Award (NRSA) zero-level stipend in effect at the time of the competing award. Support recommended for future years has been adjusted accordingly, if applicable. See the NIH GPS for full details and a link to the schedule for NRSA stipends.

SPREADSHEET SUMMARY
AWARD NUMBER: 5R01DK124895-05 REVISED

INSTITUTION: UNIVERSITY OF CALIFORNIA SANTA BARBARA

Budget	Year 5
Salaries and Wages	$138,180
Fringe Benefits	$26,184
Personnel Costs (Subtotal)	$164,364
Materials & Supplies	$35,904
Travel	$3,379
Other	$37,171
Subawards/Consortium/Contractual Costs	$71,083
Publication Costs	$2,112
TOTAL FEDERAL DC	$314,013
TOTAL FEDERAL F&A	$134,826
TOTAL COST	$448,839

Facilities and Administrative Costs	Year 5
F&A Cost Rate 1	55.5%
F&A Cost Base 1	$242,930
F&A Costs 1	$134,826

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Electronic Cover Sheet		
PI: **Weimbs, Thomas**	Title: Ketosis as a therapy for polycystic kidney disease	
Received: 07/15/2019	Opportunity: PA-19-056 Clinical Trial:Not Allowed	Council: 01/2020
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1R01DK124895-01	Dual: AT	Accession Number: 4330605
IPF: 577509	Organization: UNIVERSITY OF CALIFORNIA SANTA BARBARA	
Former Number:	Department: Neuroscience Research Inst.	
IRG/SRG: KMBD	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 287,559 Year 2: 293,914 Year 3: 349,989 Year 4: 382,142 Year 5: 372,023	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Thomas Weimbs Ph.D.	The Regents of the University of California, Santa Barbara	PD/PI
Redacted by agreement		

Additions for Review

Accepted Publication Accepted publication

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED 2019-07-15

Application Identifier Weimbs20191670NRII

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **Organizational DUNS*:** 094878394

Legal Name*: The Regents of the University of California, Santa Barbara
Department:
Division:
Street1*: 3227 Cheadle Hall
Street2:
City*: Santa Barbara
County: Santa Barbara
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 93106-2050

Person to be contacted on matters involving this application
Prefix: First Name*: Redacted by Middle Name: Last Name*: Redacte d Suffix:
Position/Title: Sponsored Projects Analyst
Street1*: Redacted by agreement
Street2:
City*: Santa Barbara
County: Santa Barbara
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: Redacted by
Phone Number*: Redacted by Fax Number: Emai Redacted by agreement

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1-956006145-A1

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Ketosis as a therapy for polycystic kidney disease

12. PROPOSED PROJECT
Start Date* 04/01/2020
Ending Date* 03/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
CA-024

Uncovered by a White Coat Waste investigation

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Thomas Middle Name: Last Name*: Weimbs Suffix: Ph.D.
Position/Title: Professor
Organization Name*: The Regents of the University of California, Santa Barbara
Department: Neuroscience Research Inst.
Division:
Street1*: LSB, Room 2113
Street2:
City*: Santa Barbara
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 93106-5060
Phone Number*: (805) 893-4144 Fax Number: 805-893-2017 Email*: weimbs@lifesci.ucsb.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,602,764.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,602,764.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Redacted by Middle Name: Last Name*: Redacted by Suffix:
Position/Title*: Sponsored Projects Analyst
Organization Name*: The Regents of the University of California, Santa Barbara
Department: Office of Research
Division:
Street1*: Redacted by agreement
Street2:
City*: Santa Barbara
County: CA
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: Redacted by
Phone Number* Redacted by agreement Fax Number: 805-893-2611 Email*: proposals@research.ucsb.edu

Signature of Authorized Representative*
Redacted by agreement

Date Signed*
07/15/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:cover_letter_11012199308.pdf

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424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	UCSB
Duns Number:	094878394
Street1*:	LSB, Room 2113
Street2:	
City*:	Santa Barbara
County:	
State*:	CA: California
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	93106-5060
Project/Performance Site Congressional District*:	CA-024

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	University of Missouri
DUNS Number:	153890272
Street1*:	900 E Campus Dr
Street2:	
City*:	Columbia
County:	MO
State*:	MO: Missouri
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	65211-0001
Project/Performance Site Congressional District*:	MO-004

Additional Location(s)

File Name:

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056. Received Date: 2019-07-15T18:19:00.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ● No

IACUC Approval Date: 01-14-2019

Animal Welfare Assurance Number D16-00497

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project_Summary_Abstract1012199301.pdf
8. Project Narrative*	Project_Narrative1012199434.pdf
9. Bibliography & References Cited	Bibliography1012199306.pdf
10.Facilities & Other Resources	Facilities___Other_Resources1012199307.pdf
11.Equipment	Equipment1012199305.pdf

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056. Received Date: 2019-07-15T18:19:00.000-04:00

Project Summary/Abstract

We have previously shown that a mild reduction in food intake strongly inhibits progression of polycystic kidney disease (PKD) in an orthologous mouse model but we did not understand the mechanism. Now, we discovered that the metabolic state of ketosis is important, not caloric restriction *per se*. Dietary interventions leading to ketosis profoundly inhibit - and even reverse - PKD progression in orthologous and non-ortholgous mouse, rat and feline models of PKD. Remarkably, treatment with the ketone β-hydroxybutyrate (BHB) alone is *almost 100% effective in preventing PKD progression*. Preliminary results suggest that BHB acts on PKD kidneys *via* its receptor GPR109a, a GPCR that suppresses cAMP signaling. Our results suggest that cyst cells in PKD are metabolically inflexible, depend on glucose and are unable to shift to utilizing fatty acids and ketone bodies. The main thrust of this proposal is to generate compelling results to justify clinical trials to investigate the efficacy of dietary interventions and/or BHB supplementation in ADPKD, and to inform the design of such trials. The main significance of this proposal is the enormous potential for clinical translation. Dietary interventions to induce ketosis are well-established. Because dietary interventions frequently fail in clinical practice due to poor adherence, our finding that BHB (an FDA-classified dietary supplement) has a dominant beneficial effect could rapidly lead to a highly feasible therapy. To achieve our goals, we will treat rodent and feline models of PKD, with dietary interventions to induce ketosis (time-restricted feeding or ketogenic diets) or mimic ketosis by supplementation with BHB. Efficacy on parameters of PKD progression and effects on molecular mechanisms will be assessed. To determine whether BHB acts via GPR109a, we have crossed Gpr109a$^{-/-}$ mice with fast- and slowly-progressing Pkd1 mouse models. We will test whether Gpr109a knock-out affects disease progression and prevents the efficacy of ketogenic dietary intervention or BHB. Successful completion of the proposed work could lead to a disruptive change in ADPKD therapy by utilizing dietary interventions and/or dietary supplements without the need for pharmacological intervention.

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Project Narrative

We have discovered that the metabolic state of ketosis profoundly inhibits - and even reverses - the progression of polycystic kidney disease (PKD) in animal models. Inducing ketosis with dietary interventions or mimicking it by administration of the natural ketone body β-hydroxybutyrate (BHB) all proved highly effective in inhibiting renal cyst proliferation and fibrosis. Here, we propose to identify the most effective treatments and study the underlying mechanisms in order to be able to design a future clinical trial to test the efficacy of dietary interventions and/or treatment with BHB, an inexpensive and safe compound that is FDA-classified as a dietary supplement.

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Facilities & Other Resources

UCSB: The proposed work at UCSB will be carried out in the laboratory of the PI on the second floor of the "Life Sciences Building" (construction completed in 2005). The building houses approximately 12 laboratories of the Department of Molecular, Cellular, and Developmental Biology and additional molecular biology labs of the Department of Ecology, Evolution, and Marine Biology. It is located adjacent to the "Bio II" building which houses most other labs of MCDB and the Neuroscience Research Institute. Numerous additional "biomedical" research labs are present nearby on campus in additional departments and units including the Department of Chemistry and Biochemistry, the Department of Psychology & Brain Sciences, the Neuroscience Research Institute, several departments in the College of Engineering (including a new Bioengineering research building), Physics, Materials, the California NanoSystems Institute and others.

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LABORATORY:
Weimbs Lab: The Principal Investigator has a well-equipped ~1,800 square foot laboratory as part of an "open floor plan" laboratory for four investigators on the same floor. The laboratory has excellent gas, water, compressed air and vacuum facilities, fume hoods and sinks for handling radioactive materials and solvents, and adequate bench space to devote to this project. Twelve desks for personnel are located adjacent to the benches. Additional space is available in adjacent rooms: a central equipment room, a microscopy room, a fully equipped cell culture room with two laminar flow hoods and five CO2 incubators, a cold room, and a room containing glass washer/dryer and autoclaves.

Redacted by agreement laboratory has expertise in the ascertainment and development of biomedical models of the domestic cat and identified the PKD1 mutation in Persian cats. She has led the development of the 99 Lives Cat Genome Sequencing Initiative that includes the genomes of ~ 200 domestic cats from the feline research community. Redacted by ither leads or has direct collaborations on ~ 114 cats in the 99 Lives project that encompasses over 40 different disease or trait projects, including an undefined model of PKD in Siberian cats. Redacted by agreement
Redacted by agreement
Redacted by agreement . This space was renovated in 2013 and Redacted by agreement
Redacted by agreement. This space includes seven desk spaces with computers at the end of the laboratory benches for staff and students. Additional space includes a tissue culture room and storage area for freezers and other larger equipment, all within the same building. Freezers have automated failure reporting systems and generator power back-up.

CLINICAL:
N/A

ANIMAL:
UCSB: The Animal Resource Center (ARC), the primary animal care facilities at UCSB, complies with all federal, state, and local regulations and guidelines for laboratory animal care and is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The combined square footage of the two ARC animal facilities is approximately 12,000 ft2 within secure perimeters. Both are conventional, single corridor animal facilities with adequately wide hallways and sanitizable construction, designed and built in the early 1960s, and housing primarily rodent species (i.e., rats and mice). In addition to the animal husbandry facilities (i.e., animal holding and cage washing areas), the ARC contains dedicated

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animal surgical suites for rabbits or rodents, which are equipped with ceiling mounted surgical lights, either downdraft tables or a stainless steel surgical table, a boom-mounted surgical microscope, gas anesthesia machines, and anesthetic monitoring equipment (e.g., pulse oximeter). The ARC facilities also contain a small diagnostic laboratory, a necropsy room, and a small pharmacy, all of which are available to Principal Investigators.

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COMPUTER:

Weimbs Lab: Approximately twelve desktop computers are available in the Principal Investigator's office and laboratory spaces. All computers have software for word processing, calculation, image processing and analysis, bioinformatics. All computers are either connected to a gigabit ethernet network or fast WIFI and are connected to a NAS drive used for data storage, file sharing and automatic data backups. The primary NAS drive is mirrored on a backup-NAS drive located in a different, locked space and additionally backed up daily using a cloud server to prevent data loss. Peripherals including black/white and color laser printers, high-resolution scanner etc are available. UCSB's Life Sciences Computing Group provides full desktop, network, server, and web support on a recharge basis.

Redacted by agreement: Approximately six PC and Mac-based desktops are available in Redacted by agreement All computers have software for word processing, calculation, image processing and analysis, bioinformatics. All computers are connected to a gigabit ethernet network and are connected to a lab server that provides file sharing and automatic data backups. Peripherals including black/white and color laser printers, high-resolution scanner are available. In addition to access to the MU computing resources, desktop computing resources for use by laboratory personnel (bioinformatics post-doctoral fellows) consist of a personal Dell Precision tower 7910 that

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contains the following specifications: 2 by 10 core Intel Xeon Processors, 4 TB disk space and 512 GB of RAM.

OFFICE:
UCSB: The Principal Investigator has an office of approximately 140 square feet adjacent to the laboratory. All laboratory personnel have a personal desk adjacent to their lab bench equipped with a networked, personal computer.

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OTHER:
UCSB: Nearby Core Facilities that are available for this project include: the UCSB Microscopy Core Facility directed by a full-time PhD-level staff member provides access to state-of the art fluorescence, laser-scanning confocal, two-photon and electron microscopes; the Genomics/Proteomics Facility of UCSB's Neuroscience Research Institute provides instrumentation and personnel for quantitative PCR and microarray screening, as well as for automated two-dimensional protein separations and peptide sequencing by mass spectroscopy; the UCSB Mass Spectrometry Facility is directed by a full-time, doctoral level staff member and equipped with several mass spectrometers and LC systems; the "Bioshop" provides a wide variety of services including maintenance and repair of equipment and custom-manufacturing of equipment.

MU: MU Campus-Wide High Performance Computing and Data Storage
The Lewis HPC is a shared cluster accessible to researchers and their collaborators at the University of Missouri and across the University of Missouri System. It is connected with 10 Gigabit Ethernet and 40 Gigabit QDR Infiniband. The environment consists of more than 5200 cores that have access to nearly 2 PB of dedicated research data storage. The cluster is connected to the Internet2 AL2S SDN network at 100Gigabits. The BioCompute partition was built in 2016 and specifically designed for processing NGS data and consists of 38 nodes, each with Intel(R) Xeon(R) CPU E5-2670 v3 @ 2.30GHz or E5-2680 v4 @ 2.40GHz, 512 GB RAM and 3 TB local SSD.

The Lewis cluster also has 500 older cores for interactive and long jobs. In addition to the Lewis Cluster, the BioCompute cluster consists of 37 Dell R630 Broadwell nodes with 28 cores, 512GB of RAM, and 3TB of local SSD storage for a total of 1036 cores, 19TB of RAM, and 104TB of local SSD storage.

MU DNA Core: The University of Missouri-Columbia also maintains several core facilities including a DNA Core (https://dnacore.missouri.edu/). The DNA core research facility provides services in: Sanger Sequencing, Fragment Analysis, Illumina (Next Gen) Sequencing, Illumina Library Construction and Affymetrix Arrays. The facility utilizes a 3730xl 96-capillary DNA Analyzer with Applied Biosystems Big Dye Terminator cycle sequencing chemistry. The 96-capillary instrument with robotic plate handler provides a high throughput capacity at minimal cost to investigators. The facility maintains two Illumina HiSeq and a single MiSeq instruments, as well as, necessary ancillary equipment. The combined data yield of these instruments allows the DNA Core to provide sequencing capacity for both the small and large project. The Illumina HiSeq 2000 is an ultra-high-throughput DNA sequencing platform capable of generating more than 600Gb of data in a single run. Library preps are performed with Illumina index adapters to take advantage of multiplexing capabilities which reduce sequencing costs by allowing multiple samples per lane. The Illumina NextSeq 500 provides high-throughtput sequencing at increased speeds and lower cost. The platform is capable of generating 120Gb of data (~800 million paired-end reads) in a single run. The system is compatible with all Illumina libraries. The faster run times are due to Illumina's new two-channel SBS technology which requires only two images to capture all four base calls. The Illumina MiSeq is a DNA sequencing platform capable of generating paired-end, 250 base read lengths. The single lane flow cell design, on-board clustering and five minute cycle times allow for rapid sequencing times (~48 hours for a 2x250 run). A 10X Genomics instrument was recently acquired and is capable of generating data for de novo assembly and various single cell genomic applications.

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Equipment

UCSB: The P.I. has an inverted spinning disc confocal fluorescence microscope (Olympus) with a sensitive cooled CCD camera which is housed in a microscope room adjacent to the laboratory. Departmental, shared equipment is available in the same building including ultra- and high speed centrifuges, microplate reader for absorption/fluorescence/luminescence, an Odyssey Infrared Imaging System (LiCor Biosciences) for quantitative western blot analysis, a Guava flow cytometer, gel documentation systems, liquid scintillation and gamma counters, lyophylizer etc. Additional shared equipment is available in the adjacent Redacted by agreement including French Press, real-time PCR, nucleofector, a KODAK In-Vivo Imaging System FX for animal imaging including fluorescence and X-ray, and a BTX electroporator with 2-Needle Array Electrode for in vivo transfections of plasmids in mice.

Redacted by agreement

Equipment includes: (3) Perkin Elmer GeneAmp PCR System 9700 Thermal Cyclers, (1) Bio-Rad DNA Engine Peltier Thermal Cycler, (3) Stratagene 96-well Gradient Robocyclers, (1) MJ Research PTC-200 Gradient Thermal Cyclers, (4) MJ Research PTC-100 Thermal Cyclers (one has a heated lid), (1) Qiagen Rotor-Gene-Q, BioRad Gel Doc 2000 gel documentation system.

The new model Agena (formerly Sequenom) MassArray System (MALDI-TOF) in 96-format was purchased in December 2015. The MassArray Analyzer 4 processes up to two multiplexed 96-well SpectroCHIP Arrays per run. Each well can multiplex a maximum of 40 assays using iPLEX primer extension chemistry. This instrument can also quantify gene expression and copy number variant analysis. DNA isolation to data acquisition can be performed by next day. Therefore, candidate variant analyses be performed within one month, consider designing of each variant primers and performing full population screens of candidates.

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OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Thomas Middle Name Last Name*: Weimbs Suffix: Ph.D.

Position/Title*: Professor
Organization Name*: The Regents of the University of California, Santa Barbara
Department: Neuroscience Research Inst.
Division:
Street1*: LSB, Room 2113
Street2:
City*: Santa Barbara
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 93106-5060

Phone Number*: (805) 893-4144 Fax Number: 805-893-2017

E-Mail*: weimbs@lifesci.ucsb.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 1993

Attach Biographical Sketch*: File Name: Biosketch_TW_2019_61012199311.pdf

Attach Current & Pending Support: File Name:

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Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Redacted by agreement Suffix:

Position/Title*: Professor
Organization Name*: University of Missouri
Department:
Division:
Street1*: Redacted by agreement
Street2:
City*: Columbia
County: MO
State*: MO: Missouri
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 65211-0001

Phone Number: Redacted by agreement Fax Number:
E-Mail*: Redacted by agreement

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1991

Attach Biographical Sketch*: File Name: Redacted by agreement .pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Redacted by agreement Suffix:

Position/Title*: Associate Professor
Organization Name*: University of Missouri
Department:
Division:
Street1*: Redacted by agreement
Street2:
City*: Columbia
County: MO
State*: MO: Missouri
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 65211-0001

Phone Number: Redacted by agreement Fax Number:
E-Mail*: Redacted by agreement

Credential, e.g., agency login: eRA Commons User Name

Project Role*: Consultant Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1991

Attach Biographical Sketch*: File Name: Redacted by agreement

Attach Current & Pending Support: File Name:

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PROFILE - Senior/Key Person

Prefix: Redacted by agreement Suffix:

Position/Title*: Post-Doctoral Fellow
Organization Name*: The Regents of the University of California, Santa Barbara
Department: Neuroscience Research Inst.
Division:
Street1*: Redacted by agreement
Street2:
City*: Santa Barbara
County: Santa Barbara
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 93106-2050

Phone Number*: Redacted by agreement Fax Number:

E-Mail*: Redacted by agreement

Credential, e.g., agency log eRA Commons User Name

Project Role*: Post Doctoral Scholar Other Project Role Category:

Degree Type: PhD Degree Year: 2017

Attach Biographical Sketch*: File Name: Biosketc Redacted by agreement pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Redacted by agreement Suffix: PhD

Position/Title*: Associate Professor
Organization Name*: Augusta University
Department: Biochemistry and Molecular Bio
Division:
Street1*: 1120 15th Street
Street2:
City*: Augusta
County:
State*: GA: Georgia
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 30912-0001

Phone Number* Redacted by agreement Fax Number: (706) Redacted by agreement

E-Mail* Redacted by agreement

Credential, e.g., agency login eRA Commons User Name

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: PhD Degree Year: 1996

Attach Biographical Sketch*: File Name: Biosketc Redacted by agreement .pdf

Attach Current & Pending Support: File Name:

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PROFILE - Senior/Key Person

Prefix: Redacted by agreement Suffix: MD

Position/Title*: Visiting Investigator
Organization Name*: Scripps Research Institute
Department:
Division:
Street1*: 10550 North Torrey Pines Road
Street2:
City*: La Jolla
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92037-0001

Phone Number*: eRA Commons User Fax Number:

E-Mail: Redacted by agreement

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: MD Degree Year: 2011

Attach Biographical Sketch*: File Name: Biosketch Redacted by agreement .pdf

Attach Current & Pending Support: File Name:

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OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

NAME: Thomas, Weimbs, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
University of Cologne, Germany	*Diplom-Chemiker (BS, MS)*	1990	Chemistry
University of Cologne, Germany	*Dr. rer. nat. (PhD)*	1993	Biochemistry
University of California at San Francisco	Postdoctoral Fellowship	1994-1999	Cell Biology

A. Personal Statement

Research in my laboratory is divided in two interrelated areas of interest. One area is the investigation of molecular mechanisms underlying polycystic kidney disease (PKD) with an emphasis towards identifying therapies. The other area is the investigation of novel functions of SNARE membrane fusion proteins and their role in membrane trafficking and epithelial cell polarity. There has been an amazing level of cross-fertilization between these areas in my lab. Our SNARE work originated from my postdoctoral training in Keith Mostov's lab at UCSF.

Our research on PKD began in 1999 when I established my first independent laboratory as an Assistant Professor at the Cleveland Clinic. In 2005, I moved my lab to UCSB. My lab is investigating the function of polycystin-1 (PC1), the protein affected in most cases of ADPKD. In particular, we wish to understand how (and why) PC1 regulates various signaling pathways and how disruption leads to renal cyst growth. In addition, we are identifying novel therapeutic approaches and investigate novel methods to deliver therapeutics to polycystic kidneys. We are very experienced with all of the methods and technologies described in this proposal. We are maintaining colonies of several PKD rodent models (mice and rats), and are very experienced with genetic crossing, pharmacological and dietary intervention studies, and analysis of animal data. Recent publications illustrating our animal work are:

a. Kipp KR, Kruger SL, Schimmel MF, Parker N, Shillingford JM, Leamon CP, Weimbs T. (2018) Comparison of folate-conjugated rapamycin versus unconjugated rapamycin in an orthologous mouse model of polycystic kidney disease. ***Am J Physiol Renal Physiol.*** 315: F395-F405.
b. Kipp KR, Rezaei M, Lin L, Dewey EC, **Weimbs** T. (2016). A mild reduction of food intake slows disease progression in an orthologous mouse model of polycystic kidney disease. ***Am J Physiol Renal Physiol.*** 310: F726–F731.

We are equally experienced with cell biological and biochemical experimentation, especially investigation of signaling pathways as illustrated in the following publications:

c. Talbot et al (2014) The cleaved cytoplasmic tail of polycystin-1 regulates Src-dependent STAT3 activation. ***J Am Soc Nephrol.*** 25(8):1737-48
d. Olsan EE, West JD, Torres JA, Doerr N, **Weimbs** T. (2018) Identification of Targets of Interleukin-13 and Signal Transducer and Activator of Transcription-6 (STAT6) Signaling in Polycystic Kidney Disease. ***Am J Physiol Renal Physiol.*** 315: F86-F96.

B. Positions and Honors

Positions

1994-1999	Postdoctoral Researcher, Department of Anatomy, School of Medicine, University of California at San Francisco, Dr. Keith E. Mostov, Principal Investigator
1999-2005	Assistant Staff, Department of Cell Biology, Lerner Research Institute, The Cleveland Clinic
2004-2005	Assistant Professor, Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

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2005-2007	Assistant Professor, Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara
2007-2014	Associate Professor (with tenure), Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara
2014-present	Professor, Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara

Secondary Appointments and Graduate Program Memberships

2001-2005	Member, Graduate Program in Cell Biology, Case Western Reserve University
2003-2005	Member, Case Comprehensive Cancer Center, Case Western Reserve University
2005-present	Neuroscience Research Institute, University of California Santa Barbara

Honors and Service

1988	Fritz ter Meer Foundation, Undergraduate Scholarship
1993	*Summa cum laude* for Doctoral Degree
1994-1996	Feodor Lynen-Fellow, Alexander von Humboldt-Foundation
1996	Irvington Institute for Immunological Research, Postdoctoral Fellowship
2005	Ad Hoc Member, "Cellular and Molecular Biology of the Kidney" Study Section, NIH
2005	Member, Ad Hoc Special Emphasis Grant Review Panel, NIDDK, NIH
2006	Member, Review Panel, Program Project Grant, NIDDK, NIH
2007	Member, Special Emphasis Panel, "Pathobiology of Kidney Disease" Study Section, NIH
2007-2009	Editorial Board, *American Journal of Physiology - Renal Physiology*
2008	Member, Special Emphasis Panel, "Pathobiology of Kidney Disease" Study Section, NIH
2012	Member, Special Emphasis Panel, "Kidney Pathobiology and Molecular Biology and Genitourinary Organ Development", NIH
2012	Ad Hoc Member, "Kidney Molecular Biology and Genitourinary Organ Development" Study Section, NIH
2012-present	Editorial Board, *Scientifica*
2012	Member, Grant Review Panel, Digestive, Kidney and Urological Systems, DKUS-(A) 11 "Small Business and Member Conflicts: Kidney Disease", NIH
2013-2019	Permanent Member, "Kidney Molecular Biology and Genitourinary Organ Development" Study Section, NIH

C. Contributions to Science

Full list of published work:
http://www.ncbi.nlm.nih.gov/sites/myncbi/thomas.weimbs.1/bibliography/40746838/public/?sort=date&direction=descending

1. Role of mTOR signaling in PKD: Early on in our PKD research, we discovered that PC1, the protein mutated in most cases of ADPKD, interacts with tuberin (TSC2), the protein affected in tuberous sclerosis. Since tuberin is a known regulator of mTOR, we quickly discovered that mTOR is strongly activated in cyst-lining cells in human ADPKD and in PKD mouse models. Using two different PKD mouse models, we showed that treatment with the mTOR inhibitor rapamycin very strongly inhibited renal cyst growth and preserved renal function. These findings were published in the following paper which is one of the most highly cited research papers in the PKD field since 2006 (>690, Google Scholar):

a. Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, Flask CA, Novick AC, Goldfarb DA, Kramer-Zucker A, Walz G, Piontek KB, Germino GG, **Weimbs** T (2006) From the Cover: The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. ***PNAS*** 103, 5466-71

Many of these findings were independently confirmed by other labs, and this work rapidly led to several clinical trials to test the efficacy of mTOR inhibitors in ADPKD. Together with my colleagues, I helped to convince Novartis to sponsor the largest of these mTOR inhibitor trials. Unfortunately, the trial results were overall disappointing, most likely because the significant extra-renal side effects of mTOR inhibitors are dose-limiting which leads to insufficient mTOR inhibition in the target organ. This failure, however, led us to pursue novel drug targeting strategies which are described below. Additional papers belonging to this contribution include:

b. Kipp KR, Kruger SL, Schimmel MF, Parker N, Shillingford JM, Leamon CP, Weimbs T. (2018) Comparison of folate-

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conjugated rapamycin versus unconjugated rapamycin in an orthologous mouse model of polycystic kidney disease. ***Am J Physiol Renal Physiol.*** 315: F395-F405.

c. Doerr N, Wang Y, Kipp KR, Liu G, Benza JJ, Pletnev V, Pavlov TS, Staruschenko A, Mohieldin AM, Takahashi M, Nauli SM, **Weimbs** T. (2016). Regulation of Polycystin-1 Function by Calmodulin Binding. ***PLoS One*** 11(8):e0161525.
d. Kipp KR, Rezaei M, Lin L, Dewey EC, **Weimbs** T. (2016). A mild reduction of food intake slows disease progression in an orthologous mouse model of polycystic kidney disease. ***Am J Physiol Renal Physiol.*** [Published 13 January 2016; Epub ahead of print]
e. Shillingford, JM, Leamon, CP, Vlahov, IR, **Weimbs**, T (2012) Folate-Conjugated Rapamycin Slows Progression of Polycystic Kidney Disease ***Journal of the American Society of Nephrology*** 23, 1674–1681
f. Shillingford, JM, Piontek, KB, Germino, GG, **Weimbs**, T (2010) Rapamycin Ameliorates PKD Resulting from Conditional Inactivation of Pkd1. ***Journal of the American Society of Nephrology*** 21, 489-97

2. Role of STAT signaling in PKD: Also early on in our PKD research we discovered that the C-terminal cytoplasmic tail of PC1 undergoes proteolytic cleavage followed by nuclear targeting, and interacts with the transcriptional co-activator P100 and the transcription factor STAT6. We demonstrated that STAT6 normally localizes to primary cilia but is displaced from cilia after disruption of fluid flow. These findings suggested a model how fluid flow and cilia can directly regulate gene expression in tubule cells. These findings were published in an influential and highly cited paper (>330, Google Scholar):

a. Low SH, Vasanth S, Larson CH, Mukherjee S, Sharma N, Kinter MT, Kane ME, Obara T, and **Weimbs** T (2006) Polycystin-1, STAT6 and P100 function in a novel pathway that transduces ciliary mechanosensation to gene expression and is activated in polycystic kidney disease. ***Developmental Cell*** 10, 57-69

Later, we reported that STAT6 is aberrantly activated in PKD due to a positive feedback loop involving IL13 and the IL13 receptor. Importantly, inhibition of STAT6 - either pharmacologically or genetically - ameliorated disease progression. Besides the mechanistic insight, there results suggest that the IL13/STAT6 pathway is a promising drug target which we are currently pursuing:

b. Olsan EE, Mukherjee S, Wulkersdorfer B, Shillingford JM, Giovannone AJ, Todorov G, Song X, Pei Y, and **Weimbs** T. (2011) Signal Transducer and Activator of Transcription-6 (STAT6) Inhibition Suppresses Renal Cyst Growth in Polycystic Kidney Disease. ***PNAS*** 108, 18067-18072
c. Olsan EE, West JD, Torres JA, Doerr N, **Weimbs** T. (2018) Identification of Targets of Interleukin-13 and Signal Transducer and Activator of Transcription-6 (STAT6) Signaling in Polycystic Kidney Disease. ***Am J Physiol Renal Physiol.*** 315: F86-F96.

Our STAT6 results prompted us to investigate STAT3 which led to the discovery that STAT3 is very strongly activated in PKD and that it is regulated by PC1 in a complex mechanism. STAT3 is a hotly pursued cancer target and our results suggest that STAT3 inhibitors may be effective in ADPKD therapy (which we are pursuing):

d. Talbot, JJ, Shillingford, JM, Vasanth, S, Doerr, N, Mukherjee, S, Kinter, M, Watnick, T, and **Weimbs**, T (2011) Polycystin-1 Regulates STAT Activity by a Dual Mechanism. ***PNAS*** 108, 7985-7990

Our important recent results indicate that STAT3 is primarily activated by Src in PKD. This is in agreement with previous results by others that showed Src activation in PKD. Src inhibitors are currently being tested in clinical trials in ADPKD and our work provides important mechanistic insights:

e. Talbot, JJ, Song, X, Wang, X, Rinschen, MM, Doerr, N, LaRiviere, WB, Schermer, B, Pei, YP, Torres, VE, Weimbs, T (2014) The cleaved cytoplasmic tail of polycystin-1 interacts with Src and amplifies STAT3 activation in response to cAMP- and EGFR-signaling. ***Journal of the American Society of Nephrology*** 25, 1737-1748

3. Development of PKD therapies: As mentioned above, our mTOR work provided the rationale and justification to pursue some of the first significant clinical drug trials in ADPKD:

a. Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, Flask CA, Novick AC, Goldfarb DA, Kramer-Zucker A, Walz G, Piontek KB, Germino GG, **Weimbs** T (2006) From the Cover: The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. ***PNAS*** 103, 5466-71

Due to the disappointing outcome of these trials, it became clearer that it will be difficult to target most of the signaling pathways implicated in PKD with systemic drugs due to the anticipated extra-renal side effects and the fact that ADPKD will have to be treated for years or decades. To overcome this problem, we developed methods that allow drug targeting specifically to polycystic kidneys. For small-molecule compounds, this was done in collaboration with a biopharmaceutical company, and the strategy involves conjugation of drugs to folate to facilitate renal uptake by the folate receptor. This work currently continues in the lab and may lead to clinical testing:

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b. Shillingford, JM, Leamon, CP, Vlahov, IR, **Weimbs**, T (2012) Folate-Conjugated Rapamycin Slows Progression of Polycystic Kidney Disease ***Journal of the American Society of Nephrology*** 23, 1674–1681
c. Kipp KR, Kruger SL, Schimmel MF, Parker N, Shillingford JM, Leamon CP, Weimbs T. (2018) Comparison of folate-conjugated rapamycin versus unconjugated rapamycin in an orthologous mouse model of polycystic kidney disease. ***Am J Physiol Renal Physiol.*** 315: F395-F405.

To target therapeutic antibodies specifically to the renal cyst lumens we invented a method that replies on transcytosis of dimeric-IgA antibodies by the polymeric immunoglobulin receptor. This strategy will enable the repurposing of thousands of existing monoclonal antibodies against molecular targets implicated in ADPKD:

d. Olsan EE, Matsushita T, Rezaei M, **Weimbs** T. (2015) Exploitation of the Polymeric Immunoglobulin Receptor for Antibody Targeting to Renal Cyst Lumens in Polycystic Kidney Disease. ***J Biol Chem.*** 290:15679–15686

UCSB has filed a patent application on this technology and we are currently planning to found a biotech company to bring this approach to the next level:

e. US Patent Application (US20170210793A1) filed 2017-07-27: “Novel Treatment for Polycystic Kidney Disease”

4. Role of SNAREs in membrane trafficking and cell polarity in epithelial cells: This work began when I was a postdoctoral fellow in Keith Mostov’s lab at UCSF and continues to this day. SNARE proteins are the machines that mediate membrane fusion in all membrane trafficking pathways in eukaryotic cells. An important early contribution that affected the entire SNARE research field was our discovery that SNARE proteins of various families are evolutionarily related to each other and belong to a large superfamily. This relationship had previously been unrecognized. Our findings also led to the discovery of conserved glutamine and arginine residues which later led to the classification of SNAREs into Q-SNARE and R-SNARE families. Our work was published in an influential, highly-cited paper (>300, Google Scholar) and follow-up papers:

a. **Weimbs,** T., Low, S. H., Chapin, S. J., Mostov, K. E., Bucher, P. and Hofmann K. (1997). A conserved domain is present in different families of vesicular fusion proteins: A new superfamily. ***Proceedings of the National Academy of Sciences of the USA*** 94, 3046-3051

Our investigation of SNAREs in polarized epithelial cells led to the first discovery that specific SNAREs are expressed and function specifically at the apical vs. the basolateral plasma membrane domains. These findings were published in several important papers. One example is this one:

b. Low, S. H., Chapin, S. J., Wimmer, C., Whiteheart, S. W., Kömüves, L. K., Mostov, K. E., and **Weimbs**, T. (1998). The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. ***Journal of Cell Biology*** 141, 1503-1513

Numerous additional papers firmly established the roles of syntaxins 3 and 4 in the establishment and maintenance of epithelial cell polarity. Examples are:

c. Reales, E, N Sharma, SH Low, H Fölsch, and T **Weimbs** (2011) Basolateral Sorting of Syntaxin 4 Is Dependent on Its N-Terminal Domain and the AP1B Clathrin Adaptor, and Required for the Epithelial Cell Polarity. ***PLoS ONE*** 6(6): e21181 (2011).
d. Giovannone AJ, Reales E, Bhattaram P, Fraile-Ramos A, **Weimbs** T. (2017). Mono-ubiquitination of syntaxin 3 leads to retrieval from the basolateral plasma membrane and facilitates cargo recruitment to exosomes. ***Mol Biol Cell*** 28, 2843-2853.
e. Giovannone AJ, Winterstein C, Bhattaram P, Reales E, Low SH, Baggs JE, Xu M, Lalli MA, Hogenesch JB, **Weimbs** T. (2018). Soluble syntaxin 3 functions as a transcriptional regulator. ***J Biol Chem.*** 293:5478-5491

D. Research Support

Ongoing

Private Source — Weimbs (PI) — 07/01/18 - 06/30/19
Title: Preclinical investigation of MNK 1 and 2 inhibitors as a potential therapeutic in polycystic kidney disease
The main objectives are to investigate the potential therapeutic efficacy of MNK1/2 inhibitors in mouse models of polycystic kidney disease.

NIH (R01DK109563) — Weimbs (PI) — 01/16/17-12/31/20
Title: Role of renal crystal deposition in the progression of polycystic kidney disease
The main objectives are to investigate how tubular microcrystals trigger cystogenesis and accelerate disease progression in polycystic kidney disease.

Private Source — Weimbs (PI) — 08/01/17-7/31/20
Title: Polycystic Kidney Disease

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Gift to support research on Polycystic Kidney Disease in Weimbs laboratory.

Completed (selected)

UCSB Academic Senate, Faculty Research Grant — Weimbs (PI) — 07/01/16-6/31/17
Title: Development of a tissue-targeted therapeutic antibody for treatment of polycystic kidney disease
The main goals are to develop an antagonistic antibody in dimeric IgA format for targeting to renal cysts for PKD therapy.

Private Source — Weimbs (PI) — 8/01/14-7/31/17
Title: Polycystic Kidney Disease
Gift to support research on Polycystic Kidney Disease in Weimbs laboratory.

Private Source — Weimbs (PI) — 5/01/13-3/31/17
Title: Pre-clinical efficacy of Folate-Conjugated mTOR Inhibitors and related compounds in Polycystic Kidney Disease
The main goals are to investigate the efficacy and increased tissue specificity of kidney-targeted drugs, particularly mTOR inhibitors.

Private Source — Weimbs (PI) — 11/13/13-11/12/14
Title: Targeting the STAT6 Pathway for Treatment of Automal Dominant Polycystic Kidney Disease
The main objectives are to evaluate the efficacy of targeting the STAT6 pathway in mouse models of Polycystic Kidney Disease

NIH (1R21DK095248) — Weimbs (PI) — 9/21/12-7/31/15 (NCE)
Title: A Novel Role of Syntaxin 3 as a Transcription Regulator
The main objectives are to investigate a novel function of the SNARE membrane fusion protein syntaxin 3 as a signaling molecule that mediates transcriptional regulation.

California Cancer Research Coordinating Committee, Cancer Research Award (SB130052) — Weimbs (PI) — 7/01/12-6/30/14
Title: A Novel Role of Syntaxin 3 as a Transcription Regulator Implicated in Epithelial Morphogenesis and Carcinogenesis
The main objectives are to investigate a novel function of the SNARE membrane fusion protein syntaxin 3 as they relate to carcinogenesis.

NIH (R01 DK078043) — Weimbs (PI) — 7/15/2007-6/30/2012 (NCE)
Title: Regulation of the mTOR pathway in polycystic kidney disease
The main objectives are to investigate the regulation of mTOR activity by polycystin-1 and apical fluid flow, the role of this pathway in polycystic kidney disease and the utility of treatment with mTOR inhibitors.

NIH (R01 DK62338) — Weimbs (PI) — 7/1/2002-3/31/2012
Title: STAT Regulation by Polycystin-1 (previous title: Epithelial Cell Polarity in Polycystic Kidney Disease)
The main objectives of this project are to investigate the role of polycystin-1 in the regulation of STAT activity, and defects in autosomal-dominant polycystic kidney disease.

Department of Defense (Peer Reviewed Medical Research Program, PRMRP) — Weimbs (PI) — 7/1/2007- 7/31/2011
Title: Investigation of the regulation of the mTOR pathway in polycystic kidney disease
The main goals of this project are to investigate the activation status of regulators and effectors up/downstream of mTOR in polycystic kidney disease, and to investigate the efficacy and specificity of a novel, tissue-targeted, ligand-conjugated form of rapamycin in polycystic mouse models.

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Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 094878394

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Thomas		Weimbs	Ph.D.	PD/PI	0.00			EFFORT	48,075.00	4,795.00	52,870.00
2 .		Redacted by agreement			PhD	Post-Doctoral Fellow		EFFORT			56,880.00	13,765.00	70,645.00
3 .					PhD	Collaborator					0.00	0.00	0.00
4 .					MD	Collaborator					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **123,515.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			50,760.00	12,284.00	63,044.00
	Graduate Students						
3	Undergraduate Students				7,850.00	150.00	8,000.00
	Secretarial/Clerical						
4	**Total Number Other Personnel**					**Total Other Personnel**	**71,044.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**194,559.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Graduate Student Fees	0.00
9 . Animal recharge costs	40,000.00
10 . User Fees, Service Contracts, Equipment or Facility Rental Fees	4,000.00
Total Other Direct Costs	**89,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**287,559.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Org Research	55	72,592.00	39,926.00
2 . On Campus Org Research	55.5	214,967.00	119,307.00
		Total Indirect Costs	**159,233.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Cora Coleman, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**446,792.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	446,792.00

L. Budget Justification* File Name: budget_justification1012199322.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 094878394

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Thomas		Weimbs	Ph.D.	PD/PI	0.00			EFFORT	48,075.00	4,844.00	52,919.00
2 .	Dr.	Redacted by agreement			PhD	Post-Doctoral Fellow		EFFORT			59,100.00	14,775.00	73,875.00
3 .					PhD	Collaborator					0.00	0.00	0.00
4 .					MD	Collaborator					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **126,794.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			52,896.00	13,224.00	66,120.00
	Graduate Students						
3	Undergraduate Students				7,850.00	150.00	8,000.00
	Secretarial/Clerical						
4	**Total Number Other Personnel**					**Total Other Personnel**	**74,120.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**200,914.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Graduate Student Fees	0.00
9 . Animal recharge costs	40,000.00
10 . User Fees, Service Contracts, Equipment or Facility Rental Fees	4,000.00
Total Other Direct Costs	**89,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**293,914.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Org Research	55.5	293,914.00	163,122.00
		Total Indirect Costs	**163,122.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Cora Coleman, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**457,036.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	457,036.00

L. Budget Justification* File Name: budget_justification1012199322.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 094878394

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Thomas		Weimbs	Ph.D.	PD/PI	0.00			EFFORT	48,075.00	4,856.00	52,931.00
2 .	Dr.	Redacted by agreement			PhD	Post-Doctoral Fellow		EFFORT			61,308.00	15,863.00	77,171.00
3 .					PhD	Collaborator					0.00	0.00	0.00
4 .					MD	Collaborator					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **130,102.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			54,756.00	14,168.00	68,924.00
	Graduate Students						
3	Undergraduate Students				7,850.00	150.00	8,000.00
	Secretarial/Clerical						
4	**Total Number Other Personnel**					**Total Other Personnel**	**76,924.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**207,026.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	67,173.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Graduate Student Fees	0.00
9 . Animal recharge costs	40,000.00
10 . User Fees, Service Contracts, Equipment or Facility Rental Fees	4,000.00
Total Other Direct Costs	**156,173.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**367,199.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Org Research	55.5	325,026.00	180,389.00
		Total Indirect Costs	**180,389.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Cora Coleman, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**547,588.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	547,588.00

L. Budget Justification* File Name: budget_justification1012199322.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 094878394

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Thomas		Weimbs	Ph.D.	PD/PI	0.00			EFFOR	48,075.00	4,892.00	52,967.00
2 .	Dr.	Redacted by agreement			PhD	Post-Doctoral Fellow		EFFORT			65,339.00	17,053.00	82,392.00
3 .					PhD	Collaborator					0.00	0.00	0.00
4 .					MD	Collaborator					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **135,359.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			56,880.00	14,846.00	71,726.00
	Graduate Students						
3	Undergraduate Students				7,850.00	150.00	8,000.00
	Secretarial/Clerical						
4	**Total Number Other Personnel**					**Total Other Personnel**	**79,726.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**215,085.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	100,860.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Graduate Student Fees	0.00
9 . Animal recharge costs	40,000.00
10 . User Fees, Service Contracts, Equipment or Facility Rental Fees	4,000.00
Total Other Direct Costs	**189,860.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**408,945.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Org Research	55.5	308,085.00	170,987.00
		Total Indirect Costs	**170,987.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Cora Coleman, (415) 437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**579,932.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	579,932.00

L. Budget Justification* File Name: budget_justification1012199322.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 094878394

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Thomas		Weimbs	Ph.D.	PD/PI	0.00			EFFORT	48,075.00	4,904.00	52,979.00
2 .	Dr.	Redacted by agreement			PhD	Post-Doctoral Fellow		EFFORT			66,646.00	17,395.00	84,041.00
3 .					PhD	Collaborator					0.00	0.00	0.00
4 .					MD	Collaborator					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **137,020.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	EFFORT			59,100.00	15,425.00	74,525.00
	Graduate Students						
3	Undergraduate Students				7,850.00	150.00	8,000.00
	Secretarial/Clerical						
4	**Total Number Other Personnel**					**Total Other Personnel**	**82,525.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**219,545.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 094878394
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Regents of the University of California, Santa Barbara

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	42,500.00
2. Publication Costs	2,500.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	85,409.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Graduate Student Fees	0.00
9 . Animal recharge costs	40,000.00
10 . User Fees, Service Contracts, Equipment or Facility Rental Fees	4,000.00
Total Other Direct Costs	**174,409.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**397,954.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . On Campus Org Research	55.5	312,545.00	173,462.00
		Total Indirect Costs	**173,462.00**

Cognizant Federal Agency DHHS, Cora Coleman, (415) 437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**571,416.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	571,416.00

L. Budget Justification* File Name: budget_justification1012199322.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

Budget Justification

Request for five years of support:
All of the proposed studies are done *in vivo* using various mouse rat and cat models of PKD. Therefore, the proposed experiments are time consuming as they require breeding to obtain animals of the required genotypes and genders, maintenance of the colonies, staggered treatment times of cohorts etc. Cat experiments are especially time consuming because of their much longer gestation period (compared to rats and mice), time to maturity and durations of intervention. Thus, to complete the goals of this project, we are requesting five years of support to allow sufficient time to conduct and analyze the proposed experiments.

Personnel

Senior / Key Personnel

Thomas Weimbs, PhD, Professor (University of California Santa Barbara, Principal Investigator EFFORT Dr. Weimbs will oversee the scientific and administrative aspects of this proposal. He will design experiments, supervise personnel, communicate with collaborators, aid in the interpretation of data, write reports and publications.

Redacted by agreement

Redacted by agreement

Redacted by agreement D (University of California Santa Barbara, Postdoctoral Fellow EFFORT Redacted by agreement has worked on PKD research in the Weimbs lab since 2010, first as a PhD graduate student and now as a postdoctoral fellow. Redacted by responsible for most of the discoveries shown as preliminary data in this application. He is highly experienced with all of the proposed experimentation and will be involved in the design and execution of rodent experiments for Specific Aims 1-3 of this proposal. Redacted by will also train new lab personnel and supervise undergraduate student research interns, and work-study students.

Redacted by agreement PhD, Associate Professor (Augusta University EFFORT D Redacted by agreement is an expert on GPR109A and has recently established the anti-tumor and anti-inflammatory potential of niacin and GPR109A in breast and colon cancers. We have an ongoing collaboration with Redacted by agreement His lab has already crossed Gpr109a-/- mice with three different Pkd1 mouse models as described in the proposal. Two of these crossed strains have already been sent to UCSB and colonies are being established. The third stain will be shipped very shortly. We will frequently consult with Redacted by agreement by email, phone or video conference, and exchange data, ideas, materials and reagents.

Redacted by agreement MD, Visiting Investigator (Scripps Research Institute EFFORT Redacted by agreement is funded by a grant from the Redacted by agreement to develop metabolic methods specifically for the analysis of polycystic kidneys in collaboration with our group and has already generated interesting initial preliminary data Redacted by agreement s a Visiting Investigator i Redacted by agreement dact Redacted by agreement a leader in developing cutting-edge methods for metabolomics analyses and is the Director of the Redacted by agreement and a Professor of Chemistry, Molecular and Computational Biology. Dr. Redacted by agreement will conduct metabolomics analyses on the rat/mouse kidneys in this study and specifically

Uncovered by a White Coat Waste investigation

investigate how dietary interventions affect metabolites of the TCA cycle, amino acid and fatty acid metabolism. He will be in close communication with the PI's laboratory at UCSB [Redacted by agreement] comes from the group of [Redacted by agreement], with whom we have a long-standing collaborative relationship on current and past projects.

Other Personnel

TBN (University of California Santa Barbara, Postdoctoral Fellow, [EFFORT]). A postdoctoral fellow is being recruited to join the Weimbs lab. This fellow will be involved in the design and execution of experiments for Specific Aims 1-3 of this proposal.

[Redacted by agreement] – Laboratory manager (University of Missou[EFFORT] [Redacted by agreement] is the laboratory and assistant colony manager for [Redacted by agreement] and will oversee the laboratory aspects including sample collection and processing [EFFORT] per week addressing the cat management for the project, including environmental enrichment. Year 3 – [EFFORT] per week during the feeding trials to support handling of diet, sampling support, acquisition of needed reagents, forms. Support colony management.

Travel:

UCSB: We are requesting funds for domestic travel to allow the PI, and a postdoctoral fellow to attend and present data at a national/international conference per year. Examples of these meetings include the annual conferences of the American Society of Nephrology or the American Society for Cell Biology.

MU: We are also requesting funds for domestic travel for each year to allow [Redacted by agreement] attend the annual conference of the American Society of Nephrology to present work and meet with Dr. Weimbs and his postdoc.

Materials and Supplies:

UCSB: We are requesting funds for general laboratory supplies and reagents. These include primary and secondary antibodies, chemicals, reagents, standards for electrophoresis, disposables, plasticware, glassware, membranes and reagents for Western blots, molecular biology reagents including supplies for PCR, histology supplies, cell culture media, supplements and supplies.

MU: We are requesting funds for materials and supplies including for Genotyping by PCR and direct Sanger sequencing, ultrasound pre-screening, CT Imaging including sedation and blood draw, bloodwork and urine analysis.

Animal feed and supplements:

UCSB: Off-the shelf and custom-manufactured rodent chow including custom ketogenic diets, and BHB for animal supplementation.

MU: cost of custom diet.

Publication Costs:

The anticipated publication costs are for publishing at least one manuscript per year describing the outcome of the study.

Animal facility charges:

UCSB: We are requesting funds to cover the cost of mouse and rat studies. The costs include per diems, technical support recharges, animal husbandry recharges and animal shipping costs. Proposed experiments involve a rat model (Han:SPRD rat), a mouse model (Pkd1-nestrinCre), and three additional Pkd1 mouse models crossed (or not) with a GPR109a null strain (the Ksp, Pkhd1 and ER models). All models must be maintained and bred separately to generate the correct genotype, sex, and age as outlined in the proposal. Feeding/supplementation studies require individual cages per cohort or animal. Between breeding, maintenance and feeding cages, we estimate an average of ~100 cages per day for 365 days. Per diem charges at UCSB are $1.34/cage/day for rats and $0.96/cage/day for mice.

Uncovered by a White Coat Waste investigation

MU: MU per diem includes normal husbandry, normal feed, and standard veterinary care. Breeding stock are male PKD+ and female PKD- cats. Average litter size from breeding of a PKD+ male with PKD– female is 4 kittens, one - two litters per year (16 – 32 kittens). Expect a 50% distribution of males to females and PKD+ to PKD-. Gestation is 63 – 65 days (2 months). Queens are with kittens for 2 months until weaning. Feeding trials: sixteen PKD+ adult (~ year) trial cats (n = 16). All PKD+ cats will have cysts by 8 months of age, severity is variable and determined and monitored by US and CT.

User Fees, Service Contracts, Equipment or Facility Rental Fees:
We request funds to cover recharge costs for microscopy use and image analysis in the UCSB microscopy core facility, and qPCR and flow cytometer instrument recharges.

Other costs:
UCSB: Salaries for work-study students to support the general laboratory operation by making common buffers and solutions, electrophoresis gels, stocking of reagents and supplies, glassware washing and autoclaving, and general lab upkeep. The lab employs several undergraduate students (generally biology majors) who are eligible for support by the Federal Work-Study program and each spend ~10 hours per week on routine lab work. In our experience, employing work-study students is more cost-effective than employing a full-time technician, and additionally promotes the education of undergraduate students by offering hands-on laboratory experience.

Uncovered by a White Coat Waste investigation

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		652,790.00
Section B, Other Personnel		384,339.00
Total Number Other Personnel	20	
Total Salary, Wages and Fringe Benefits (A+B)		1,037,129.00
Section C, Equipment		
Section D, Travel		20,000.00
1. Domestic	20,000.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		698,442.00
1. Materials and Supplies	212,500.00	
2. Publication Costs	12,500.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	253,442.00	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2	200,000.00	
10. Other 3	20,000.00	
Section G, Direct Costs (A thru F)		1,755,571.00
Section H, Indirect Costs		847,193.00
Section I, Total Direct and Indirect Costs (G + H)		2,602,764.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		2,602,764.00

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 1538902720000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Missouri

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				Co-Investigator		EFFORT			0.00	0.00	0.00
2.						Consultant					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
	Total Equipment

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	0.00

L. Budget Justification*	File Name: MU_Budget_Justification1012199310.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 1538902720000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Missouri

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				Co-Investigator		EFFORT			0.00	0.00	0.00
2.						Consultant					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*

Total Number Other Personnel **Total Other Personnel**

Total Salary, Wages and Fringe Benefits (A+B) **0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
Total Other Direct Costs	

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	0.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
		Total Indirect Costs	

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	0.00

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	0.00

L. Budget Justification*	File Name: MU_Budget_Justification1012199310.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 1538902720000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Missouri

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				PD/PI	Institutional Base Salary	EFFORT			9,615.00	3,433.00	13,048.00
2.						Co-I					0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **13,048.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Specialist	EFFORT			3,901.00	1,393.00	5,294.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,294.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**18,342.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,250.00
2. Foreign Travel Costs	
Total Travel Cost	**1,250.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . PKD Genotyping/US Pre-screen/CT imaging/Sedation	2,100.00
9 . Bloodwork	9,600.00
10 . Animal Care per diem	18,671.00
Total Other Direct Costs	**30,371.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**49,963.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	55	31,291.00	17,210.00
		Total Indirect Costs	**17,210.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services(DHHS), Arif Karim (214) 767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**67,173.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	67,173.00

L. Budget Justification*	File Name: MU_Budget_Justification1012199310.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 1538902720000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Missouri

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				PD/PI	Institutional Base Salary	EFFORT			9,615.00	3,535.00	13,150.00
2.						Co-I					3,771.00	1,387.00	5,158.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **18,308.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Specialist	EFFORT			4,018.00	1,477.00	5,495.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,495.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**23,803.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,250.00
2. Foreign Travel Costs	
Total Travel Cost	**1,250.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	4,380.00
2. Publication Costs	100.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . PKD Genotyping/US Pre-screen/CT imaging/Sedation	11,700.00
9 . Bloodwork	7,500.00
10 . Animal Care per diem	25,324.00
Total Other Direct Costs	**49,004.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**74,057.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	55	48,733.00	26,803.00
		Total Indirect Costs	**26,803.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services(DHHS), Arif Karim (214) 767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**100,860.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	100,860.00

L. Budget Justification*	File Name: MU_Budget_Justification1012199310.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 1538902720000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: University of Missouri

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Redacted by agreement				PD/PI	Institutional Base Salary	EFFORT			9,615.00	3,641.00	13,256.00
2.						Co-I					3,884.00	1,471.00	5,355.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **18,611.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Specialist	EFFORT			4,139.00	1,567.00	5,706.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**5,706.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**24,317.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	1,250.00
2. Foreign Travel Costs	
Total Travel Cost	**1,250.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 1538902720000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: University of Missouri

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	4,380.00
2. Publication Costs	100.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . PKD Genotyping/US Pre-screen/CT imaging/Sedation	9,600.00
9 . Bloodwork	7,500.00
10 . Animal Care per diem	12,331.00
Total Other Direct Costs	**33,911.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**59,478.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Modified Total Direct Cost	55	47,148.00	25,931.00
		Total Indirect Costs	**25,931.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services(DHHS), Arif Karim (214) 767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**85,409.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	85,409.00

L. Budget Justification*	File Name: MU_Budget_Justification1012199310.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Uncovered by a White Coat Waste investigation

UNIVERSITY OF MISSOURI BUDGET JUSTIFICATION (Years 3-5)

Salary Escalation Rates: Based on historical increases, all salary rates have been escalated by 3% per year (unless otherwise noted) to accommodate annual cost of living increases. This escalation has also been applied to current base salaries and stipends in order to estimate anticipated rates in year 1 of the project.

Fringe Benefit Rates: The University of Missouri (MU) has a federally negotiated rate for fringe benefits. For budgeting purposes, rates are projected to increase 3% each year.

A. SENIOR / KEY PERSONNEL

Redacted by agreement	EFFORT	Redacted by agreement
Redacted by agreement		
Redacted by agreement		

B. OTHER PERSONNEL

Redacted by agreement

C. EQUIPMENT

D. TRAVEL - $1,250 each year to attend Kidney Week to present work and meet with collaborators.

E. E. PARTICIPANT /TRAINEE SUPPORT COSTS

None

F. OTHER DIRECT COSTS

F.1 Materials and Supplies

Cost of diet in year 2 – 3 - estimated cost of ketogenic diet feeding trials. 8 cats on trial x $3.00 per day per can food x 12 months (365 days). $8,760 distributed over years 4-5.

The canned diets discussed that are low in carbohydrate, high in fat, and adequate in protein are in cans and sold in cases:

1. Royal Canin Veterinary Diet® Feline Renal Support D cat food
    a. % ME: protein, 24; fat, 60; carbohydrate, 16
    b. kcal/kg: 1,142
    c. Case of 24, 5.5 (0.156 kg) ounce cans is $48.34 (autoship) or $50.88 (one case)
    d. $11.31 per 1000 kcal

2. Hill's® Prescription Diet® d/d® Feline Duck and Pea Formula
    a. % ME: protein, 29; fat, 54; carbohydrate, 17
    b. kcal/kg: 1,209
    c. Case of 24, 5.5 ounce (0.156 kg) cans is $63.64 (autoship) and $66.99 (one case).
    d. $14.06 per 1000 kcal

3. Estimate of cost of diet to be hand-made - $15.00 per 1000 kcal

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F.2 Publication Costs

$100 /year, years 4-5, is requested to disseminate the results of this research in publications and poster presentations at scientific meetings. Manuscripts will be produced by the PI. Year 4 and Year 5

F.8 PKD Genotyping/US Pre-Screen/CT imaging/Sedation

Genotyping – 32 kittens DNA isolation, PCR, direct sanger sequencing $500 Year 3 and Year 4

US pre-screen 16 x $200 = $3200 distributed over Years 3-4

CT Imaging: 16 x 3 x $600 = $28,800 distributed over 3 years, including sedation, blood draw

F.9 Bloodwork

Bloodwork/urine/SDMA/ketones: 16 x 4 x $234 = $15,000 distributed over years 4-5

F.10 Animal Care per diem

MU per diem includes normal husbandry, normal feed, and standard veterinary care. Breeding stock is three male PKD^+ and four female PKD^- cats. Average litter size from breeding of a PKD^+ male with PKD^- female is 4 kittens, one - two litters per year (16 – 32 kittens). Expect a 50% distribution of males to females and PKD^+ to PKD^-. Gestation is 63 – 65 days (2 months). Queens are with kittens for 2 months until weaning. Feeding trials: sixteen PKD^+ adult (one year) trial cats and sixteen PKD^+ control cats (n = 32). Produce 16 kittens per breeding cycle in order to match gender and PKD severity. PKD^- cats used as controls. All PKD^+ cats will have cysts by 8 months of age, severity is variable and determined and monitored by US and CT.

Maintain 3 males at conditioned cost 365.25 x 4.22 = $1,542/year 3

4 females x 4.22/day x 245 days = $4,136/year 3

4 queens with litter at 2 months (April - May) 4 x 6.84 x 60.89 days = $1,666/year, years 3-4

Weaned kittens: 16 x 4.22 x 243.5 days = $16,442 over years 3-4

16 wildtype adoption 16 x 4.22 x 3 mos (92 dys) = $6,212 over years 3-4 (adoptions, re-assignment will be as rapidly as possible) – over 3 years

MU Office Animal Resources PER DIEM & SERVICE RATES effective February 1, 2019

Cat-conditioned	4.22
Cat-quarantined	7.81
Cat-queening w/ litter	6.84
Professional/Technical Service Charges	
OAR Tech Time/hr	23.34
OAR Tech OT/hr	35.01
Vet Tech Time/hr	31.00
Vet Tech OT/hr	46.50
After-hours veterinary research support	59.26

H. Indirect Costs:

The University of Missouri has a federally-negotiated indirect cost rate of 55% applied to modified total direct costs. Animal care per diem costs are excluded from the indirect costs calculation.

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RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		49,967.00
Section B, Other Personnel		16,495.00
Total Number Other Personnel	3	
Total Salary, Wages and Fringe Benefits (A+B)		66,462.00
Section C, Equipment		
Section D, Travel		3,750.00
1. Domestic	3,750.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		113,286.00
1. Materials and Supplies	8,760.00	
2. Publication Costs	200.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	23,400.00	
9. Other 2	24,600.00	
10. Other 3	56,326.00	
Section G, Direct Costs (A thru F)		183,498.00
Section H, Indirect Costs		69,944.00
Section I, Total Direct and Indirect Costs (G + H)		253,442.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		253,442.00

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Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	287,559	293,914	349,989	382,142	372,023	**1,685,627**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056 . Received Date: 2019-07-15T18:19:00.000-04:00

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Uncovered by a White Coat Waste investigation

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims1012199303.pdf
3. Research Strategy*	Research_Strategy1012199438.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals1012199309.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Consortium_Contractual_Arrangements1012199321.pdf
9. Letters of Support	
10. Resource Sharing Plan(s)	Resource_Sharing_Plan1012199323.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Biological_Che1012199429.pdf
Appendix	
12. Appendix	

Uncovered by a White Coat Waste investigation

Funding Opportunity Number: PA-19-056. Received Date: 2019-07-15T18:19:00.000-04:00

Specific Aims

Autosomal dominant polycystic kidney disease (ADPKD) is a very common genetic disease leading to renal failure. Only one FDA-approved drug is available to a limited fraction of patients, has only modest efficacy, significantly impacts patients' quality of life due to side effects and toxicities, and is offered at an extreme cost. An effective, safe and widely accessible therapy is urgently needed. This proposal is based on our preliminary results demonstrating that the metabolic state of ketosis profoundly inhibits PKD progression in animal models. Successful completion of the proposed work could lead to a disruptive change in ADPKD therapy by utilizing dietary interventions and/or dietary supplements without the need for pharmacological intervention.

We recently reported that a mild reduction in food intake strongly inhibits PKD progression in an orthologous mouse model. At the time, we did not understand the underlying mechanism. Now, we have found that the metabolic state of ketosis - not caloric restriction *per se* - is critical for the observed beneficial effect. During ketosis - the normal physiological response to periods of starvation - the body replaces glucose with fatty acids and ketone bodies as the primary fuel for most cells. We show that ketosis induced by dietary interventions (time-restricted feeding or a ketogenic diet) effectively inhibits mTOR activation and proliferation of cyst-lining epithelial cells, prevents myofibroblast expansion and fibrosis, and halts - and even reverses - overall disease progression. Remarkably, supplementation with the ketone β-hydroxybutyrate (BHB) alone - in the context of normal rodent chow - is *almost 100% effective in preventing PKD progression*. Preliminary results suggest that BHB acts on PKD kidneys *via* its receptor GPR109a, a GPCR that suppresses cAMP signaling.

Our results suggest that cyst cells in PKD are metabolically inflexible, depend on glucose and are unable to shift to utilizing fatty acids and ketone bodies. This view is consistent with recent results by several groups suggesting a metabolic defect in PKD. The goal of this proposal is to complete and expand our preliminary results and investigate the underlying mechanisms to understand how ketosis suppresses renal cyst growth. The main thrust of this proposal is to generate compelling results to justify clinical trials to investigate the efficacy of dietary interventions and/or BHB supplementation in ADPKD, and to inform the design of such trials. The main significance of this proposal is the enormous potential for clinical translation. Dietary interventions to induce ketosis are well-established. Because dietary interventions frequently fail in clinical practice due to poor adherence, our finding that BHB (an FDA-classified dietary supplement) has a dominant beneficial effect could rapidly lead to a highly feasible therapy.

Aim 1: To test the hypothesis that dietary interventions leading to ketosis will inhibit and/or reverse the progression of PKD.

The non-orthologous Han:SPRD rat model and an orthologous Pkd1 mouse model will be treated with regimens of a high-fat/low-carb ketogenic diet or by time-restricted feeding (TRF) using normal rodent chow. Efficacy on parameters of PKD progression and effects on molecular pathways will be assessed. To investigate whether the ketogenic diet primarily acts via lipotoxicity or via a BHB effect we will alter the composition of saturated vs. unsaturated vs. medium-chain fatty acids. Finally, the most effective dietary intervention based on rodent models will then be tested in an orthologous feline ADPKD model that most closely resembles the human disease, as a stepping stone towards clinical trials.

Aim 2: To test the hypothesis that treatment with BHB will decrease PKD progression.

PKD rodent models will be treated with a BHB salt formulation in the drinking water. The effects on PKD progression and molecular mechanisms will be investigated as in Aim 1. Because the BHB dose tested so far was almost 100% effective in preventing PKD progression, we will determine the minimum dose needed for efficacy in a "reversed" dose-finding study.

Aim 3: To test the hypothesis that BHB ameliorates PKD progression *via* its receptor GPR109A.

Our preliminary results indicate that crossing Gpr109a$^{-/-}$ mice with a Pkd1 mouse model accelerates disease progression. We will expand this investigation by using two rapidly-progressing Pkd1 mouse models (Ksp-Cre and Pkhd1-Cre) and one slowly progressing Pkd1 model (Cre-ER^{T2}). We will test whether Gpr109a knock-out prevents the efficacy of ketogenic dietary intervention or BHB.

This is a **low-risk/high-reward** proposal. Our strong preliminary data suggest that most of the experiments will be successful. An emerging effective, safe and available therapy will have an extremely high impact.

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Research Strategy

a. Significance

ADPKD is a common, life-threatening genetic disease that causes immense human suffering and a significant economic burden on the health care system[1]. Most patients will require dialysis or kidney transplantation. The vasopressin receptor antagonist tolvaptan has been FDA-approved as the first, and so far only, therapy for ADPKD in April 2018. However, tolvaptan is only moderately effective, problematic due to side effects including polyuria and potential liver toxicity, and its high cost has called the cost-effectiveness into question[2]. Many other potential pharmacological approaches are being developed/tested and are promising in short-term preclinical studies using rodent models. However, translation of such approaches to the clinic has proved challenging because ADPKD is a slowly progressing disease that likely requires pharmacological intervention for years and decades, starting long before patients may even exhibit significant symptoms[3]. None of the molecular drug targets implicated in ADPKD are unique to polycystic kidneys. Therefore, potentially effective drugs also tend to cause extra-renal, long-term effects that would be prohibitive in clinical practice[3]. A prime example are the mTOR inhibitors that are highly effective at high doses in PKD rodent models[4,5] but failed in ADPKD clinical trials due to dose-limiting adverse effects[6].

To circumvent this problem, our lab has developed methods for tissue-targeting to renal cysts of small molecule drugs *via* the folate receptor[7,8] and of monoclonal antibodies *via* the polymeric immunoglobulin receptor[9]. Development of tissue-targeted drugs continues in the lab and is hoped to lead to therapeutics with minimized extra-renal adverse effects suitable for long-term treatment.

Even better would be an effective therapy that is free of significant adverse effects to begin with. Our recent results suggest that such a therapy may be possible. As detailed in Preliminary Data below, our results indicate that ***induction of the metabolic state of ketosis leads to strong inhibition of renal cyst growth, and even regression of existing cyst burden***. Ketosis is the natural response of the body to periods of fasting that involves a shift in the utilization of fuels for energy. Glucose is replaced by fatty acids and ketone bodies as the primary fuel for most cells during ketosis. We recently reported that a mild reduction in food intake surprisingly strongly inhibits PKD progression in a Pkd1 mouse model[10], and an independent study came to the same conclusion[11]. Follow-up studies now revealed that the beneficial effect was actually not due to caloric restriction *per se* but rather to the effects of ketosis.

We found that ketosis, induced either intermittently by time-restricted feeding or continuously by a ketogenic diet, leads to strong inhibition of PKD progression. In addition, acute fasting leads to rapid induction of death in cyst-lining cells, disruption of the epithelial barrier of cysts, loss of cyst volume and decrease of total kidney mass. These striking results suggest that cyst-lining cells are metabolically inflexible and unable to effectively utilize fatty acids and ketones during periods of ketosis. Our results are consistent with recent findings by others suggesting that renal cysts exhibit metabolic changes including increased glycolysis, and disrupted mitochondrial function and fatty acid oxidation similar to the Warburg effect in cancer[12-14].

Our surprising finding that the apparent metabolic defects of cyst cells can be so effectively exploited by inducing ketosis to inhibit and reverse cyst growth is of enormous significance due to the very high translational potential. Time-restricted diets and ketogenic diets are widely used, even among lay people, to control obesity and diabetes with few, if any, adverse effects. The ketogenic diet has been clinically used as an effective and safe therapy for epilepsy in children for nearly 100 years[15].

We propose here to complete, solidify and expand our preliminary results, and to define the effects of ketosis on PKD. We also propose to investigate the possible efficacy of compounds that mimic ketosis, such as beta-hydroxy butyrate, as an alternative therapy to dietary restriction. While we also propose to investigate underlying mechanisms to begin to explain the effects of ketosis, the main thrust of this proposal is to generate compelling results to justify clinical trials to investigate the efficacy of dietary interventions in ADPKD, and to inform the design of such trials.

Achieving the aims of this proposal could lead to a disruptive and fundamental change in the treatment of ADPKD. The outcome could literally be “disruptive" because a safe and effective therapy based on dietary intervention could undermine very substantial investments world-wide by the biopharmaceutical industry, funding agencies, patient advocacy organizations, and academic investigators to develop drugs for ADPKD therapy.

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We feel that there is an urgency to the completion of our proposed research. Most ADPKD patients in the US consume a high carbohydrate diet throughout their waking hours, as is typical in industrialized societies. Therefore, most patients essentially never experience periods of ketosis. Our results suggest that such dietary habits promote renal cyst growth. Nutritional studies by well-meaning investigators recommend diets with even increased carbohydrate content for ADPKD patients[16] and this diet is currently promoted by the PKD Foundation in the form of a cookbook[17]. A plethora of additional cookbooks, websites, online forums etc advocate various diets for ADPKD, and many of these recommendations are counter to our results and may worsen PKD progression. Experimentally based insights are urgently needed, leading to scientifically validated dietary interventions with the potential to become the standard of care for ADPKD.

b. Innovation

The main innovative aspect of this proposal is conceptual. Dietary restriction is perhaps the simplest possible intervention and could have been tested ever since the PKD research field was born. However, it took until 2016 when our group and a group at the Mayo Clinic independently reported the effect of food restriction on PKD progression in mouse models[10,11]. Even at that time, we had not realized that the effect is due to ketosis, as we now shown in our preliminary results. Based on everything we have recently learned about metabolic changes in PKD, however, this should probably not have come as a surprise. Mechanistically, our findings make sense, and successful completion of this project will better enable us to put various known concepts together into new models and hypotheses. Can fasting be regarded as an "innovative" approach? Clearly not fasting *per se* because fasting may be the oldest form of medical therapy going back to prehistoric times. However, the application of fasting to the problem of ADPKD, and the mechanistic insights that will be gained, are highly innovative. An experimental innovation in this proposal is the use of feline ADPKD as an orthologous model system to test the efficacy of dietary interventions. In collaboration wit[Redacted by agreement] (U. Missouri), we have successfully utilized cats with a mutation in Pkd1 in a small longitudinal study (see Preliminary Results). It can be argued that cats with a natural mutation in Pkd1 are by far the closest animal model to human ADPKD due to the identical underlying genetic mechanism of gene inactivation. Our study is the first interventional study in feline ADPKD and may serve as a model for future investigations with the aim to validate results derived from rodent models prior to the initiation of clinical trials.

c. Approach

Background

We recently published that a mild reduction (by 23%) in food intake ameliorated disease progression in an orthologous PKD mouse model leading to inhibition of mTOR signaling and a marked decrease in cyst growth, kidney weight and improved kidney function[10]. An independent study came to the same conclusion, and these investigators found that even a 10% reduction in food intake was still beneficial[11]. The surprising efficacy of such a minor level of food reduction led us to further investigate the potential underlying mechanism. In our study, we noticed a behavioral difference between animals in the reduced-food-intake (RFI) and *ad libitum* (AL) cohorts. Whereas AL-fed controls consumed their food in small portions frequently throughout the day, mice in the RFI cohort consumed their entire daily pre-weighed food allotment shortly after feeding, usually within one hour. This behavioral difference makes it difficult to separate effects of caloric restriction and those of time-restricted feeding. We found that the blood levels of the ketone body β-hydroxybutyrate (BHB) from the animals of our previous study was significantly elevated in the RFI cohort compared to the AL cohort (not shown) suggesting that these animals had indeed experienced intermittent fasting leading to ketosis.

Preliminary Data (note: additional preliminary data are shown in context in Aim 3)

Time-restricted feeding (TRF) reduces cystic disease progression. To test whether the beneficial effect on renal cyst growth may be due to the effects of intermittent fasting, rather than reduced food intake *per se*, we placed Han:SPRD rats on a TRF regimen with nightly access to food for an 8-hour period (Fig. 1) from postnatal weeks 3-8. (Note that in this well-established, non-orthologous rat model male Cy/+ animals exhibit more severe PKD than females[18]). TRF animals consumed a comparable number of calories and gained weight similar to the AL-fed controls (not shown) indicating that TRF does not lead to caloric restriction. TRF led to strikingly reduced renal cystic disease progression compared to controls with marked reductions in the 2-kidney/body weight ratio and cystic index (Fig.1A,B). Increased levels of BHB (Fig.1C) and reduced blood

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glucose (Fig.1D) indicated that TRF led to increased utilization of ketones and fatty acids as energy source at the expense of glucose.

mTORC1 activity (Fig. 2A) and proliferation (Fig. 2D) were strongly inhibited in cyst-lining epithelial cells in TRF animals and interstitial fibrosis was strongly reduced (Fig. 2B). Pericystic myofibroblasts are abundant in AL-fed animals but almost absent in TRF-animals (Fig. 2C) suggesting that their loss is responsible for the improvement in fibrosis. These results suggest that the metabolic changes leading to ketosis induced by TRF have a profound inhibitory effect on renal cyst growth and fibrosis. This effect appears to be specific to polycystic kidneys because TRF did not affect normal kidneys, other organs or total body weight.

The ketogenic diet reduces cystic disease progression. To test whether the beneficial effect of TRF may be due to ketosis as opposed to other possible consequences of TRF (e.g. alterations in circadian rhythms) we explored the ketogenic diet. Han:SPRD rats were given *ad libitum* access to a high fat, very low carbohydrate ketogenic diet (KD; caloric ratio of ~91% fat, ~2% carbohydrates and ~5% protein) and compared to animals on normal chow (NC). Animals were again treated from postnatal weeks 3-8. KD-treated rats exhibited much more exaggerated increases in BHB levels and decreased blood glucose (Fig. 3C,D) compared to animals on the previous TRF regimen, indicating that the KD leads to a profound metabolic switch. Renal cystic disease progression was strongly inhibited in animals on the KD regimen compared to those on NC, with reductions in the 2-Kidney/Body Weight ratios, cystic index, renal fibrosis and cyst cell proliferation (Fig. 3).

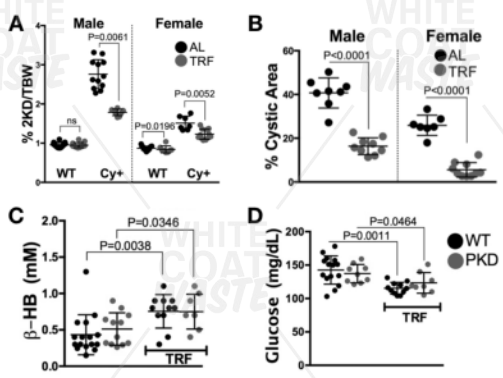


Fig. 1. Time-restricted feeding (TRF) vs. *ad libitum* feeding (AL) in Han:SPRD rats. Cy/+ rats were treated from postnatal weeks 3-8. TRF animals had access to food for an 8-hour period within their 12-hour dark cycle. TRF animals show a significant decrease in their 2-kidney/body weight ratio (**A**) and the cystic index (**B**). TRF leads to a moderate increase in BHB blood levels (**C**) and decrease in blood glucose (**D**).

Animals on the KD appeared phenotypically normal, did not exhibit any signs of distress, and consumed comparable calories to NC controls relative to their body weight. However, KD-treated animals exhibited reduced bodyweight gain (Fig. 3G). During postnatal weeks 3-8, rats are rapidly growing juveniles and we attributed the reduced body weight gain to protein restriction due to the KD diet. To exclude possible confounding factors related to the suppression of body weight gain we next treated Han:SPRD rats at an older age (postnatal week 8-12) with the KD vs NC. During this 4-week treatment period, these early adult rats exhibit only a modest body weight gain on NC, and this gain was mildly affected in rats on the KD but was not different between wild-type or cystic animals (Fig. 4D). Remarkably, whereas the total mass of polycystic kidneys slightly increased in animals on NC between week 8-12, it ***decreased*** by 1.3 grams in animals on KD (Fig. 4D). This effect of KD on organ size disproportionally affected polycystic kidneys because wild-type kidneys or other organs, such as the heart, were only mildly affected or unaffected (Fig. 4D and not shown). KD-treatment resulted in a significant decrease in the 2-kidney to bodyweight ratio (Fig.4A) and similar changes on BHB and blood glucose (Fig. 4B,C) as in younger animals. These striking results suggest that metabolic changes induced by the ketogenic diet

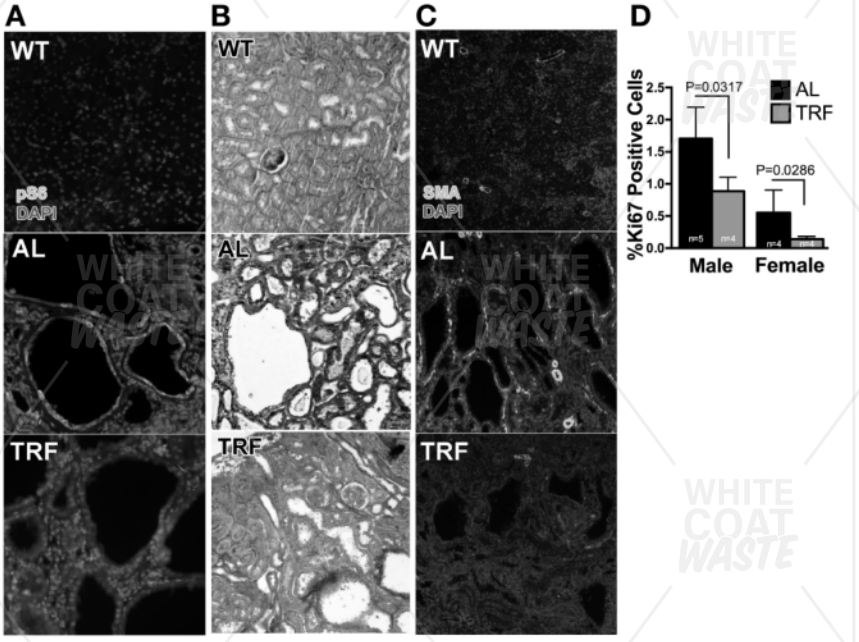


Fig. 2. Inhibition of mTOR, fibrosis and proliferation by time-restricted feeding in Han:SPRD rats. Experiment as in Fig. 1. Note that TRF leads to inhibition of mTORC1 activity (**A**: pS6, green), fibrosis (**B**: Sirius red stain), pericystic myofibroblasts (**C**: smooth muscle actin staining, green), and cell cycling (**D**: Ki67-positive cyst cells).

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can lead to ***reversal*** of existing renal cystic disease.

Acute fasting leads to reversal of cystic burden. These observed strong effects of dietary interventions occurred during the course of several weeks. To investigate whether ketosis has any fast-acting effects on renal cysts we subjected Han:SPRD rats and a well-characterized, orthologous mouse model ($Pkd1^{cond/cond}$:Nes^{cre})[5,9,10,19-22] to ***acute ketosis*** by fasting for 48 or 24 hours, respectively, with free access to water. Fasting led to a modest decrease in body weight as expected due to depletion of fat reserves and liver glycogen but had no effect on heart weight or the weight of normal kidneys (not shown). Strikingly, acute fasting led to a ~20% reduction in kidney mass in the cystic animals both in rats and mice with PKD (Fig. 5A and not shown) that could be accounted for by a decrease in the cystic volume (Fig. 5B). Histological analysis revealed frequent cell death of cyst-lining cells leading to sloughing off into the cyst lumen, the appearance of denuded cyst areas (Fig. 5E) and an increase in TUNEL-positive cells (Fig. 5F,G). The results were identical between the non-orthologous rat model and the orthologous mouse model and suggest that extended fasting leads to cell death in PKD cysts causing draining of cyst fluid and overall reduction of cystic burden and TKV. Remarkably, cyst-lining cells in fasted animals exhibited extreme loading with intracellular oil droplets (Fig. 5H). This result suggests that cystic cells can still efficiently take up fatty acids but are unable to metabolize them which necessitates their deposition into lipid droplets as a stress response similar to what has been observed in cancer cells[23]. Therefore, the observed increased cell death of cyst cells during acute ketosis may mechanistically be due to lipotoxicity.

To extend these studies, we conducted a longitudinal experiment in cats with mutations in PKD1 (10063C>A) that cause feline ADPKD[24]. ADPKD is common in domestic cats and can be regarded as the animal model that best recapitulates the human disease due to the essentially identical underlying genetics (second hit mechanism). Four cats with confirmed PKD1 mutations were studied by measuring total kidney volumes (TKV) ~17 months prior to the study, immediately prior to fasting and immediately after fasting for 72 hours. Fasting led to decreased TKV in all cats (Fig. 5J) and visible size decreases of large cysts (Fig. 5I).

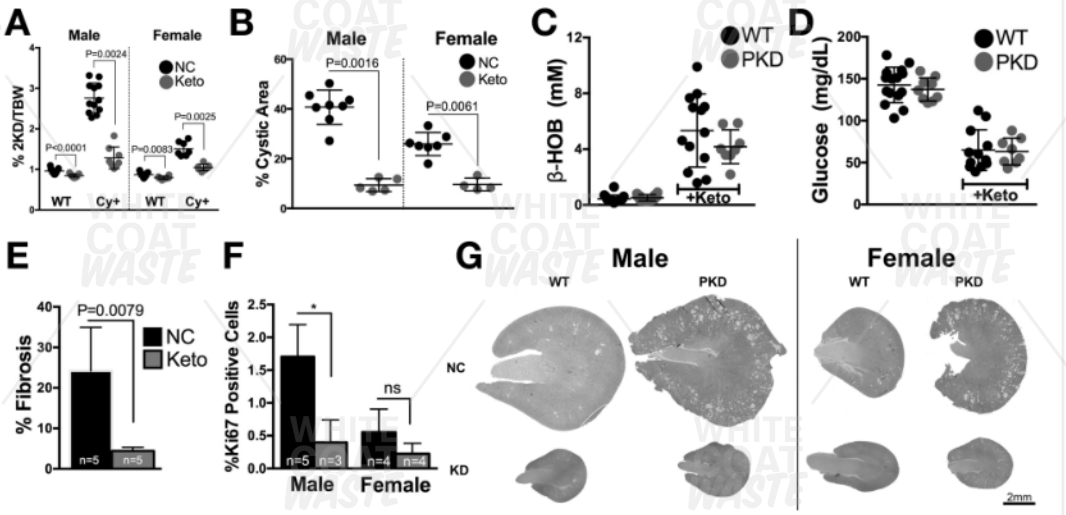


Fig. 3. The ketogenic diet inhibits renal cystic progression in juvenile Han:SPRD rats. Cy/+ rats were treated from postnatal weeks 3-8 with *ad libitum* access to either normal chow (NC) or a ketogenic diet (KD). KD animals show a significant decrease in their 2-kidney/body weight ratio (**A**) and cystic index (**B**). KD leads to a strong increase in BHB blood levels (**C**), and strong decreases in blood glucose (**D**) fibrosis (**E**) and cyst cell proliferation (**F**). However, note that the KD also impacts normal body weight gain in these juvenile rats as illustrated by smaller kidneys in wild-type animals (**E**).

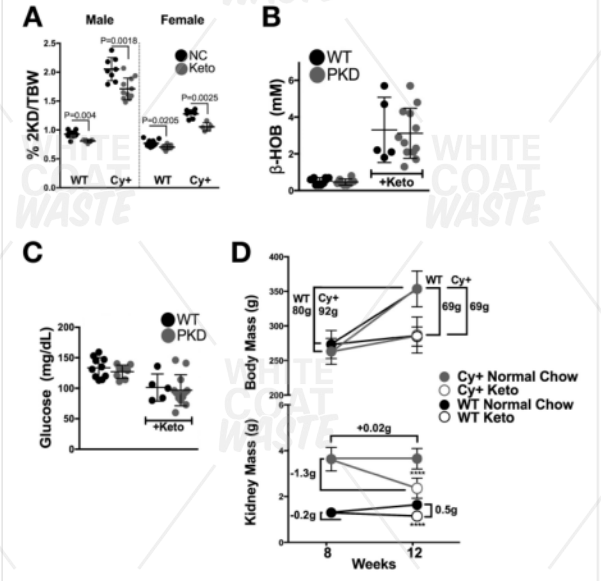


Fig. 4. The ketogenic diet reverses renal cystic progression in early adult Han:SPRD rats. Cy/+ rats were treated as in Fig. 3 but a later age, from postnatal weeks 8-12. KD animals exhibit a significant decrease in their 2-kidney/body weight ratio (**A**), strong increase in BHB blood levels (**B**), and decrease in blood glucose (**C**). Note that these early adult animals exhibit little body weight gain during this time (**D**). Strikingly, the total kidney mass in cystic rats on KD substantially decreases at week 12 compared to week 8, suggesting reversal of renal cystic disease (**D**).

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BHB treatment prevents PKD progression. Finally, we hypothesized that BHB itself may play a role in our observed effects of ketosis. In addition to being an energy source for cells, BHB also has complex effects on cellular signaling and metabolism that are only partially resolved to date including effects on signaling pathways implicated in PKD such as mTOR, AMPK and HDACs[25-28] . We treated Han:SPRD rats with BHB from postnatal weeks 3-8 in the context of AL-feeding with normal chow. BHB was administered in the drinking water *ad libitum* at a dose equivalent to the daily dose recommended as a dietary supplement. BHB treatment had no effect on water consumption, body weight or blood glucose levels. The effect of 5 week BHB-treatment was truly remarkable and the kidneys of PKD animals were nearly indistinguishable from kidneys of wild-type animals, both at the gross and histological level (Fig. 6A, B). BHB-treatment caused dramatic reductions in the 2-kidney to bodyweight ratio (Fig. 6C), cystic area (Fig. 6D), and fibrosis (Fig. 6B) compared to controls. Neither wild-type kidneys nor other organs were affected by BHB treatment indicating that the effect is specific to polycystic kidneys. These results demonstrate that BHB acts in a dominant fashion as a suppressor of PKD progression, even in animals that are fed a high carbohydrate diet and have unaltered blood glucose levels. Altogether, our results suggest that inducing the state of ketosis, either by dietary intervention or by mimicking its effects with BHB, may be an effective treatment for ADPKD in humans.

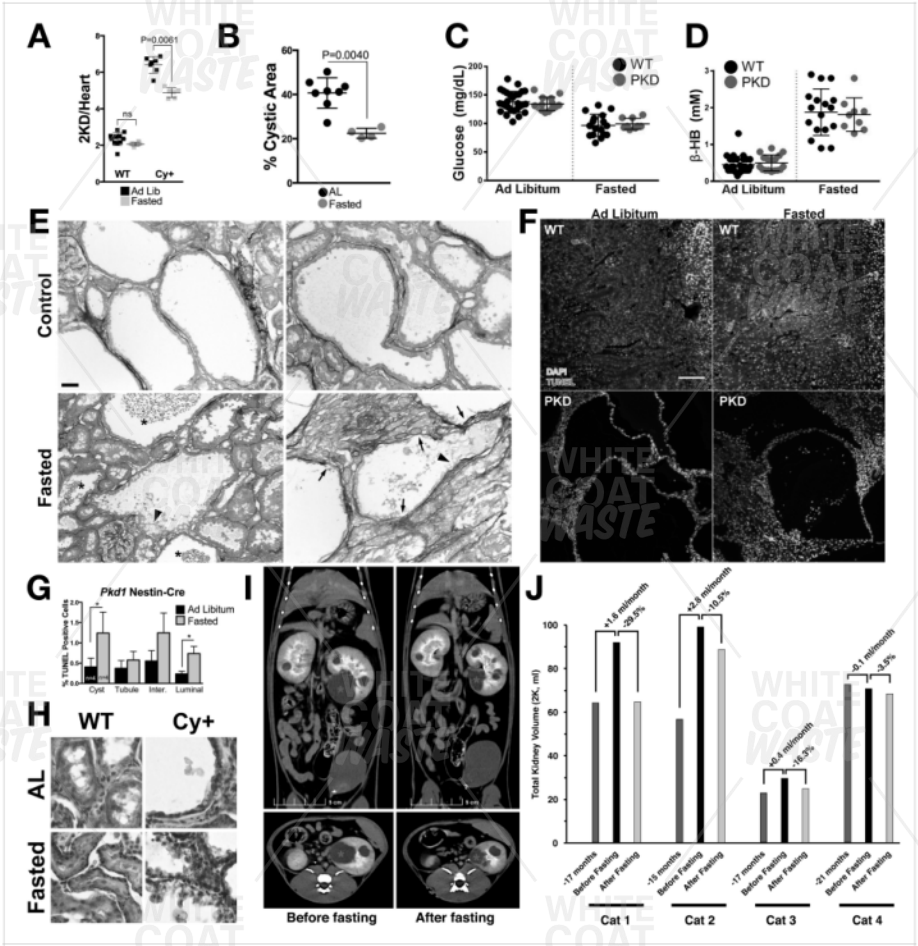


Fig. 5. Extended fasting reverses cystic burden in Han:SPRD rats, *Pkd1*$^{cond/cond}$:Nes^{cre} mice, and PKD1-cats. 8-week old Han:SPRD rats (**A-E, H**) and *Pkd1*$^{cond/cond}$:Nes^{cre} mice (**F-G**) were fasted for 48 or 24 hours, respectively. Adult cats with confirmed PKD1 mutations were fasted for 72 hours (**I-J**). Note the significant decrease in 2-kidney/heart weight ratio (**A**), cystic index (**B**) and blood glucose (**C**), and increase in blood BHB (**D**) in fasted Han:SPRD rats. Sirius red/fast green stain (**E**) revealed significant cell death of cyst-lining cells in fasted animals leading to sloughing off of cells (arrowheads) and denuded cyst areas (arrows). Increased cell death and accumulation of dead cells in cyst lumens was also seen by TUNEL staining in fasted *Pkd1*$^{cond/cond}$:Nes^{cre} mice (**F, G**). Fasting led to extreme accumulation of intracellular oil droplets in cysts as shown by Oil Red O staining (**H**). A longitudinal study in cats with PKD was conducted by measuring total kidney volumes (TKV) ~17 months prior to the study, immediately prior to fasting and immediately after fasting. Note that fasting led to decreased TKV in all cats (**J**) and visible size decreases of large cysts (**I**, asterisks).

A personal note: My lab has been involved in PKD research for almost 20 years, our work has led to clinical trials (mTOR inhibitors), and I have reviewed countless manuscripts and grant proposals in the field and have been serving for the last 6 years as a permanent member on the NIH-KMBD study section, which reviews most of the grants in the PKD field. I can confidently state that I have never seen any treatment in PKD animal models (by us or others) that is as profoundly effective as the treatment with BHB shown here. And most remarkable is that this treatment appears to be devoid of any detectable adverse effects.

Hypotheses

These striking preliminary data suggest a model that is consistent with recent findings suggesting that renal cysts exhibit metabolic abnormalities including increased dependence on glycolysis, and disrupted mitochondrial function and fatty acid oxidation similar to the Warburg effect in cancer[12-14]. Treatment of a PKD mouse model with the glycolytic inhibitor 2-deoxy-glucose inhibited renal cyst growth[29] suggesting that cysts may be dependent on glucose as their energy source. Cyst cells have recently been shown to have

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fragmented, defective mitochondria[14] and an intrinsic fatty acid oxidation defect[13]. Altogether, these results and our preliminary data suggest the ***overarching hypothesis that cyst-lining epithelial cells in PKD are metabolically inflexible, and are incapable of switching their metabolism to utilizing ketones and fatty acids during periods of ketosis.*** Consequently, in the state of ketosis cyst-lining cells are starved of fuel for energy generation, leading to inhibition of signaling pathways critical to sustain cell growth and proliferation (e.g. mTOR). In acute ketosis (e.g. extended fasting), this effect can even lead to significant cell death, causing disruption of epithelial integrity, draining of cyst fluid (presumably by interstitial/lymphatic absorption) and reversal of cystic burden. These considerations lead to the ***next overarching hypothesis that the metabolic inflexibility of cyst-lining cells in PKD can be exploited for therapy using dietary interventions that induce the state of ketosis.*** Mechanistically, we hypothesize that the observed effects of ketosis on renal cysts are due to **(a)** restricting glucose availability in cells that are dependent on glucose, **(b)** signaling effects of BHB, **(c)** lipotoxicity due to increased fatty acid uptake and simultaneous inability to catabolize fatty acids, or **(d)** a combination of all.

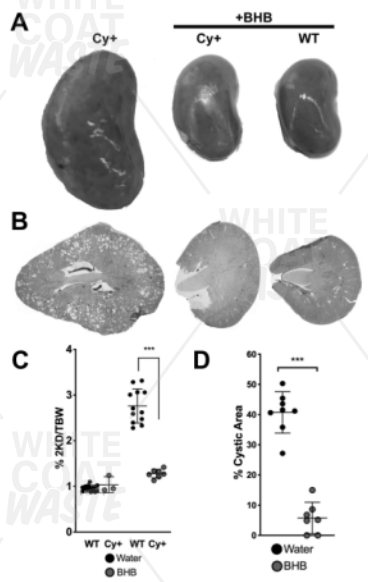


Fig. 6. Effect of BHB. Han:SPRD rats (only males shown) were given 150 mM BHB in the drinking water between postnatal weeks 3-8. **A**, gross. **B**, fibrosis by Sirius red stain. **C**, 2-kidney to body weight ratio. **D**, cystic index of Cy/+ kidneys.

Scientific Premise: Statistical significance for our key preliminary data was established as indicated in the figures. A wide variety of experimental approaches yielded results that are all consistent with our hypotheses. For example, experiments using a non-orthologous rat model and two orthologous Pkd1 models (mice and cats) are all in agreement. The key observation that food restriction ameliorates PKD progression was made by us[10] and another lab independently[11] using two different orthologous models. Our results and hypotheses are consistent with published findings by several other labs regarding metabolic changes in PKD. Our conclusions are also consistent with the clinical observations that hyperglycemia[30] and obesity[31] correlate with faster ADPKD disease progression. All of these data together lead to a very strong scientific premise.

Scientific Plan

Scientific Rigor, Reproducibility and Transparency: All experiments in this proposal will have appropriate positive/negative controls to ensure scientific rigor. Quantitative data based on image analysis of stained tissue sections (cystic index, proliferation, cell death, phospho-specific immunostaining, myofibroblast counts, fibrosis etc) will be obtained by morphometric analysis of grid intersection points or automated image analysis using Image J using a sufficiently large number of microscopy fields per sample or after whole-slide-scanning as appropriate. Analysis will be performed in a blinded fashion by highly trained investigators able to identify the features of interest (e.g. cyst-lining vs. interstitial cells) and reject non-relevant features (e.g. smooth-muscle cells of blood vessels vs. pericystic myofibroblasts). Fluorescence images of all samples of an experiment will be acquired in the same microscopy session using identical microscope and camera settings, and if necessary, the histograms will be linearly adjusted equally in Photoshop. If the data indicate that additional replicates are needed for sufficient power, new experiments will be performed with adequate power.

To guard against the possibility that effects of interventions observed in one PKD animal model may represent merely a peculiarity of that model, we will conduct all experiments in at least two independent rodent models (the non-orthologous Han:SPRD rat model and the orthologous Pkd1:Nestin^{Cre} mouse model). Some interventions will additionally be tested in the orthologous feline model. If results from multiple animal models are consistent it makes a strong case that the observed effects will be predictive for human ADPKD as well. The minimal animal numbers needed to obtain conclusive and statistically significant data will be used for the proposed intervention studies. In our preliminary studies consisting of the same cohorts of animals, we were able to detect highly significant effects of dietary interventions using 5-8 animals per treatment group. We therefore anticipate that similar sample sizes will provide sufficient power to detect significant differences in the proposed experiments. Equal numbers of male and female animals will be randomized into the treatment groups, and data will be initially analyzed separately for each gender to detect possible effects of sex as a biological variable. If sex is found to affect any of the outcomes, sufficient numbers of male and female animals

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will be used to achieve statistically significant gender-specific results. If sex is not found to affect any outcomes, data from male and female animals will be pooled. In the case of the Han:SPRD rat model, male/female cohorts will always be analyzed separately because of the known gender effect in this model.

To ensure transparency, all original, unaltered data (e.g. micrographs, immunoblots, data tables) will be immediately stored after acquisition on a NAS drive that is mirrored on a backup-NAS drive and backed up daily using a cloud server to prevent data loss. Original data will be reviewed between the PI and lab personnel, and made available upon request by scientific journals during manuscript review.

Aim 1: To test the hypothesis that dietary interventions leading to ketosis will inhibit and/or reverse the progression of PKD.

Rationale: Our preliminary data make a strong case that the hypothesis will be confirmed. Compelling data from animal experiments, using multiple models, are needed to justify long-term clinical trials to test the efficacy of ketogenic dietary interventions in ADPKD patients. We will therefore complete and expand the experiments described in Preliminary Results, and begin to explore the mechanisms involved. *Please note that our preliminary data are already very extensive and will be part of an initial publication that may appear before the review of this proposal. This does not mean that the proposed work has already been accomplished. We need to conduct all of the proposed work to investigate mechanisms, test an orthologous model etc. We anticipate that there will be many follow-up papers to our initial publication.*

Experiment #1: **Time restricted feeding (TRF).** We will complete our studies using Han:SPRD rats shown in Fig. 1 and 2, and additionally use an orthologous mouse model with a mosaic deletion of Pkd1 utilizing Cre driven by the Nestin promoter ($Pkd1^{fl/-}$:Nes^{Cre}). This model exhibits variegated renal Pkd1 deletion to mimic human ADPKD and has been well characterized in our lab[5,9,10,19,20,32-34].

To preserve normal circadian rhythm, animals will be fed normal chow *ad libitum* (Pico Lab Rodent Diet 20) beginning 3 hours after lights-out with their food removed 1 hour before lights-on (8 hours fed) and restricted from food for 16 hours from week 3 to week 8 of age. Food will be weighed prior to feeding and upon withdrawal to determine overall caloric intake. At the end of the study, animals will be euthanized, their organs removed, weighed and subjected to biochemical and histological examination. Standard parameters to assess ketosis and PKD progression will be similar to those shown in preliminary data and include blood glucose and BHB levels, BUN, 2-kidney/body weight ratio, renal cystic index, and renal fibrosis (Sirius red stain).

A likely mechanism of TRF on PKD progression is the expected reduction of mTOR activity during the fasting period. This will be investigated by monitoring the levels of P-S6 and P-4EBP1 by immunoblotting and IF-microscopy as shown before[4,5,7,10,19,34] and in Fig. 2. We will similarly analyze associated cellular pathways involved in energy metabolism including AMPK, Erk, and Akt, as well as effects on STAT3 activity as previously shown[5,7,10,19,20,35]. Cell cycle entry leading to possible hypertrophy will be investigated by IF-staining for Ki-67. Proliferation will be assessed using the mitotic marker P-histone H3. TUNEL staining will be used to evaluate effects on dell death. Depending on results obtained, other markers may be added to delineate signaling pathways. In all cases, these markers will be investigated by IF-microscopy and effects will be stratified (by co-staining) based on the affected cell types, including cyst-lining epithelial cells, normal tubule cells, interstitial cells, macrophages and myofibroblasts (see below).

We hypothesize that the observed inhibition of fibrosis by TRF treatment (Fig. 2B) may be due to inhibition of pericystic myofibroblasts (Fig. 2C) and/or cyst-associated macrophages. Myofibroblasts are known to be pro-fibrotic in PKD and their regulation may be an important determinant in disease severity[36]. The role of macrophages in inflammation and promoting fibrosis in PKD is suggested by recent results as summarized by us[37]. Fasting has previously been shown to reduce inflammation[38]. Effects of TRF on the abundance of myofibroblasts and macrophages will be determined by IF-staining for SMA-1 (Fig. 2C) and F4/80, respectively. Depending on results, markers against macrophage subtypes (M1- vs. M2-like) can be used. If a role of macrophages is indicated we may further investigate effects on inflammatory regulators/markers by qPCR and ELISA (e.g. IL6, TNF-alpha and C-reactive protein) found within the kidney and the serum.

To investigate changes in energy metabolism we will investigate pathways associated with the regulation of fatty acid metabolism such as PPAR-α and -γ, and their downstream effectors which includes effects on mitochondrial biogenesis (assessed by mitochondrial number), ketone metabolism (HMG-CoA synthase and monocarboxylate transporter-1, MCT-1), fatty acid transportation (carnitine palmitoyl transferase, CPT1-α) and

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fatty acid utilization in the citric acid cycle (pyruvate dehydrogenase, electron transport complexes 2 and 4). These analyses will be performed using IF-microscopy, immuno-blotting, and/or qPCR.

Anticipated results, pitfalls, future experiments: Based on our extensive preliminary results we expect that this experiment will conclusively prove that TRF - in the absence of caloric restriction - has a profound beneficial effect on PKD disease progression. This is the primary outcome and would have immediate implications for translation into a clinical therapy. A secondary outcome will be the identification or rejection of possible mechanisms involved in the beneficial effect of TRF that will help to shape future mechanistic investigations. A potential pitfall is that the orthologous Pkd1 mouse model may respond differently to TRF than the non-orthologous rat model. Given that both models (and the orthologous feline model) respond identically to acute fasting (Fig. 5) we consider this pitfall unlikely. Furthermore, we[10] and the Mayo Clinic group[11] have already published that TRF (with only mild caloric restriction) effectively inhibits PKD progression in our and their Pkd1 mouse models. However, if necessary, we may utilize other mouse models that we can obtain, e.g. the $Pkd1^{RC/RC}$ mouse model and others available *via* the national PKD centers (Mayo, Kansas, UAB, Baltimore) that the PI is a member of.

In collaboration with Redacted by agreement Scripps Research Institute) we will conduct metabolomics analyses on the rat/mouse kidneys in this study. Redacted by agreement was awarded a grant from the Deutsche Forschungsgemeinschaft to develop metabolomic methods specifically for the analysis of polycystic kidneys in collaboration with our group and has already generated interesting initial preliminary data. We will specifically investigate how dietary interventions affect metabolites of the TCA cycle, amino acid and fatty acid metabolism. Results may allow us to delineate specific metabolic defects in PKD kidneys and suggest which defects lead to metabolic inflexibility and susceptibility to ketosis. We have a long-standing collaboration with Redacted by agreement and he has recently discussed [39,40] the methods that will be employed. No funds for Redact Redacted by agreement or his lab are requested. Please regard this as an "extra bonus." The metabolomic investigation is relatively open-ended and may yield unexpected results to be followed up on in future experiments.

***Experiment #2:* Standard ketogenic diet.** Effects of ketosis induced by TRF (above) will be directly compared to ketosis induced by a standard, high-fat/low-carb ketogenic diet administered *ad libitum* to exclude any potential confounding effects of disruptions of circadian rhythm. Experiments shown in Fig. 3+4 will be completed in Han:SPRD rats as described, and also extended to $Pkd1^{fl/-}$:Nes^{Cre} mice. Both models will be treated as juveniles from postnatal weeks 3-8, and as adults from postnatal weeks 8-12, respectively. Due to the observed (Fig. 3) stunting effect on juvenile rats we will first conduct a pilot experiment to establish the required protein amount needed to avoid effects on body weight gain. This has similarly been done recently by other investigators[41] in rats and we expect that a similar modification will prevent stunting. Once established, all ketogenic diets will be formulated with the appropriate protein content for all subsequent experiments. All ketogenic diets will be custom formulated by Envigo (www.envigo.com) to maintain standards amongst experimental groups. Formulations will be modified based on the standard ketogenic diet (Bioserv F3666) utilizing established protocols[42,43]. Control animals will be fed normal chow *ad libitum*. All food will be weighed to determine caloric intake. Animals will be weighed weekly with blood collected to record ketone and glucose levels. At the end of the study, the same analyses will be conducted as for Experiment #1.

***Experiment #3:* Different fatty acid compositions in ketogenic diet.** We hypothesize that ketosis effectively inhibits renal cyst growth due to either **(1)** a lipotoxic effect due to increased supply of circulating fatty acids in combination with a defect of fatty acid oxidation in cyst-lining cells and/or **(2)** effects of BHB which is known to regulate several signaling pathways[26,27] important in PKD. These mechanisms would be directly affected by the fatty acid composition in the ketogenic diet. Unsaturated fatty acids (UFA) are expected to increase effects of lipotoxicity due to the generation of reactive oxygen species during their catabolism and lipid peroxidation[44,45]. In contrast, medium-chain triglycerides (MCT; carbon lengths between 8-10) are expected to increase effects of BHB due to their preferential conversion to ketones by the liver[46] leading to increased ketosis and effects on fat metabolism including PPAR-γ[3,47-49]. The "standard" ketogenic diet used in Experiment #2 and in preliminary data contains a mixture of corn oil, butter and lard used commonly in animal experimentation. *Prior to testing the efficacy of ketogenic diets in long-term clinical trials it will be extremely important to know which fats should be present/avoided in the prescribed diets. Unnecessary risks should be avoided, for example increased oxidative stress due to high content of unsaturated fatty acids.*

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To determine the effects of altered fatty acid composition, we will repeat Experiment #2 using three modified forms of the ketogenic diet. Each diet will be custom formulated by Envigo and matched isocalorically (identical caloric ratios of protein:carbohydrate:fat) with varying ratios of corn oil, lard or MCTs (from fractionated coconut oil), to assess the effects of UFA, long chain saturated (LCSF) and MCTs, respectively, on PKD progression. LCSF and MCT diets will contain 10% of calories from UFA with the remaining calories from LCSFs or MCTs, respectively. The only fat source in the UFA diet will be corn oil (contains ~10% saturated fat). In addition to the analyses described in Expt #2, we will evaluate how fatty acid composition affects oxidative damage by immunohistochemical staining for 4-hydroxy-2-nonenal (4-HNE, a marker of lipid peroxidation) and 8-oxo-dG (a marker for oxidative DNA damage)[50,51] as well as heme oxygenase-1, a marker of oxidative stress[52] and glutathione peroxidase 4 (GPX4), an inhibitor of lipid-based reactive oxygen species that cause ferroptosis, a form of non-apoptotic cell death[45].

Anticipated results, interpretation: Experiments examining the use of supplemental fatty acids on the progression of PKD have been performed previously with mixed or contradicting results depending on sex, model used, and the type or quantity of fatty acid supplemented[13,53-56]. All of these studies however were performed in the context of a high carbohydrate diet, and therefore in the absence of ketosis. Despite their almost exclusive use in today's animal experimentation, standard laboratory rodent chows are considered an aberration due to their extremely high carbohydrate content[57]. Since ketosis may be a critical component of the effectiveness of the treatment, and without controlling for carbohydrate content, the effect of fat intake on disease outcome cannot be made. Our experiments would provide critical insight into the most effective dietary composition for translation to clinical trials.

***Experiment #4:* Feline ADPKD model.** All rodent models of PKD are imperfect. The genes affected in non-orthologous models, such as the Han:SPRD rat, are not a known cause of ADPKD in humans. Genetically engineered orthologous mouse models do not necessarily replicate the second/third hit mechanisms that are thought to underlie human ADPKD[58]. In contrast, feline ADPKD is a higher mammal model with a naturally occurring, heterozygous mutation in the orthologous Pkd1 gene that leads to a high prevalence of ADPKD in cats of Persian cat descent as discovered by our co-investigator Redacted by agreement. Arguably, this feline model most closely approximates human ADPKD because the underlying genetics appear to be identical. Our preliminary study (Fig. 5H, I) is the first intervention study in feline ADPKD. Due to heterogeneity in the strain background, the rate of disease progression in feline ADPKD is naturally variable, as in human ADPKD, which necessitates a longitudinal study design. After completing Expts #1-3 in rodents, we will select the most effective dietary intervention and adapt it for testing in feline ADPKD. TRF would be straight-forward to test in cats. A ketogenic diet would require some adaptation due to the typically higher protein content of feline diets. Redacted by agreement nd our Redacted by agreement are very experienced with dietary studies in cats (see letters), and will formulate and produce the appropriate diet - if necessary - based on the outcomes of our rodent experiments. Note that ketogenic feline diets have recently become available and are promoted as healthy pet foods (e.g. www.ketogenicpetfoods.com). Therefore, we are confident that any dietary intervention suggested from our rodent experiments can be feasibly adapted for the feline ADPKD model.

The longitudinal design will minimize the number of animals needed to observe significant effects and minimize any confounding effects due to differences in disease progression between individuals. The experiment will be conducted as described in Preliminary Results (Fig. 5I,J). A cohort of PCR-confirmed, male and female, desexed ADPKD cats will be obtained by breeding. Baseline TKV of all adult (~1 year old) animals will be determined by CT scan ~6 months prior to, and immediately prior to the dietary intervention. This will yield the estimated rate of 6-months disease progression in each animal. Clinically normal animals with no evidence of renal failure (creatinine, urine specific gravity) will be randomized by sex and rate of progression. The control cohort will be maintained on *ad libitum* normal feline diet whereas the experimental cohort will be adjusted to the experimental dietary regimen. Both cohorts will be continued for one year, and CT scans will be obtained at 6 months and 12 months. Full blood analysis will be conducted at these time points including glucose and BHB to assess the effectiveness of the diet, and creatinine and BUN to assess renal function. TKV will be determined from CT scans by semi-automated segmentation using 3D Slicer software (www.slicer.org) as shown in Fig. 5I,J. After 12 months, all animals will be euthanized and their kidneys subjected to the same analyses as detailed in experiments #1-3. Based on our previous experience with the rate and heterogeneity of progression in feline ADPKD and the anticipated magnitude of efficacy of dietary

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intervention we have conducted a power analysis[59] and estimate that cohorts of 8 animals will be sufficient for approximately 80% power to detect a between-group difference with $p<0.05$.

Anticipated results, pitfalls, future experiments: We anticipate that the dietary intervention will be similarly effective in the feline model as in the rodent models. The longitudinal design will enable us to determine if the intervention leads to regression of existing cystic disease. A potential problem may be that cats may be reluctant to accept a new diet. In this case, we may need to include an adaptation period of slowly introducing the new regimen which would somewhat extend the overall study. This study could lay the groundwork for future studies to routinely assess the efficacy of interventions in feline ADPKD prior to clinical trials.

Aim 2: To test the hypothesis that treatment with BHB will decrease PKD progression.

Rationale: In clinical practice, dietary interventions require the patient to consistently impose self-control on eating. For countless physiological and social reasons, dietary interventions typically fail due to lack of adherence. Most patients would rather "pop a pill" than adhere to a diet. Given this reality, it would be highly advantageous if the beneficial effects of ketosis on PKD progression could be realized by a pharmacological agent or, better, a nutritional supplement as a method to elevate blood ketone concentrations and mimic a ketogenic diet without the need for dietary changes. This goal will be explored in this Aim.

Ketogenic dietary interventions, including intermittent fasting and the ketogenic diet, ameliorate the pathogenesis of Alzheimer's disease in mouse models[60]. More recent data suggest that the ketone BHB may be directly responsible for the neuroprotective effects of such diets because dietary supplementation with the synthetic ketone ester (R)-3-hydroxybutyrate-(R)-1,3-butanediol monoester ("BHB-monoester"), a precursor of BHB, was sufficient to suppress AD-like pathology and behavioral abnormalities[61]. It is clear that BHB is more than just an energy source for cells, and has highly complex molecular effects on cellular signaling and metabolism[26,27]. Importantly, BHB affects numerous signaling pathways that are (or may be) implicated in PKD including mTOR, AMPK, NLRP3, HDACs and other pathways[25-28,62,63]. It is therefore conceivable that the beneficial effect of ketosis on PKD progression may be (partially) due to direct effects of BHB on cystic epithelial cells (or other cell types involved in PKD pathogenesis such as myofibroblasts or macrophages).

As shown in Fig. 6, treatment of Han:SPRD rats with a commercial salt formulation of BHB (FDA-classified as a dietary supplement) in the drinking water from postnatal week 3-8 ***proved extraordinarily effective and almost completely suppressed renal cyst growth and fibrosis*** and had no apparent adverse effects.

***Experiment #1:* Treatment with BHB in the drinking water.** The experiment shown in Fig. 6 will be completed as described using Han:SPRD rats, and extended to $Pkd1^{fl/-}$:Nes^{Cre} mice. Rats/mice will be treated as juveniles from postnatal weeks 3-8, or as adults from weeks 8-12, respectively, with a BHB salt formulation (Na^+/K^+, KetoSports) delivered in the drinking water at 150 mM final concentration, offered *ad libitum* together with normal chow. To guard against a possible confounding effect of increased intake of Na^+/K^+ with the BHB-salt administration we will administer the equivalent dose of Na^+/K^+ as Cl^- salts to an additional control cohort (note: this was done in Fig. 6 and no salt effect was detected. Not shown). The same analyses as described in Aim 1 will be conducted. The mechanism of BHB action in PKD will be further investigated in Aim 3.

***Experiment #2:* BHB dose finding study.** Because the administered dose (150 mM in water) of BHB almost completely stopped PKD progression (Fig. 6), we assume that the chosen dose exceeded that needed for efficacy. We will successively investigate the efficacy of two-fold, step-wise reductions in the BHB dose: 75 mM, 38 mM, 19 mM… This will be done using the robust Han:SPRD rat model. To keep animal numbers to a minimum, we will analyze results from the first dose reduction before moving on to the next lower dose. We will test the lowest effective dose additionally in the $Pkd1^{fl/-}$:Nes^{Cre} mouse model for verification. This "reverse" dose-finding study is important in order to conduct the subsequent experiments with an appropriate dose of BHB, and to inform the design of clinical trials.

Pitfalls, alternatives, future experiments: While we did not observe any adverse effects in the salt-only control cohort, the increased intake of Na^+/K^+ may be confounding or undesirable for future clinical trials. Depending on the outcome of our dose-finding study, we may wish to test a BHB-monoester which does not lead to increased consumption of inorganic cations and has been used in clinical trials[61,64,65]. Another alternative is

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1,3-butanediol which can be administered with the food, is oxidized to BHB and has been used to study effects of BHB in mouse kidneys[66]. Compared to the BHB-monoester, the BHB-salt formulation may be superior in clinical practice because it is widely available and inexpensive. Furthermore, a recent study found that a 10-fold increase in dietary sodium intake has no significant effect on PKD progression in the PCK rat model[67].

Aim 3: To test the hypothesis that BHB ameliorates PKD progression *via* its receptor GPR109A.
Rationale and preliminary data: BHB has numerous molecular effects, many of which are poorly understood. Originally, BHB has been thought to act *via* direct inhibition of several HDACs, and/or indirectly due to its catabolism to acetyl-CoA and ATP. More recently, BHB has been identified as the physiological ligand of the G_i-coupled receptor GPR109A[68]. GPR109A activation decreases cAMP levels by inactivating adenylate cyclase[69]. Interestingly, increased cAMP signaling is a known driver of PKD progression[70,71]. The EC50 for activation of GPR109A by BHB is ~0.7 mM, a level reached in human serum after 2–3 days of fasting or upon administration of a ketogenic diet[72]. GPR109A is also activated by unphysiologically high concentrations of niacin which can be achieved by administering megadoses of this B3-vitamin. Megadoses of niacin - or its analog niacinamide - are taken by millions of Americans as a lipid-lowering drug since more than 50 years, and the molecular action of this effect is now understood to be due to activation of GPR109A in adipocytes. In addition, GPR109A is widely expressed in other tissues including the kidneys. Interestingly, administration of megadoses of niacinamide has previously been shown to ameliorate PKD in a Pkd1 mouse model although the effect was believed to be due to direct inhibition of SIRT1 activity at the time[73]. Based on this study, a pilot clinical trial (Niacinamide in Polycystic Kidney Disease, ClinicalTrials.gov Identifier: NCT02558595) is currently being conducted. To our knowledge, no results of this trial are yet available and the possible involvement of GPR109A has not been considered.

To investigate whether GPR109A may be involved in the inhibitory effect of BHB on PKD progression, we have begun to collaborate with Redacted by agreement (Augusta University, see letter). He is an expert on GPR109A and has recently established the anti-tumor and anti-inflammatory potential of niacin and GPR109A in breast and colon cancers[74,7576-78]. Redac Redacted by agreement lab has now crossed Gpr109a$^{-/-}$ mice with three different Pkd1 mouse models as described below. Striking preliminary data obtained with the first model are shown in Fig. 7. The loss of GPR109A leads to accelerated PKD progression indicating that GPR109A is normally a suppressor of cyst growth. Importantly, niacin treatment ameliorates PKD progression in mice expressing GPR109A but not in mice lacking GPR109A (Fig. 7D). These preliminary data strongly suggest that niacin inhibits renal cyst growth by activating GPR109A. Importantly, given that BHB is the physiological ligand of GPR109A, these data also ***strongly suggest that BHB inhibits renal cyst growth by activating GPR109A***. We will explore this mechanism in this Aim.

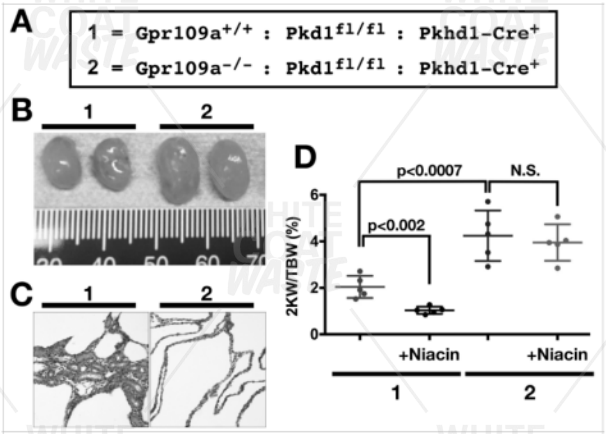


Fig. 7. Knock-out of Gpr109a worsens PKD progression and prevents amelioration by niacin treatment. Genotypes of mice used in this study (**A**). Loss of Gpr109a leads to increased kidney (**B**) and cyst growth (**C**). Niacin treatment (10 mg/kg, daily ip, from postnatal day 8-21) ameliorates PKD progression in mice expressing Gpr109a but not in mice lacking Gpr109a (**D**). Unpublished data courtesy of our collaborator Redacted by agreement

***Experiment #1:* Effect of Gpr109a knock-out on PKD progression.** The following orthologous models will be compared and referred to by the following short names:

"**Pkhd1 Model**" (Pkd1$^{fl/fl}$:Pkhd1-Cre)	"**Ksp Model**" (Pkd1$^{fl/fl}$:Ksp-Cre)	"**ER Model**" (Pkd1$^{fl/fl}$:Cre-ER^{T2})

Ksp-Cre and Pkhd1-Cre drivers lead to overlapping gene inactivation in distal tubule segments and collecting ducts, respectively, and have been extensively used for PKD mouse models[79-81]. The Cre-ER^{T2} driver (estrogen receptor) enables temporal control of floxed gene expression *in vivo* by tamoxifen administration and has also been extensively used in PKD mouse models[82,83]. Gpr109a$^{-/-}$ mice have no obvious phenotypic abnormalities under normal conditions[84]. All mice are in the C57BL/6 background. Gpr109a$^{-/-}$ mice have already been crossed with all three PKD models in Redacted by agreement lab. The crosses with the Pkhd1 and Ksp

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models have already been obtained at UCSB and colonies are being established. The Gpr109a$^{-/-}$ cross with the ER model will be received shortly.

The exact timing of this experiment for each model will depend on preliminary observation of the PKD severities. Therefore, the proposed timings are only approximate. In all experiments, we will compare cohorts that are either Gpr109a$^{+/+}$ or Gpr109a$^{-/-}$. For the rapidly progressing constitutive models (Pkhd1 and Ksp), we will investigate PKD progression at postnatal day ~21. For the slowly progressing tamoxifen-inducible model, mice will be injected i.p. with tamoxifen (125 mg/kg) on 2 sequential postnatal days (P31 and P32) to induce Pkd1 deletion which will lead to cystic progression during the course of 3-6 months[83]. PKD severity will be investigated using the same analyses as described in Aim 1.

Anticipated results: Based on our preliminary data (Fig. 7) we anticipate that the PKD phenotype will be more severe in mice that lack Gpr109a than those that are wild-type for Gpr109a. This result would suggest that GPR109A normally suppresses renal cyst growth. It will be highly interesting to determine exactly which PKD phenotypes will be affected: e.g. cyst-cell proliferation vs. mTOR activity vs. myofibroblasts vs. overall fibrosis etc. These distinctions could be highly revealing because Gpr109a is knocked-out in all cells whereas Pkd1 knock-out would only affect tubule epithelial cells in the Pkhd1- and Ksp models. It could also be highly revealing whether - or not - Gpr109a knock-out promotes cyst growth equally in the rapidly progressing models (Pkhd1 and Ksp) and the slowly progressing ER model. Based on the results, important follow-up experiments will likely suggest themselves, and there are currently too many possibilities to put down on paper.

Experiment #2: **To test whether Gpr109a knock-out prevents the efficacy of BHB.** The preliminary experiment shown in Fig. 7 will be repeated with all three Pkd1 models with/without Gpr109a, but we will use BHB instead of niacin. The timing of BHB treatment in the rapidly progressing constitutive models (Pkhd1 and Ksp) will have to be early (postnatal days ~8-21) which means that we will need to administer BHB by i.p. injection (instead of the drinking water) in these pre-weaned mice. Alternatively, we could administer BHB with the drinking water to the mothers. BHB is known to cross efficiently into milk in ketotic mammals as shown in dairy cows[85,86]. To our knowledge, this has not been done before in mice and we will determine the feasibility in a pilot experiment. If drinking water-administration proves unfeasible, i.p. administration should work well, similar to the i.p. administration of niacin shown in Fig. 7. For the slowly progressing ER model, we will administer BHB with the drinking water starting at postnatal month ~2.

Experiment #3: **To test whether Gpr109a knock-out prevents the efficacy of ketogenic dietary intervention.** Based on our experience in Aim 1, we will select the most effective dietary intervention (ketogenic vs. time-restricted diet), treat the slowly progressing ER model and compare the efficacy in cohorts expressing or lacking GPR109A. Results from Experiment #2 will guide the exact timing of this experiment.

Anticipated results: Based on our preliminary data (Fig. 7) we anticipate that BHB treatment and the ketogenic dietary intervention will NOT be effective in mice lacking GPR109A. This would be very strong evidence implicating this receptor mechanistically, and suggest that the beneficial effect of ketosis is primarily due to the BHB/GPR109A axis. If the knock-out of Gpr109a only partially prevents the beneficial effect of BHB, this would suggest that additional mechanisms (glucose restriction and/or lipotoxicity) also play a role.

Additional and future experiments: To elucidate the signaling pathways triggered by BHB in renal cysts, we may monitor the most plausible candidate pathways. We could do this by treating a severe PKD model (e.g. Pkhd1) with a one-time dose of BHB and assess which signaling pathways are affected in a rapid fashion (e.g. after 2-8 hours). We expect that GPR109A activation by BHB will lead to inhibition of cAMP signaling which we could assess by monitoring the levels/phosphorylation status of cAMP-regulated proteins such as CREB and GSK3β[87,88]. Any such effects should NOT be seen in mice lacking GPR109A. To test if BHB directly inhibits HDACs we would monitor the acetylation status of the most prominent substrate histone H3 (H3ac)[27,6289]. This mechanism should still occur in mice lacking GPR109A. These experiments are all feasible and may suggest themselves during the course of this project. They may be carried out during this project or in future projects. In addition Redacted by agreement lab will be investigating aspects of GPR109A regulation in the kidney (e.g. regulation of expression, cell-type specific expression etc). We will be in close contact with Redacted by agreement throughout the course of this project and exchange information that may give us additional leads to be investigated on a collaborative basis.

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002
Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

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Vertebrate Animals

1. Concise description of the proposed use of the animals in the work outlined in the Research Strategy section.

Mice and rats will be utilized at UCSB. Cats will be used at the University of Missouri. All experiments will be performed according to protocols approved by the Institutional Animal Care and Use committees (IACUC) of the respective institution and the NIH guidelines.

Mouse and rat models of PKD will be utilized in dietary intervention studies or supplemented with BHB. The exact mouse genotypes are listed in detail in the Research Strategy. Both sexes of rodent models will be investigated and data analyzed initially separately for each gender to determine if sex is a biological variable for the purposes of this study. In all studies, animals will have free access to water (or medicated water) at any time. For time-restricted feeding experiments, animals will have nightly access to food for an 8-hour period from postnatal weeks 3-8 or 8-12. For treatments with ketogenic diets, animals are given *ad libitum* access to high fat, very low carbohydrate ketogenic diets that are either a standard ketogenic diet or custom manufactured ketogenic diets as specified in the Research Strategy. Animals are treated from postnatal weeks 3-8 or 8-12.

Each treatment/control cohort will consist of approximately 6-8 animals. Based on our experience with these models, and based on the anticipated magnitude of effects as observed in preliminary results, these cohort numbers will be sufficient to obtain statistically significant between-cohort differences. For all proposed experiments, we estimate a need for a total of approximately 1,800 animals (mice and rats) which includes animals needed for breeding and maintenance of the colonies.

At the end of each treatment regimen, mice/rats will be anesthetized with ketamine:xylazine and weighed. Blood will be removed by retro-orbital puncture using heparinized capillary tubes and transferred to a plasma separator tube. Plasma will be separated and the plasma level of urea, an indicator of kidney function, will be determined using an in-house colorimetric assay. Animals will be euthanized by cervical dislocation while still under anesthesia, and kidneys and other organs removed and weighed. Dissected kidneys will be bisected longitudinally; one half will be frozen in liquid nitrogen and the other half will be fixed in 10% neutral buffered formalin. Total cellular lysates will be prepared from frozen kidneys and analyzed by western blotting using a variety of antibodies that will determine the activity of components the signaling pathways of interest. Formalin fixed tissues will be embedded in paraffin and sectioned onto glass slides. We will then determine histology, cellular apoptosis, proliferation and signaling pathway activity using immunohistochemical assays.

Cats will be fed a commercial diet (Royal Canin Persian) and provided water *ad libitum* for standard husbandry. The cat PKD1 variant is lethal in the homozygous state therefore, all matings will be between a PKD^+ cat to a PKD^- mate. No sex bias has been noted in the research colony, no sex bias has been noted in PKD^+ offspring and litters are generally ~ 4 kittens per queen. We expect 50% of kittens to be PKD^+ and 50% PKD^- with an even sex distribution in both groups. Queens can be bred twice per year. Kittens can be genotyped for the PKD1 variant postnatal week 1 using DNA collected via buccal swabbing. Development of cysts can be monitored by ultrasonography to determine rate of progression of disease at the MU VHC.

The current cat breeding stock includes 3 males (toms) and 4 females. Toms can breed throughout their lifetime. The toms have been imaged at least twice by US, CT and MRI as part of a PKD imaging study, thus, their severity has been defined and disease rate of progression is known. These cats will be re-imaged prior to breeding to evaluate the current disease status and progression. The goal with the cat selection is to use PKD^+ cats with approximately the same severity of disease and rate of progression during the feeding trial. We will attempt to use a single tom for breeding to control for genetic background and variation, however, three toms will be maintained until matings are completed in case some females are not receptive to a particular tom. The toms and queens will be retained for potential future studies to retain common genetic backgrounds and produce less genetic variability in future studies. Each of the toms inherited the PKD^+ variant from the same founder PKD^+ Persian cat. The four PKD^- females are also from the longer-term breeding colony.

Mating timing - Toms will be housed separately from females. Females can be gang housed whereas toms can be pair or small group housed depending on temperament. The photoperiod will be maintained at 15 hrs lights on from January – September, to induce and maintain estrous cycling in the females. Females will be

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checked for estrous daily after the photoperiod is changed after the winter solstice. Although cats have no exposure to sunlight, they still respond to the natural photoperiod. A male can service 1 – 2 females simultaneously. Females displaying estrous will be housed with the tom for 1 week. Mounting and copulation will be monitored by remote video camera if not immediate at pairing. Pregnancy will be confirmed by palpation and ultrasound. After breeding, toms will be returned to individual housing and females will be gang housed until ~ 1 week before queening. One week prior to queening (59 – 65 days), the females will be confined individually to a queening cage. Queening will be monitored by remote video camera and at least three times per day. Queens and kittens are housed in the queening cage until kittens are robust and ambulatory at ~4 weeks. Queens will begin weaning kittens naturally at ~ 8 weeks postnatal. After parturition, females may come into estrous rapidly, however, a second breeding will be conducted after weaning to support the nutritional needs of the kittens and once the pre-pregnancy weight of the female has been regained. Queens and kittens will be offered dry kitten food (Royal Canin) for the first 12 months of kittenhood, during pregnancy and until the normal, pre-pregnancy weight has been regained for the queen. Kittens will be allowed to mature until adulthood, at least age 1 year, before initiating the feeding trial.

Like humans, the disease is variable in cats. However, the colony has been established for ~20 years from 5 foundation Persian cats. Thus, the genetic background was more limited than random bred cats, because of the cats being of the same breed, and also, only 5 cats were used as founders of the PKD1 variant. Therefore, on one hand, genetic variation is more diverse than in the mouse/rat strains used in this proposal, but not as diverse as humans. Power calculations are difficult to estimate because of this variation. Our recent imaging study suggested the fractional cyst volume progressed at ~0.26%/mo for the majority of cats and was suggested to be linear during the early stages of cyst development in young cats (< 5 years). Of eleven cats in the imaging study, one was suggested to be more slowly progressing and one was suggested as a very fast progressing cat. Of ~20 PKD^+ kittens produced in this study, we therefore expect 2 – 4 to have slow or fast progression and a majority (16+) expressing a standard rate of progression of cystic development for inclusion in the feeding trial.

Cats will be fasted for 12 hours prior to diagnostic CT imaging and placed under general anesthesia using clinical protocols as deemed appropriate by the anesthesia service of the Veterinary Health Center at MU. Alfaxalone (1-4 mg/kg IV or IM), dexmedetomidine (0.005-0.020 mg/kg IM) buprenorphine (0.005-0.01 mg/kg IM), Propofol (1-8 mg/kg) or isoflurane (inhaled to effect) may be used for sedation/general anesthesia protocols. Cats will be intubated, and anesthesia maintained using isoflurane gas, and a catheter placed in the right brachial vein. For CT imaging during the fasting trial, the cats will be sedated with 20 mg/kg dexmedetomidine (Zoetis, Parsippany, New Jersey), i.m. Cats are recovered from sedation with 350 - 400 mg/kg atipamezole (Zoetis), i.m. After the final post-fasting CT imaging cats will not be recovered from sedation and euthanized by barbiturate overdose to obtain kidneys for pathological and histological examinations.

A total of 16 cats will be used in this trial (8 control and 8 for dietary intervention).

2. Rationale for Using Animals, the choice of species, and the numbers to be used.

It is of paramount importance that we use mammalian animal models of polycystic kidney disease as well as wild-type control animals in our research for a number of reasons. For example, while cell culture experiments can provide useful biochemical information they do not recapitulate the course of the disease state in a tissue-specific, *in vivo* context. Therefore the use of an appropriate animal model of PKD will provide us with unique *in vivo* information related to disease onset, development and progression that we cannot obtain in vitro. Furthermore, dietary intervention studies cannot be conducted in cell culture because the effects depend on the interplay of multiple organ systems (adipose, liver etc). In this regard, non-mammalian species are too different from humans to be valid model systems in the proposed studies. Mice are the most feasible species for some our proposed studies because genetically engineered strains are available (e.g. Gpr109-null). The proposed rat model is robust and has already yielded highly promising preliminary results that we need to compare against.

An experimental innovation in this proposal is the use of feline ADPKD as an orthologous model system to test the efficacy of dietary interventions. We have already successfully utilized cats with a mutation in Pkd1 in a small longitudinal study (see Preliminary Results). It can be argued that cats with a natural mutation in Pkd1

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are by far the closest animal model to human ADPKD due to the identical underlying genetic mechanism of gene inactivation. Our study is the first interventional study in feline ADPKD and may serve as a model for future investigations with the aim to validate results derived from rodent models prior to the initiation of clinical trials.

Based on our experience with these models and power calculations we will use 6-8 animals per treatment group for rodents, and 8 animals per treatment group for cats. Based on our published and preliminary data, these numbers are sufficient to demonstrate statistically significant effects of various treatments affecting cystic disease progression. Overall, our studies require that we maintain considerable numbers of different strains of rats and mice which need to be maintained as separate colonies. Depending on the genetic model to be used, only a fraction of the animals in each litter will have the genotype needed for experimentation.

3. Veterinary care

Rodents will be housed and treated in the animal resource center at UCSB that is directed by a full-time veterinarian to encompass all aspects related to animal husbandry, ordering and procurement of animals, quarantine procedures, perioperative animal care, health surveillance and consultation on animal models and procedures. Animals will receive water and are housed in a room with a 12h light and dark cycle. If any animals should become visibly sick during the course of treatment they will be removed from the study and cared for according to the advice of the veterinarian.

Cats will be Redacted by agreement

Redacted by agreement

Redacted by agreement There is an onsite Supervisor which reports directly to the Office of Animal Resources (OAR) Facility Manager. There is surgical, radiology, and procedural room space in the vivarium so most animal work is done onsite. A climate-controlled loading dock is located at the eastern side of the building and is used for loading and unloading animals for transport to other buildings, Redacted

Redacted by agreement The animal facility is a double corridor type typically used to maintain conventional animals. Each animal room is equipped with environmental control sensors that will alert staff immediately when temperature or humidity levels fall outside the programmed range. These alerts are delivered by text and email to facility management and EMO (Energy Management Operations) 24 hours/day. Two redundant air handlers provide 100% fresh air to this facility. There is an on-site emergency generator capable of servicing the entire facility.

4. Minimization of Pain and Distress:

If any animals appear to be suffering, they will be treated as per the advice of the veterinarian. At the end of each treatment regimen, rodents will be anesthetized with a mixture of sterile saline:ketamine:xylazine (2:1:1) prior to euthanasia. Euthanasia will be by cervical dislocation of anesthetized mice. This methods is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Mice or rats will be euthanized at the end of the study as detailed above. For minimization of pain and distress during CT imaging, cats will be sedated/anesthetized as described under point #1 above.

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Consortium/Contractual Arrangements

Experiments using the feline ADPKD model will be carried out as a subaward with Redacted by agreement at the College of Veterinary Medicine, University of Missouri – Columbia (MU). Redacted by agreement serves as a co-investigator on this proposal and is supported by Redacted by agreement as a consultant at the same institution.

Dr. Weimbs already has an established collaboration with Redacted by agreement and they have together conducted the experiment shown as preliminary data (Fig. 5 I, J) which will be part of a collaborative manuscript that is in preparation for publication.

Redacted by agreement is uniquely qualified to conduct the proposed experiment. In fact, to our knowledge, her laboratory is the only laboratory worldwide to systematically investigate feline ADPKD. Redacted by agreement has discovered the mutation in the Pkd1 gene underlying ADPKD in cats which makes this an orthologous model that very closely resembles the human disease, in fact closer than any other animal model.

Redacted by agreement is a leading expert in feline nutrition, a clinical nutritionist and a researcher whose career has focused substantially on health problems related to the nutrition of cats, in particular with regard to kidney disease. He is also uniquely qualified to act as a consultant on this proposal.

Redacted by agreement maintains a colony of cats with confirmed heterozygous Pkd1 mutation. These animals have been carefully selected and bred to produce a population with reduced heterogeneity of disease progression as compared to randomly selected cats. These animals will be the breeding stock for our proposed experiments.

During years 3-5 of this proposal, Redacted by agreement will lead the cat experiments at MU. Based on our results obtained using rodent PKD models during years 1-2, we will determine the optimal dietary intervention that will then be adapted to the feline model. Redacted by agreement will be invaluable as a consultant to design the most feasible dietary intervention and support this trial on an ongoing basis. Redacted by agreement will direct all animal breeding and treatments with support of a laboratory manager who has many years experience with cat experimentation, and the personnel of the animal facility at MU. Redacted by will also coordinate all procedures necessary for CT scans, the required anesthesia etc.

During the course of this work, Redacted by agreement and Dr. Weimbs will be in frequent communication by email, phone and video conference. They will also both travel annually to the meeting of the American Society of Nephrology (Kidney Week) and meet there in-person, along with at least one postdoc from Dr. Weimbs' laboratory. Due to their existing collaboration, Dr. Weimbs and Redacted by agreement already have an excellent working relationship and have proven that they can productively collaborate, with support of their institutions, despite the physical distance.

The proposed structure as a subaward will give Redacted by agreement autonomy and flexibility to use the funds for the various expenses associated with this project, without creating extra administrative burden and inefficiencies.

Both, Redacted by agreement and Dr. Weimbs are committed to establishing the feline ADPKD model as a feasible model system to test new therapies as a stepping stone between rodent experiments and clinical trials. We believe that the availability of this model will reduce the costs of drug development for PKD by avoiding clinical testing of candidate drugs that may appear promising in rodent models but fail in the feline model.

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Resource Sharing Plan

We are proposing to use only strains of animals that are already available. Therefore, no new model organisms are expected to be generated. Neither are we proposing to generate genomic or other large data sets. In the unlikely event that such resources should be generated, we will follow the NIH policies and guidelines regarding the dissemination of biomedical research resources to ensure that such resources be made readily available for research purposes to qualified individuals within the scientific community.

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Authentication of Key Biological and/or Chemical Resources

Animals

All mice and rats for this study will be housed in the Animal Resource Center at UCSB, a facility with restricted access. All animal cages have a proper description of animal strain, age, sex and any treatments. Animals are maintained in a conventional rodent facility housed in sanitized individually-ventilated microisolator cages (IVCs) with autoclaved bedding, and fed irradiated rodent chow and micro-filtered drinking water (Hydropac®). Cage changes are performed in either an animal transfer station (horizontal or vertical laminar flow clean bench) or biosafety cabinet at least once every 14 days. At the time of the cage change, sentinel animals are exposed to any unwanted infectious agents that may be present in the principal colony through the transfer of soiled bedding from the principal animals to the sentinel animals. These sentinels are tested on quarterly (prevalent pathogens) or annual basis for the presence of a select list of adventitious rodent pathogens. The sentinel monitoring program is augmented with direct non-invasive sampling and testing of principal animals, and environmental sampling and testing of the dust or debris that accumulates on the surfaces of the exhaust manifolds or air filters of IVC racks to compensate for the limitations of the sentinel monitoring program (i.e., false-negative results for pathogens that cannot be transmitted by soiled-bedding transfer).

For experiments that exclusively use “wild-type” rats and mice, Sprague Dawley rats and C57BL/6 mice of the appropriate age will be purchased from the Proprietary Info

All animals from new litters of mutant or genetically modified mouse/rat strains are genotyped using DNA isolated either from ear punches or from tail snips. Genotyping is done by analytical PCR using established primers that specifically detect the presence/absence of the mutated genes and/or transgenes. The complete genotypes, together with other pertinent information, of all animals are entered into a database that is accessible to all investigators involved in these experiments. The genotyped animals in any given litter are marked by a standard ear punch/notch numbering system to prevent misidentification.

Antibodies

Antibodies are purchased from reputable vendors (e.g. R&D Systems, Cell Signaling Technology etc), and include antibodies against signaling proteins of interest such as S6, STAT3, Src, Erk, Akt, AMPK, and their respective phosphorylated forms (if applicable). Any new antibodies (or new lots of antibodies) are first validated by immunoblotting using lysates of cell lines or tissues. Validation is done by verifying that the observed molecular weight matches the expected size. For phospho-specific antibodies, we will determine that activation and/or inhibition of a certain signaling pathway (e.g. IL6 stimulation for STAT3 activation, or rapamycin treatment for mTOR inhibition) leads to the expected increase/decrease of the appropriate band on immunoblots. Validation of immunohistochemistry-staining involves ensuring that any signal increases/ decreases observed in tissue sections match corresponding increases/decreases of band intensities in immunoblotting analysis of the same tissues.

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