

IACUC Application (Version 1.6)

1.0 General Information

***Please enter the full title of your study:**

Pathogenic Mechanisms of Oral Bacteria

***Please enter the Short Study Title you would like to use to reference the study:**

Oral Bacteria

* This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.

2.0 Add Department(s)

2.1 List the departments associated with this study. Add the Principal Investigator's department as the **PRIMARY DEPARTMENT**. For research conducted at a Norton facility add: Norton Healthcare. For research conducted at a Jewish Hospital/KyOne facility (e.g. Frazier, St Mary's, etc) add: Ky One Health. For research conducted at University Hospital /James Graham Brown Cancer Center add: University Hospital:

Primary Dept?

Department Name

☐

U of L - 41 - Dental-Oral Immun & Infect Dis

3.0 Assign key study personnel (KSP) access to the project

3.1 *Please add a Principal Investigator for the study:

Lamont, Richard J

3.2 In this section, please add any project personnel that needs to have access to the submission or will need to approve the submission. In the case of the Undergraduate Research Symposium, your mentor and co-authors would be included in this section.

A) Additional Investigators

B) Research Support Staff

Jin, Shunying
Research Associate
Pandey, Satya Deo, Ph.D.
Research Associate
Stocke, Kendall S
Research Associate
Yakoumatos, Lan Chen
Research Associate

3.3 *Please add a Study Contact. The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact.

Adding someone here does not add them as study personnel.

Lamont, Richard J
Stocke, Kendall S

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact.

3.4 Please select the Designated Approvers:

Lamont, Richard J
Combined_Chair-Scientific_Reviewer

Add the name of the individuals authorized to approve and sign off on this protocol.

4.0 IACUC Form Type

4.1 Please indicate the type of form you are submitting

- ☒ Live Animals (including non-observational field studies)
☐ Tissue Only
☐ Field Investigations – Observational Only

- **Live Animals** - Use this form if you will be using any live animals at UofL, including description of Core Animal Laboratories. Field studies that involve capture, an invasive procedure, harms or materially alters/influences the behavior or activities of a wild animal should use this form.
- **Tissue Only** - Use this form if you will be using **fresh** or **frozen** (not fixed) tissues, organs, or carcasses obtained from animals outside of UofL or from animals assigned to, and euthanized by, PIs with other IACUC *Proposals*. If you plan to handle live animals in any way, a *Live Animal* form must be used.
- **Field Investigations – Observational Only** – Use this form if you will be conducting a field investigation limited to observation of free-living, wild **vertebrate** animals in their natural habitat and there will be no manipulation of the animal or its environment. **Note:** Field studies that involve capture, an invasive procedure, harms, or materially alters or influences the behavior or activities of a wild animal **must use the Live Animals form**.

5.0 Emergency Contacts

5.1 Indicate the Key Study Personnel who will act as emergency contacts

Study Personnel	Phone Numbers
Lamont, Richard J	During Work Hours <input type="text" value="852 2112"/>
	After Hours <input type="text" value="356 3990"/>
Stocke, Kendall S	During Work Hours <input type="text" value="852 2644"/>
	After Hours

6.0 Welcome Page

6.1

Welcome to **IACUC** @ Louisville **Replacement Reduction Refinement Responsibility** Do I need an IACUC protocol? For best viewing of form materials, it is recommended that you expand your window as much as possible.

7.0 Proposal Purpose / 3 Year Renewal

7.1 Proposal Purpose (Check all that apply)

- ☒ Research using live animals
☐ Teaching and Training
☐ Core Animal Laboratory Proposal

Is this a 3-Year Renewal?

☒ Yes ☐ No

7.2 Previous Proposal number:

21869

Do you currently have animals in-house in ongoing studies under your expiring Proposal that will be transferred to this Proposal?

☐ Yes ☒ No

7.3 Scientific Review and Funding Source(s)

Scientific review has been, or will be, performed by an internal or external review panel before experimentation begins. Select all that apply:

- ☒ Federal Agency
☐ State Agency
☐ Private Foundation
☐ U of L Review Panel
☐ Industry Sponsor
☒ Department Chair or Designee
☐ Other

List any funding sources applicable to this Proposal

Grant No.	Sponsor	Title
DE012505	NIH	Molecular Aspects of Oral Plaque Formation
		P. gingivalis interactions with

DE011111	NIH	gingival epithelial cells
N/A	OIID department	N/A

8.0 Species to be used in this Proposal (Only ONE Allowed)

8.1 Select species in drop down list below. Note: If the species you would like to use is not listed, please contact the IACUC Office (IACUC@Louisville.edu, 852-7307) so that it may be added to the form.

Select Species to be used in the Proposal (for Field Study, select "Wild Caught Species")

Mouse (Laboratory)

8.2 Justification of Selected Species

Give rationale for the selection of this species. In all cases, a "lower" species should be given primary consideration.

Mice are proposed for use because they display gingival anatomy and periodontal disease characteristics that resemble those of humans. Moreover, mice and humans display similar innate immune mechanisms. The availability of high-quality murine immunochemical reagents render mice a convenient model, in addition to their being relatively inexpensive compared to larger animal models. The suitability of the mouse model in periodontal and inflammation research is well established in the literature; see for example Graves DT, et al. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. Journal of Clinical Periodontology 2008: 35: 89-105. The only other reliable models for periodontal disease progression, for which there is enough information, are the rat, dog and the monkey, which however are much more expensive; less convenient for use, and are "higher" species.

8.3 List strains, lines, stocks, breeds, etc. in the table below. - NOTE: it is acceptable to group multiple strains/lines on a single row AS LONG AS the description of potential adverse phenotypes is the same for all strains/lines included in that row. For example, you may be using a number of transgenic lines. You are not required to list every line as long as the expected phenotypes are the same.

General Information	Strain Origin	Description of Strain and Adverse Phenotypes (if any)
Strain or Lab Name <input type="text" value="BALB/c"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input checked="" type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>
Strain or Lab Name <input type="text" value="C57BL/6"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input checked="" type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>
Strain or Lab Name <input type="text" value="B6_5XFAD"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input checked="" type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>

Strain or Lab Name <input type="text" value="B6_5XFAD_BLT1-/-"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input checked="" type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>
Strain or Lab Name <input type="text" value="B6_3XTG"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input checked="" type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>
Strain or Lab Name <input type="text" value="B6_3xTG_BLT1-/-"/> Is strain irreplaceable? <input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Commercial Vendor <input type="radio"/> Import from Colleague <input checked="" type="radio"/> Existing colonies bred at UofL <input type="radio"/> New genetically-modified line created at UofL <input type="radio"/> Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) <input type="text" value="n/a"/>

8.4 Are any of the strains/lines above genetically-modified (transgenic, knock-out, knock-in, etc.)?

☒ Yes ☐ No

8.5 Will any of the transgenic animals need housing at ABSL2 or higher?

☐ Yes ☒ No

8.6 Will the transgene or product of the transgene interact with any agent administered to the animal that may result in the need to house the animals at ABSL2 or higher? For example, a transgene may produce a viral protein, such that when the animal is infected with a non-replicative virus, the combination can result in production of virus.

☐ Yes ☒ No

8.7 Are you willing to share tissues from animals or transfer live animals to other colleagues at U o f L?

☒ Yes ☐ No

8.8 Related Proposals Are the experiments similar or related to those described in another proposal for a different species? For example, multiple species may be needed to satisfy regulatory requirements.

☐ Yes ☒ No

9.0 Lay Project Summary (250 words max)

9.1 Provide a non-technical summary of the proposed research, using language that a person not trained in biomedical sciences can understand. Describe the significance of the project and the reasons for which it has been proposed. This description should allow the reader to weigh the potential human or other animal health benefits against animal welfare concerns.

Lay Summary (250 words maximum):

Periodontal (gum) diseases are one of the most common bacterial infections of humans and impose a significant burden on the health care system. Periodontal diseases and periodontal bacteria are also associated with a number of serious systemic conditions including Alzheimer's Disease (AD). which may ensue from systemic spread of the bacteria. Recent advances have shown that the bacteria that cause periodontal disease comprise a community that includes a number of different species, and it is the community that is responsible for disease. Little is known, however, about how these communities assemble, how they transition to being able to cause disease, the effect they have on host inflammatory responses and their role in facilitating AD. To begin to answer these questions, we will test colonization and survival of defined communities of bacteria in experimental animals and subsequent initiation of disease. We will use both oral infection which results in gum destruction, and under the skin inoculation which causes a local abscess. We will test different combinations of organisms as well as mutants which lack defined properties that may be involved in community development and disease. We will also introduce defined bacterial communities into the mouths of mice that are genetically modified to be susceptible to AD and measure the effect on mouse behavior. These studies will generate data that will begin to establish the rules that govern the assembly of pathogenic multispecies communities and their impact on oral and systemic diseases. Ultimately, the knowledge gained could be translated into novel diagnostic, therapeutic or preventive strategies for periodontal diseases and for AD.

10.0 Technical Summary (800 words max)

10.1 Technical Summary Describe specific aim(s) and the long-term goals of the project.

This project involves the study of bacteria that are associated with periodontal disease and potentially with Alzheimer's Disease (AD). The long term goals of the project are to bring molecular definition to the process of pathogenic oral microbial community formation, the associated host immune responses and any effects on behavior that are associated with AD. Two animal models are proposed: the abscess model, and the oral inoculation model.

In the abscess model, animals are challenged s.c. on the posterior dorsolateral surface, or the thigh, with bacteria. Lesions will be allowed to develop and the animals euthanized and abscess material collected for culture of bacteria. This allows for a determination of the ability of the bacteria to survive and replicate in vivo. A second use of the abscess model will be to inoculate combinations of organisms and test for systemic spread of the organisms using in vivo imaging technology. Finally we shall also introduce tracking dyes in order to image recruitment of neutrophils or macrophages.

In the oral inoculation model, bacteria are introduced into the oral cavity in carboxymethylcellulose (CMC). After several weeks the mice are euthanized and jaws collected. The amount of alveolar bone loss is then recorded. We will introduce tracking antibodies in order to image recruitment of neutrophils post-mortem. We will also test mouse behaviors which are associated with AD

The specific aims are 1) to examine the in vivo pathogenicity of bacterial wild type (WT) strains, mutants in specific genes, and combinations of oral organisms, along with the associated host inflammatory responses; 2) to determine if synergistically pathogenic combinations of organisms can spread systemically after sc inoculation; 3) to determine if oral bacteria may be associated with the onset of AD

11.0 Justification for Animal Use

11.1 Justification for Animal Use Describe why the Proposal requires the use of animals, as opposed to *in vitro* or *in silico* approaches. (250 words or less)

We have developed this project to the extent possible without the use of animals, i.e. using in silico approaches to identify putative virulence factors, and using human *ex vivo* (primary epithelial cells) cells. Furthering the principal objectives of our project now requires experiments to be performed *in vivo*. Animal models (mice, rats, dogs and monkeys) have been used extensively to study aspects of periodontal disease, host-pathogen interactions, and Alzheimer's Disease. Knowledge from these studies has been essential for understanding the pathogenesis of human periodontitis; the virulence of specific organisms and combinations of organisms; the pathogenesis of AD; and the development of therapeutic approaches. In this context, we must now further characterize in vivo pathogenicity of defined communities of bacteria.

12.0 Assurance of Non-Duplication

- 12.1 Assurance of Non-Duplication** Provide a *written assurance* that the proposed activities do not unnecessarily duplicate previous or ongoing experiments. Describe methods and sources (journals, abstracts, *etc.*) to support this assurance. Include the date the search was performed, years included in the search, and keywords used. [Examples can be found in the help button to the right. Links to Databases: [PubMed](#); [Google Scholar](#)]

Database Info	Keywords and Description of Results
<div>PubMed</div> <div>04/14/2025</div> <div>Years Searched</div> <div>All in database</div>	<div>List Keywords</div> <div>oral polymicrobial community periodontal mouse oral polymicrobial community abscess mouse gingivalis alzheimer's 5XFAD gingivalis alzheimer's 3XTG</div> <div>Describe Results</div> <div>'oral polymicrobial community periodontal mouse' returned 11 hits, 3 were from our group and one was a review. The others did not examine the same organisms or immune responses to be tested here. 'oral polymicrobial community abscess mouse' returned 6 hits. Three were from our group, one was a methods paper, and the others did not examine the same organisms or immune responses to be tested here. 'gingivalis alzheimer's 5XFAD' returned one hit but that study did not use germ-free animals. 'gingivalis alzheimer's 3XTG' did not return any hits</div>
<div>Google Scholar</div> <div>04/14/2025</div> <div>Years Searched</div> <div>All in database</div>	<div>List Keywords</div> <div>oral polymicrobial community periodontal mouse oral polymicrobial community abscess mouse alocis sputigena nucleatum gordonii gingivalis polymicrobial community mouse</div> <div>Describe Results</div> <div>'oral polymicrobial community periodontal mouse' and 'oral polymicrobial community abscess mouse' returned thousands of hits. When sorted by relevance none of the top 50 were relevant to this study. Focusing on specific organisms we we will use, F. alocis and S. sputigena, F. nucleatum, P. gingivalis, and S. gordonii, returned 67 hits, none of which used the same approaches we propose here. 'gingivalis alzheimer's 3XTG' and 'gingivalis alzheimer's 5XFAD' no hits using germ-free mice and behavior test</div>

13.0 Experimental Groups

- 13.1 Describe Experimental Groups in Table Below** Provide a description of each experimental group in enough detail that reviewers can understand what happens to each animal assigned to that group. - **Group Name or Number:** May be a user defined number or brief descriptive name. *Example:* heart transplant; dietary restriction. - **Pain Class:** Class 0 - Animals will be acquired/held, but not used or manipulated in any way. - Class I - Animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. - Class II - There is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include induction of cancer/tumors, biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic

catheters, as well as survival and non-survival surgery. - Class III - Animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section. - **Treatment/Description:** Devise a brief descriptive title for each procedure and describe the treatments each animal in this group will receive, including the time period between procedures. For studies in which the exact sequence or number of procedures cannot be determined, include a range of potential time periods and note the maximum potential procedures to be performed on the animals in that group. **Example:** This group will receive heart transplant followed by stem cell treatments. The stem cells will be given IV by tail vein injection 10 days after heart transplant surgery. In later sections you will describe "heart transplant" (Survival Surgery), "stem cell (IV) injection," etc. in the "*Procedures Table*" below. **Note:** You may also include in each group a small number of variables as long as it is very clear from the description what will happen to the animals in these groups and the sample size used. **Example:** Following an acclimation period of 14 days, animals will receive treatment with XJ-47 in the drinking water at 3 levels (0, 15, and 30 mg/ml) for 30 days. At this point, we will perform intrasplenic implantation of WKW-95 (or control group) and follow groups of animals for an additional 30, 60, or 90 days until euthanasia and tissue collection. 10 animals /group x 3 dose groups x 2 implantation groups x 3 time points = 180. - **Number of Animals in This Group:** The number of animals needed in the group is generally the sample size ("n"). If multiple variables were included in the "Treatment/Description," then this number may be a multiple of the sample size.

Group Name or Number and Pain Classification	Treatment/Description	Number of Animals in This Group
<p>Group Name or Number</p> <p>Abscess</p> <p>Pain Class (See definition above)</p> <p>Class II</p>	<p>Abscess formation The mice will be fed 5V75 LabDiet for the duration of the study. 10-12 week old Balb/c mice will be given one sc inoculation with bacteria. Lesions will be allowed to develop for up to 6 days. Mice will be injected ip with luminol or with lucigenin. The mice will be imaged, euthanized and abscess material surgically removed along with liver, heart, kidneys and spleen. P. gingivalis wild type strain: 10 mice. P. gingivalis 10 mutant strains: 100 mice. Community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 10 mice. P. gingivalis wild type strain with community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 10 mice. 10 P. gingivalis mutants with community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 100 mice. Sham: 10 mice. X2 with luminol x2 with lucigenin</p>	960
<p>Group Name or Number</p> <p>Oral</p>	<p>Alveolar bone loss 10-12 week old Balb/c mice will be orally inoculated with P. gingivalis +/- community of S. gordonii, S. sputigena, F. nucleatum and F. alocis, in CMC at 2-day intervals over a 10-day period. At 1 day, 14, 28, and 47, days post-infection, mice will be anesthetized with isoflurane (conventional animals) in order to sample the oral microbial population. On the final day of the experiment (day 47) mice will be inoculated (iv) with Ly-6G</p>	

<p>Pain Class (See definition above)</p> <p>Class II</p>	<p>antibody, once only. After 2-4 h, the mice will be euthanized and jaws will be collected. P. gingivalis wild type strain: 10 mice. P. gingivalis 10 mutant strains: 100 mice. Community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 10 mice. P. gingivalis wild type strain with community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 10 mice. 10 P. gingivalis mutants with community of S. gordonii, S. sputigena, F. nucleatum and F. alocis: 100 mice. Sham: 10 mice. 1 repeat</p>	<p>480</p>
<p>Group Name or Number</p> <p>AD</p> <p>Pain Class (See definition above)</p> <p>Class II</p>	<p>Alzheimer's Disease. Mouse strains: C57BL/6 B6_5XFAD B6_5XFAD_BLT1-/- B6_3XTG B6_3xTG_BLT1-/- 10-12 week old will be orally inoculated with P. gingivalis in CMC at 2-day intervals over a 10-day period. Animals in this group will undergo behavioral testing including possibly all of the following: Morris Water Maze, Barnes Maze Test, Y maze, Elevated Plus Maze and Novel Object Recognition. Mice will be assessed at 150 days and 240 days of age. No more than 1 test will be performed on any one day. Once an animal has completed its required test(s), the animal will be euthanized at either 150 or 240 days Pg infection: 10 mice x 2 sexes x 5 strains = 100 mice Control: 10 mice x 2 sexes x 5 strains = 100 mice</p>	<p>200</p>
<p>Group Name or Number</p> <p>Breeding</p> <p>Pain Class (See definition above)</p> <p>Class 0</p>	<p>Breeding group: 1 female will be paired with one male. Pregnant females will be separated if more than one female is pregnant with the male prior to parturition. 5 strains x 6 breeding pairs /strain = 30 animals Retired breeders will be kept and analyzed at the 240 day timepoint for behavioral testing and tissue analysis similar to progeny experimental animals. Animals not required for experimental endpoints will be identified and euthanized via approved methods</p>	<p>30</p>

13.2 Will You Maintain a Breeding Colony?

☒ Yes ☐ No

13.3 Breeding Colony Details

- ☒ Monogamous pair mating
- ☐ Harem Breeding (more than 1 female per male). Pregnant females will be separated to prevent multiple litters per cage.
- ☐ Other (Describe below)

13.4 Total Number of Animals Requested Remember to include those from Breeding colony if relevant.

1670

13.5 Animal Number Justification *Provide specific justification for the number of animals to be used in each group i.e., the sample size. This must address statistical significance as it relates to experimental design. Please be as detailed as possible. Statistical power analysis or calculation given a known or expected error/failure rate and difference between groups is preferred, but experience with the model may be acceptable. [IACUC Fact Sheet] For teaching or training, the number of "students" per animal and expected number of training exercises may suffice. Note: Details of treatments, procedures, and other experimental variables should be included in the "Treatment/Description" column in the table above.*

Results from the past three years showed that the abscess model was preferable to the chamber coil model, so this latter model is no longer included, reducing the total number of mice to be used. We also found that neutrophil and macrophage responses are important, so we are proposing additional experiments to further characterize these.

Abscess model

From previous results with neutrophil recruitment levels we will require 10 mice per group for p-value <0.05.

Alveolar bone loss model

From previous results the average bone loss values were 55% for the control and 47% for the experimental with a 4.5 standard deviation. For a p-value <0.05 between groups we need 10 mice per group

Alzheimer's Disease

These are pilot experiments to determine extent of effect

14.0 Field Studies

14.1 Does proposal involve field studies?

☐ Yes ☒ No

15.0 Procedures

- 15.1 Procedures Checklist** Indicate the types of procedures used in this proposal (check all that apply). Definitions:
- Minor:** Any surgical intervention that does not expose a body cavity and causes little or no physical impairment. Example: laparoscopy; wound suturing; peripheral vessel cannulation; percutaneous biopsy; routine farm-animal procedures such as dehorning, castration; prolapse repair; and most procedures done on an "outpatient" basis in veterinary clinical practice.
- Major:** Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for inducing permanent physical or physiologic impairment; and/or any procedure associated with orthopedics or

extensive tissue dissection or transection. - For **Multiple Survival Surgical Procedures**, i.e., surgical procedures that will be performed under separate anesthetic periods from which the animal will recover from each anesthetic period, describe each surgical procedure separately (there must be two separate procedures in the "Procedures Table"). - Be sure to save often!

- ☐ None: Animals will be acquired/held and/or bred, but not used or manipulated in any way (exceptionally rare)
- ☐ Genotyping
- ☐ Individual Animal Identification
- ☒ Non-Surgical: may require anesthesia, but do not involve a surgical incision. Examples include test article administration, behavioral assessment, tissue collection (prior to euthanasia), imaging, irradiation, etc.
- ☐ Surgery - Non-Survival: a surgical procedure, performed under anesthesia, from which the animal does not recover from the anesthetic (also known as terminal or acute surgery)
- ☐ Surgery - Survival: a surgical intervention from which the animal is expected to recover from the anesthesia
- ☐ Surgery - Multiple: TWO or more survival surgical procedures (major or minor) between which the animal recovers from anesthesia

15.4 **Procedures** **Note** when anesthetics or analgesics will be used, **DO NOT** provide dose, route, frequency, etc., as these must be included in a later section.

Procedure Type and Name (descriptive title used in EXPERIMENTAL GROUP section above)	Description. Provide sufficient detail such that the reviewer can understand exactly what will occur and the potential impact of the procedure on the health and well-being of the animal. For surgical procedures include incision site, all tissue manipulations, temporary wound closures, etc. Indcate when anesthetics or analgesics will be used, but DO NOT include doses - these will be described in a later section.
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Abscess formation</div>	<p>Anesthetize animals with isoflurane, and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex.</p> <p>Remove a single mouse from the isoflurane box and using a fresh cotton swab brush Nair with Softening Baby Oil onto the inner thigh and surrounding abdomen, making sure Nair thoroughly coats the hair. Wait 30 sec (maximum) and then wipe liberally with pieces of paper towel soaked in water. Hair removal is necessary to obtain subsequent images of sufficient resolution and sensitivity Place animal in a clean cage. Load syringe (28G 100U-insulin needle) with inoculant, wipe inoculation site with alcohol wipe. Hold the animal by grasping the skin along its back. Insert needle at base of skin fold between thumb and finger. Pull back the syringe plunger to aspirate the syringe. Any blood indicates improper needle placement, and needle must be repositioned. Administer substance in a steady, fluid motion Observe the mice until all have regained consciousness and are fully ambulatory. Record on animal card the date and time of inoculation and contents of inoculant</p>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>IP injection</div>	<p>Restrain the animal by grasping the skin along its back. Tilt the mouse with its head slightly toward the ground so that its head is lower than its hind end. Clean injection site with alcohol. Insert needle, direct tip toward head, bevel up, at 15-30 degree angle, to depth of ~5mm. Pull back the syringe plunger to</p>

	aspirate the syringe. Administer reporter agent in a steady, fluid motion.
<div>Non-Surgical</div> <div>Procedure Name</div> <div>imaging</div>	<div>Anesthetize animal with isoflurane and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Place animal on heated stage in AmiHT IVIS imager with nose cone for further isoflurane delivery as necessary to maintain in stable plane. Image for 1 - 15 min and euthanize while under anesthesia.</div>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>oral inoculation</div>	<div>Restrain the mouse by grasping the skin along its back. Place tip of needle in animal's mouth. Do not aspirate the syringe. Once needle is properly placed, administer 100 µl of the substance into the mouth using a ball-ended 2.25 mm feeding needle.</div>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>IV infection</div>	<div>Intravenous injection of Ly-6G antibody: immerse tail in warm water (100F or less) for 3-5 min. Place animal in restraint. Clean injection area with alcohol. Grasp the tail at mid-length and place the index and middle fingers of the non-dominant hand around the tail above where the needle will be inserted. Hold the lower part of the tail between the thumb and ring finger below the injection site. Put slight tension on the tail by applying pressure with both sets of fingers. Release pressure to the proximal fingers before administering the reporter agent into the vein. Insert the needle, bevel up, into the vein towards the direction of the head. Keep the needle and syringe parallel to the tail and deliver 200 microliters.</div>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>oral sampling</div>	<div>Anesthetize animals with isoflurane, and wait until the animal reaches a stable plane of anesthesia as observed by lack of pedal reflex. Roll a 15-cm sterile polyester-tipped applicator along the gingivae and molars.</div>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Morris water maze</div>	<div>Stoelting water maze (4ft diameter) assays cognitive impairment and measures hippocampal-dependent spatial learning and memory. Mice are placed in the pool filled with opaque water (colored with white tempera paint, Crayola) and must learn to find a submerged platform based on spatial clues. Water temperature is kept between 75-80 degrees F. The lighting is dimmed to ~ 100 lux. Animals are recorded and monitored continually while undergoing this assay. Any mouse showing distress or falling below the water surface will be immediately removed. Mice will be dried with paper towels before being returned to normal caging. Mouse is placed into tank and given 60s to find the platform. Habituation and training may be performed up to 4 repeats per day for 5 days with at least 15 minutes between trials. On test day, the platform is removed, the mouse</div>

	is given 60s to swim in the tank and its path monitored to determine speed, distance, and platform crossover number.
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Barnes maze test</div>	<p>Barnes Maze Test (Stoelting) is a table with 20 evenly spaced peripheral holes with only one escape box. The mice are put on the table with a light above to encourage the mouse to escape. Cues are given on the walls of the room to orient the mouse towards the escape hole. The mouse is placed under a bucket for 15s. When the bucket is removed, the recording begins and the mouse must make their way to the escape hole. After 90s, if the mouse has not entered the escape hole, it is guided there and the entrance is covered. The mouse is left in the escape box for 60s. Mice are trained twice a day for 4 days and then probed. Mice are recorded and monitored continually while undergoing this assay.</p>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Novel object recognition</div>	<p>Stoelting "any-box" assays for recognition memory. The mouse is allowed to investigate 2 objects in a testing arena. One object is replaced with a novel object and the mouse is scored for how long it interacts with the novel object over the memory object. Mice are recorded and monitored continually while undergoing this assay and lighting must be turned off in the room and supplemental lighting provided to emit ~20 lux in the testing arena. Mouse is placed inside for 10 minutes for exposure to 2 items, then replaced in its home cage for about 30 minutes up to 24 hours. One object is changed and the mouse is placed back in the box and monitored for 10 minutes calculating how long the mouse interacts with both objects.</p>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Y maze test</div>	<p>Y maze test (Stoelting) is a platform with three equidistant arms. Mice will be placed in the middle of the platform and allowed to investigate the arms for 8 minutes while video recording is taken from above. Spontaneous alternation is measured by noting which arms the mice enter in sequence. Alternative methods include closing one arm, habituation of mice for 8 minutes followed 2-24 hours later with opening of the third arm and investigation over 8 minutes. A combination of this platform and the NOR method could also be undertaken where mice are acclimatized two 2 identical objects in 2 different arms and one object is replaced with a new one.</p>
<div>Non-Surgical</div> <div>Procedure Name</div> <div>Elevated plus maze</div>	<p>The elevated plus maze (Stoelting) is a platform with four equally spaced arms. Two of the arms have walls and the other two are open. The lights are dimmed to ~ 15 lux in the testing arena. A mouse is placed in the center of the plus platform facing an open arm and allowed to search for 5 minutes. The amount of time they spend in closed arms (walled) or open arms is tabulated.</p>

Project Participants

Provide name(s) (in order of greatest involvement) of individual(s) participating in or involved with this *Proposal* (experimental procedures, animal observation or care, *etc.*) and describe their role in the proposed study. PI and Co-PIs should also be included. The experience described for each person should match their role in the studies described in this *Proposal*. If not, please indicate how they will obtain sufficient training.

Name	Role (List Specific Procedures to be performed)	Experience
Lamont, Richard J	Overall PI. Will not perform any hands-on work with animals	see online training log
Stocke, Kendall S	Perform experimental procedure, perform euthanasia	see online training log
Pandey, Satya Deo, Ph.D.	Perform experimental procedure, perform euthanasia	see online training log
Yakoumatos, Lan Chen	Perform experimental procedure, perform euthanasia	see online training log
Jin, Shunying	Perform experimental procedures, perform euthanasia	see online training log

If there are any non-UofL personnel who will be handling animals or performing any procedures as part of this Proposal, please provide their names, the functions they will perform, and the relevant experience /training they have in performing those functions.

Name	Procedures Performed and Experience
No records have been added	

- ☒ As Principal Investigator (or designee), I attest that the Key Study Personnel selected for this study have, or will obtain, the necessary experience, training, and are proficient, or will be proficient, in performing all of the procedures listed above.

16.0 Anesthetics, Analgesics, and Other Therapeutic Agents

- 16.1 List ALL pre-anesthetic, anesthetic, analgesic, tranquilizing agents, surgical support fluids, antibiotics, and other veterinary medical therapeutics to be used (even if their use has been described elsewhere in this *Proposal*). Examples include not only peri-operative drugs, but also such drugs as insulin for diabetic animals, or hot/cold packs.**

Analgesics "PRN" or "as needed"

The USDA Research Facility Inspection Guide states that "PRN" or "as needed" frequency of administration is not acceptable unless there are detailed instructions and criteria for determining administration of the drug. Non-pharmacological methods, such as hydrotherapy and hot/cold packs, should also be described. Availability of experienced personnel, especially at night and on weekends, should also be assured in protocol review.

IM = intramuscular, IP = intraperitoneal, IV = intravenous, PO = per os (by mouth), SC = subcutaneous

Be sure to save often!

Click "Save and Continue" button at top right of screen. (You will move to the next section. You can return to "Anesthetics, Analgesics, and Other Therapeutics" by clicking the appropriate section on the left. This annoying feature will be fixed in future releases!)

Name and Purpose (check labels)	Dose (mg/kg) and Route (check labels).	Frequency (e.g., twice daily) and Duration (e.g., once, three weeks) - check labels
Name <input type="text" value="isoflurane"/> Purpose <input type="text" value="Anesthetic"/> Other Use, Explain <input type="text"/>	Dose <input type="text" value="0.5 - 3%"/> Route <input type="text" value="Inhalation"/> Other Route: <input type="text"/>	Frequency <input type="text" value="once"/> Duration <input type="text" value="30 min"/>
Name <input type="text" value="Puralube"/> Purpose <input type="text" value="Anesthetic/Surgical Support"/> Other Use, Explain <input type="text"/>	Dose <input type="text" value="as need to cover eye"/> Route <input type="text" value="Topical"/> Other Route: <input type="text"/>	Frequency <input type="text" value="once"/> Duration <input type="text" value="during anaesthesia"/>

In the text box below, provide additional information on the use of the agents listed above. Examples may include use of certain analgesics pre-emptively, clarifying different anesthetic regimens for specific procedures, anesthetics used in combination, decision making process used to determine frequency or duration, etc.

17.0 Anesthesia and Anesthetic Monitoring

17.1 Will animals be anesthetized for any reason OTHER THAN Euthanasia?

☒ Yes ☐ No

17.2 Animal Preparation for Anesthesia Select those that apply

- ☒ Observation for normal behavior
- ☐ Pre-anesthetic diagnostics (e.g., hematology, serum blood chemistry panel)
- ☐ Overnight fasting (NON-RODENT MAMMALS ONLY).
- ☐ Use of sedatives (describe in Anesthetics, Analgesics, and Other Therapeutics table).
- ☒ Placement of non-medicated ophthalmic ointment in eyes.
- ☐ Other (describe below):

17.3 Monitoring Anesthetic Depth Select those that apply

- ☐ Body temperature measurement and support using temperature-measuring probe.
- ☐ Use of intra-procedural fluids (describe in Anesthetics, Analgesics, and Other Therapeutics table).
- ☒ Anesthetic depth checked at intervals no less than 15 minutes (describe other intervals below).
- ☒ Anesthetic depth verified by withdrawal reflex (toe/tail pinch).

☐ Other methods of anesthetic monitoring and animal support during anesthesia and/or surgery:

Provide additional information if needed.

-

18.0 Surgical Preparation and Support

18.1 Will animals undergo surgical procedures?

☐ Yes ☒ No

19.0 Privately-Owned Animals

19.1 Privately-Owned Animals

Does this Proposal include the use of any privately-owned animals?

☐ Yes ☒ No

20.0 Non-Standard Housing, Food and Water (or Other Special Considerations)

20.1 Indicate which of the following, if any, pertain to this proposal

- ☐ Animals require special housing conditions (e.g., individual housing, special caging).
- ☒ Animals receive special food
- ☐ Animals receive special drinking water
- ☐ Animals will experience food or drinking water restriction or regulation
- ☐ Use of non-sterile or expired medical materials (disposable surgical supplies)
- ☐ Animals will be physically restrained for prolonged periods of time. Brief manual restraint for the purpose of performing routine clinical or experimental procedures (< 15 minutes for rodents, <30 minutes other mammals) need not be described unless the procedures will cause pain or distress.

20.3 Special Diets Use of feeds beyond manufacturer-recommending expiration date should be included. Note: drugs, chemical agents, and other test articles also should be included in the Chemical Agents section later.

Name/Manufacturer	Justification/Description
<div>Diet Name</div> <div>5V75 LabDiet</div> <div>Manufacturer</div> <div>Purina</div>	<div>Justification for Diet</div> <div>The standard 5010 LabDiet (Purina) feed used at UofL has very high background levels of luminescence. 5V75 LabDiet represents a nutritionally complete feed that has very low luminescent backgrounds for use in the Abscess/Imaging studies throughout the course of the experiment</div> <div>If diet doesn't meet the nutritional needs for this species, provide a description of how the animals will otherwise be supported.</div>

21.0 Collaborating Institutions

21.1 Select all that apply:

- ☐ This project involves the use of animals at another institution. Example: A colleague at another institution performs a procedure on animals that have been or will be used in a study at UofL.
- ☐ This project has contracted the production of custom monoclonal or polyclonal antibodies at a company or another institution. Custom polyclonal and monoclonal antibodies are those produced either from antigen provided by the contracting investigator ("custom" antibodies) or through the generation of a specific polypeptide that is then used to immunize animals to produce antibodies.
- ☐ This project is funded through a subaward, subgrant, or subcontract from another institution to perform some or all of the animal work described within this Proposal.
- ☒ None of the above.

Note: this section does not include collaborations in which you import a new strain from a collaborator.

22.0 Biological Agents

22.1 Indicate which Types of Biological Agents that will be administered to animals. - Examples: Mammalian cell lines; bacteria; other microbes; viruses; materials of human or non-human primate origin (e.g. antibodies etc.); toxins of biological origin (e.g., Complete Freund's Adjuvant, pertussis toxin). These tables will be reviewed by the Biological Safety Office to determine the need for IBC Registration and/or applicable SASPs. - Select ALL that Apply

- ☐ Not Applicable: No Biological materials will be used in live animals
- ☒ Microbial Agents or Parasites (bacteria, viruses, protozoa, etc.)
- ☐ Cells or Tissues (cell lines, primary tissues or cells, etc.)
- ☒ Other Biological Material (antibodies, rDNA, toxins of biological origin such as Complete Freund's Adjuvant, pertussis toxin, etc.)

22.3 Microbiological Agents or Parasites (bacteria, viruses, protozoa, etc.) Include viral vectors, fungi, and parasites.

Name and ABSL	Description of organism and use	Select Agent
<div>Description of Organism:</div> <div>Inhabitant of the human oral cavity. Anaerobe that rapidly dies upon exposure to air</div> <div>Name:</div> <div>Porphyromonas gingivalis</div> <div>Animal Biosafety Level</div> <div>1</div>	<div>Description of Organism:</div> <div>Inhabitant of the human oral cavity. Anaerobe that rapidly dies upon exposure to air</div> <div>Describe use, (and note if the recipient is a Genetically-Modified Strain).</div> <div>Will be used in the oral infection, abscess and AD models. 10E9 cfu in 0.1 ml will be used in each inoculation for the oral infection. 10E8 cfu in 0.1 ml will be used in each inoculation in the abscess model. We will also use engineered strains of P. gingivalis that have a deletion in a gene we have identified as a potential virulence factor.</div>	<div><input type="radio"/> CDC</div> <div><input type="radio"/> USDA</div> <div><input checked="" type="radio"/> Not Applicable</div>
	<div>Description of Organism:</div>	

<p>Name:</p> <div>Filifactor alocis</div> <p>Animal Biosfatey Level</p> <div>1</div>	<p>Inhabitant of the human oral cavity. Anaerobe that rapidly dies upon exposure to air</p> <p>Describe use, (and note if the recipient is a Genetically-Modified Strain).</p> <div>Will be used in the oral infection and abscess models. 10E9 cfu in 0.1 ml will be used in each inoculation for the oral infection. 10E8 cfu in 0.1 ml will be used in each inoculation in the abscess model.</div>	<p> <input type="radio"/> CDC <input type="radio"/> USDA <input checked="" type="radio"/> Not Applicable </p>
<p>Name:</p> <div>Streptococcus gordonii</div> <p>Animal Biosfatey Level</p> <div>1</div>	<p>Description of Organism:</p> <div>Commensal in the human oral cavity.</div> <p>Describe use, (and note if the recipient is a Genetically-Modified Strain).</p> <div>Will be used in the oral infection and abscess models. 10E9 cfu in 0.1 ml will be used in each inoculation for the oral infection. 10E8 cfu in 0.1 ml will be used in each inoculation in the abscess model. In the abscess model we will also use a strain engineered to produce the luciferase enzyme</div>	<p> <input type="radio"/> CDC <input type="radio"/> USDA <input checked="" type="radio"/> Not Applicable </p>
<p>Name:</p> <div>Fusobacterium nucleatum</div> <p>Animal Biosfatey Level</p> <div>1</div>	<p>Description of Organism:</p> <div>Inhabitant of the human oral cavity. Anaerobe that rapidly dies upon exposure to air</div> <p>Describe use, (and note if the recipient is a Genetically-Modified Strain).</p> <div>Will be used in the oral infection and abscess models. 10E9 cfu in 0.1 ml will be used in each inoculation for the oral infection. 10E8 cfu in 0.1 ml will be used in each inoculation in the abscess model.</div>	<p> <input type="radio"/> CDC <input type="radio"/> USDA <input checked="" type="radio"/> Not Applicable </p>
	<p>Description of Organism:</p> <div>Inhabitant of the human oral cavity. Anaerobe that rapidly dies upon exposure to air</div>	

Name:

Selenomonas sputigena

Animal Biosafety Level

1

Describe use, (and note if the recipient is a Genetically-Modified Strain).

Will be used in the oral infection and abscess models. 10E9 cfu in 0.1 ml will be used in each inoculation for the oral infection. 10E8 cfu in 0.1 ml will be used in each inoculation in the abscess model.

- ☐ CDC
☐ USDA
☒ Not Applicable

22.4 Other Biological Materials

Describe the use of other biological materials, such as: antibodies, recombinant nucleic acids (plasmids, genes), biological toxins or products (pertussis, CFA), or other materials.

Name and Type	Description
Name: Ly6G antibody	Description of Material Rat anti-mouse Ly-6G, clone 1A8, Alexa Fluor 647 tag
Type Antibody	Description of use (and note if the recipient is a Genetically-Modified strain).
Animal Biosafety Level: N/A	iv injection of 0.1 mg in the oral infection model to track neutrophil recruitment post mortem.

22.5 Testing of Biological Agents

NOTE: Policy, “[Testing of Cell Lines and Other Biological Materials for Rodent Pathogens](#).” Are any of the above agents of rodent origin or may have been passaged through rodents?

☒ Yes ☐ No

22.6

If any agents are of rodent origin, or may have been passaged through rodents, then the materials must be tested for potential rodent pathogens by an RRF-approved vendor prior to IACUC approval. Please describe results of testing, including dates, or other assurance that the materials are free of rodent pathogens.

Rodent pathogen testing performed by manufacturer

23.0 Chemical Agents

23.1

Will animals be exposed *in vivo* to ANY other chemical agent not included in the “Anesthetics, Analgesics, and Other Therapeutic Agents” table above? This includes investigational drugs, test articles, or any other chemical agent introduced into the animal other than biological or radiological materials.

☒ Yes ☐ No

23.2

List all Chemical Agents administered to animals that were not included in the Anesthetics, Analgesics, and Other Therapeutics table. Include all non-biological and non-radiological agents, regardless of the method of delivery (injection, food or water, inhalation, etc.). - Please note that biologically-derived toxins (e.g., pertussis toxin, CFA) and/or biohazardous agents (biologically-derived agents, and other agents that require IBC review) must be listed in the Biological Agents table to avoid delays in the review process. - A description of laboratory procedures involving the preparation of these chemical agents must be included in your laboratory's Chemical Hygiene Plan.

Uses may include test articles, agents used to induce disease (e.g., cancer), and investigational drugs.

Agent	Administration	Description
<p>Name:</p> <div>carboxymethyl cellulose</div> <p>CAS Number</p> <div>9004-32-4</div> <p> <input type="checkbox"/> Carcinogen/Mutagen /Teratogen <input type="checkbox"/> Reproductive Toxin <input type="checkbox"/> Toxicant <input type="checkbox"/> Neurotoxin <input type="checkbox"/> Irritant <input type="checkbox"/> Investigational Drug/Agent <input type="checkbox"/> Potential Hazards Not Yet Determined <input checked="" type="checkbox"/> Not Hazardous <input type="checkbox"/> Other (include in description of use) </p>	<p>Route</p> <div>Other (include in description of use)</div> <p>Dose (mg/kg)</p> <div>2%</div> <p>Concentration (mg/mL) of the chemical in solution</p> <div>2%</div> <p>Approximate Volume (mLs)</p> <div>0.1 ml</div> <p>Frequency (e.g.twice daily)</p> <div>5 times</div>	<p>Duration (e.g once, every day for 3 weeks etc.</p> <div>5 times over 10 day period</div> <p>Description of Use</p> <div>used for bacterial inoculations in the oral cavity to aid retention</div>
<p>Name:</p> <div>Phosphate buffered saline</div> <p>CAS Number</p> <div>7647-14 -5</div> <p> <input type="checkbox"/> Carcinogen/Mutagen /Teratogen <input type="checkbox"/> Reproductive Toxin <input type="checkbox"/> Toxicant <input type="checkbox"/> Neurotoxin <input type="checkbox"/> Irritant <input type="checkbox"/> Investigational Drug/Agent <input type="checkbox"/> Potential Hazards Not Yet Determined <input checked="" type="checkbox"/> Not Hazardous <input type="checkbox"/> Other (include in description of use) </p>	<p>Route</p> <div>Other (include in description of use)</div> <p>Dose (mg/kg)</p> <div>0.1 ml</div> <p>Concentration (mg/mL) of the chemical in solution</p> <div>5-10 %</div> <p>Approximate Volume (mLs)</p> <div>0.1 ml</div> <p>Frequency (e.g.twice daily)</p> <div>for each inoculation</div>	<p>Duration (e.g once, every day for 3 weeks etc.</p> <div>once with every inoculation</div> <p>Description of Use</p> <div>bacteria will be suspended in PBS and 0.1 ml used for oral inoculation or subcutaneous injection.</div>
<p>Name:</p> <div>Lucigenin</div> <p>CAS Number</p> <div>2315-97-1</div> <p> <input type="checkbox"/> Carcinogen/Mutagen /Teratogen <input type="checkbox"/> Reproductive Toxin <input type="checkbox"/> Toxicant <input type="checkbox"/> Neurotoxin <input checked="" type="checkbox"/> Irritant <input type="checkbox"/> Investigational Drug/Agent </p>	<p>Route</p> <div>IP</div> <p>Dose (mg/kg)</p> <div>25 mg/kg</div> <p>Concentration (mg/mL) of the chemical in solution</p> <div>2.5 mg/ml</div> <p>Approximate Volume (mLs)</p> <div>0.2</div>	<p>Duration (e.g once, every day for 3 weeks etc.</p> <div>once</div> <p>Description of Use</p> <div>Injection prior to imaging</div>

<input type="checkbox"/> Potential Hazards Not Yet Determined <input type="checkbox"/> Not Hazardous <input type="checkbox"/> Other (include in description of use)	Frequency (e.g.twice daily) <input type="text" value="for each inoculation"/>	
Name: <input type="text" value="Nair with baby oil"/> CAS Number <input type="text" value="29820-13-1, 1305-62-0, 1310-73-2"/> <input type="checkbox"/> Carcinogen/Mutagen /Teratogen <input type="checkbox"/> Reproductive Toxin <input type="checkbox"/> Toxicant <input type="checkbox"/> Neurotoxin <input checked="" type="checkbox"/> Irritant <input type="checkbox"/> Investigational Drug/Agent <input type="checkbox"/> Potential Hazards Not Yet Determined <input type="checkbox"/> Not Hazardous <input type="checkbox"/> Other (include in description of use)	Route <input type="text" value="Topical"/> Dose (mg/kg) <input type="text" value="fixed volume"/> Concentration (mg/mL) of the chemical in solution <input type="text" value="proprietary"/> Approximate Volume (mLs) <input type="text" value="0.2"/> Frequency (e.g.twice daily) <input type="text" value="once on one day"/>	Duration (e.g once, every day for 3 weeks etc.) <input type="text" value="once"/> Description of Use <input type="text" value="remove hair prior to injection for abscess formation"/>
Name: <input type="text" value="luminol"/> CAS Number <input type="text" value="521-31-3"/> <input type="checkbox"/> Carcinogen/Mutagen /Teratogen <input type="checkbox"/> Reproductive Toxin <input type="checkbox"/> Toxicant <input type="checkbox"/> Neurotoxin <input checked="" type="checkbox"/> Irritant <input type="checkbox"/> Investigational Drug/Agent <input type="checkbox"/> Potential Hazards Not Yet Determined <input type="checkbox"/> Not Hazardous <input type="checkbox"/> Other (include in description of use)	Route <input type="text" value="IP"/> Dose (mg/kg) <input type="text" value="300"/> Concentration (mg/mL) of the chemical in solution <input type="text" value="50"/> Approximate Volume (mLs) <input type="text" value="0.12"/> Frequency (e.g.twice daily) <input type="text" value="once on one day"/>	Duration (e.g once, every day for 3 weeks etc.) <input type="text" value="once"/> Description of Use <input type="text" value="injection prior to imaging"/>

24.0 Radiation or Other Physical Hazards

24.1 Will animals be exposed to radiation (e.g., isotopes, lasers, irradiators) or other physical hazards (e.g., loud noises)?

- ☐ Radioactive Material
☐ Cs-137 irradiator
☐ X-Ray radiation
☐ Other (magnet, lasers, noise, etc.)

25.0 Agent Administration and Return to RRF

25.1 Will live animals be returned to the RRF after exposure to hazardous substances (biological, chemical, or radioactive)?

- ☐ Not Applicable. No hazardous agents used or animals are not returned to the RRF
- ☒ Biological Exposure then return to RRF
- ☐ Chemical Exposure then return to RRF
- ☐ Radioactive Material Exposure then return to RRF

25.2 Biological Exposure and Return to RRF Indicate below the PREFERRED animal facility for housing following exposure:

If Yes, indicate below which animal facility is PREFERRED for housing following exposure:

- ☐ A-Tower
- ☐ B1-BF
- ☒ B2V
- ☐ CII
- ☒ CTRV
- ☐ DS-324
- ☐ LSB
- ☐ MDR
- ☐ RBL
- ☐ RRC

Describe other housing arrangements below:

Will also use germ-free facility in CTRV

26.0 Euthanasia or Other Disposition

26.5 Euthanasia or Other Disposition - rodent

Select Euthanasia method(s) that will be used. Describe other methods in text box below.

-

Rodents, Adults (> 21 days of age)

- ☐ Barbiturate injection (IP), overdose to effect. Death will be ensured by an adjunctive physical method such as cervical dislocation, bilateral thoracotomy, or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.
- ☒ General anesthesia as described in "Anesthetics, Analgesics, and Other Therapeutics," followed by an adjunctive physical method, such as bilateral thoracotomy, exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.
- ☒ Carbon dioxide, supplied from a cylinder or tank in a chamber fitted with an appropriate pressure-reducing regulator and flow meter or equivalent equipment to ensure a gradual displacement of 30-70% volume/minute. CO2 flow will be maintained for at least 1 minute after respiratory arrest, and death will be ensured by an adjunctive physical method such as cervical dislocation, bilateral thoracotomy, or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.
- ☐ Cervical dislocation without prior anesthesia (only animals <200 g) by luxation of cervical vertebrae without primary crushing of vertebrate and spinal cord and performed only by personnel with proficiency demonstrated with anesthetized or dead animals.
- ☐ Decapitation without prior anesthesia, by personnel with proficiency demonstrated with anesthetized and/or dead animals; commercially-available guillotine preferred.

Rodents, Neonates (< 21 days of age)

- ☐ Carbon dioxide, supplied from a cylinder or tank in a chamber fitted with an appropriate pressure-reducing regulator and flow meter or equivalent equipment to ensure a gradual displacement of 30-70% volume/minute; an adjunctive physical method (e.g., cervical dislocation or decapitation) will be performed after neonates are nonresponsive to painful stimuli.
- ☐ Cervical dislocation without anesthesia via pinching and disrupting the high cervical spinal cord.
- ☐ Decapitation without anesthesia (animals < 7 days only) with sharp scissors or blades.
- ☐ Gradual cooling (animals < 7 days only) using measures to prevent direct contact with ice or precooled surfaces. Death will be ensured by an adjunctive method (e.g., cervical dislocation or decapitation) after neonates are nonresponsive to painful stimuli.
- ☐ Rapid freezing in liquid nitrogen (animals < 5 days only).
- ☐ General anesthesia as described in "Anesthetics, Analgesics, and Other Therapeutics," followed by an adjunctive physical method, such as bilateral thoracotomy, exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.

26.8 If methods of euthanasia other than those listed above will be employed, please describe their use in detail.

Will use General anesthesia when animals have been anesthetized for imaging in abscess model. Will use CO2 for oral and AD models

Adjunctive physical method will be cervical dislocation

26.9 For animals that will not undergo euthanasia at the end of these studies, provide a description of their final disposition. If this includes assignment to another *Proposal*, identify the other *Proposal* (if known) and estimate the minimum time period before using the animal(s) in subsequent procedures.

27.0 Non-Pharmaceutical-Grade Agents

27.1 Are ANY of the agents, substances, drugs, test articles, etc. to be used in live animals chemical grade, that is, not pharmaceutical grade?

☒ Yes ☐ No

27.2 Describe the use and quality assurance practices for all non-pharmaceutical-grade agents. In the justification, please explain why pharmaceutical grade materials cannot, or should not, be used. Examples may include unavailability of an equivalent veterinary or human drug, a need for higher concentration, interference of vehicles/diluents, etc. Cost alone may not be a satisfactory reason. [IACUC Policy](#)

Chemical	Justification
<p>Agent:</p> <p>Carboxymethyl cellulose</p> <p>Describe formulation including purity, methods of ensuring sterility, and physiological compatibility (pH, pyrogenicity, osmolality, etc.):</p> <p>Carboxymethyl cellulose sodium salt pH 6.5 - 8.5. LD50 oral 27,000 mg/kg. Stable at room temperature and 37C, CMC will be dissolved in sterile Phosphate Buffered Saline and autoclaved.</p> <p>Expected shelf-life (stability, expiration/discard timeframe that will be used):</p> <p>1 year</p>	<p>Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.</p> <p>Pharmaceutical grade not available. Can not be filter sterilized due to viscosity</p> <p>Is this chemical available as a pharmaceutical or USP grade?</p> <p><input type="radio"/> Yes <input checked="" type="radio"/> No</p>
<p>Agent:</p>	

luminol

Describe formulation including purity, methods of ensuring sterility, and physiological compatibility (pH, pyrogenicity, osmolality, etc.):

3-Aminophthalhydrazide, 5-Amino-2,3-dihydro-1,4-phthalazinedione. Will be obtained from Sigma Aldrich as powder and dissolved in PBS, and filter sterilized.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1 year

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

Pharmaceutical grade not available.

Is this chemical available as a pharmaceutical or USP grade?

☐ Yes ☒ No

Agent:

lucigenin

Describe formulation including purity, methods of ensuring sterility, and physiological compatibility (pH, pyrogenicity, osmolality, etc.):

bis-N-methylacridinium nitrate. Will be obtained from Sigma Aldrich as powder and dissolved in PBS, and filter sterilized.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1 year

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

Pharmaceutical grade not available

Is this chemical available as a pharmaceutical or USP grade?

☐ Yes ☒ No

28.0 Non RRF Study Site(s)

28.1 Will animals be transported to and used in rooms outside of the RRF?

☒ Yes ☐ No

28.2 Describe where animals will be taken, including the location(s), procedures to be performed there, and the length of time that individual animals will be retained in those rooms.

Location	Procedures Performed	For Surgical Location ONLY
Building: <input type="text" value="CTR"/> Room Number: <input type="text" value="642F"/> ----- Duration: <input checked="" type="checkbox"/> Day Use	<input type="checkbox"/> Euthanasia and Tissue Collection <input type="checkbox"/> Behavior Assessment <input checked="" type="checkbox"/> Imaging <input type="checkbox"/> Hazardous Agent Use or Administration (describe) <input type="checkbox"/> Non-Survival Surgery <input type="checkbox"/> Survival Surgery <input type="checkbox"/> Other Non-Surgical	Frequency of use: <input type="checkbox"/> Heavy (daily) <input type="checkbox"/> Moderate (weekly) <input checked="" type="checkbox"/> Light For locations where surgeries will be performed only: Describe surgical support equipment available (e.g., gas anesthetic machines,

- ☐ Overnight
☐ 24-72 Hours
☐ >72 Hours

Procedures (describe)

ventilators, body temperature support).

28.3 If animals will be housed outside the RRF overnight or longer, provide a justification and brief description of practices employed. *Note that for locations where animals may be kept over 72 hours, a Satellite Housing Area Description (SHAD) is required. If you already have an approved SHAD for the location(s) you will need to attach it as a "Study Document" in the Initial Review Submission Packet.*

Note: Justification and a description of practices employed with animals retained outside of the RRF for over 12 hours must be described in the Special Housing section.

29.0 Consideration of Alternatives

29.1 Indicate HIGHEST Pain Classification of Procedures in this protocol.

- ☐ Class 0 - Animals will be acquired/held, but not used or manipulated in any way.
- ☐ Class I - Studies in which animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will therefore receive no pain-relieving agents.
- ☒ Class II - Studies in which there is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic catheters, as well as survival and non-survival surgery.
- ☐ Class III - Studies in which animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section.

29.2 List of Procedures List all procedures potentially associated with more than minor pain or distress (e.g., nephrectomy, craniotomy, forced exercise, use of Complete Freund's Adjuvant). This is meant to help you identify keywords needed in literature database searches for alternatives to the potentially painful or distressful procedures.

Abscess formation
 Oral model
 AD model

29.3 Consideration of Alternatives Provide a written description of the methods (e.g., literature database search) and sources (e.g., databases, review articles, scientific meetings) used to determine that alternatives to painful procedures were not available. *Note: Unless a compelling justification can be made without it, support your assurance by conducting a literature database search. - Note: the USDA Research Inspection Guide states that teaching exercises involving potential pain and distress (e.g., non-survival surgery) using animals should also consider alternatives such as veterinary mannequins, live tissue alternatives, and mechanical teaching devices. Protocols involving toxicity studies should also consider alternatives such as local lymph node assay, up-and-down procedures (see <http://iccvam.niehs.nih.gov/about/overview/htm>).*

The abscess model is the most effective way to measure and correlate periodontal bacterial virulence and host innate immune responses. A search of PubMed and Google Scholar revealed only the subcutaneous chamber model as an alternative which would require survival surgery.

The oral model allows measurement of oral bacterial induction of loss of the periodontal bone. A search of PubMed and Google Scholar revealed only the ligature model as a possible alternative. However the ligature model is technically challenging and often causes damage to the gingival soft tissues, and pain. Additionally, it is unclear if the ligature model is in reality a damage model rather than a bacterial infection model.

iv or intracerebroventricular inoculation is a possible alternative for the AD model, however this is more invasive than oral inoculation

USDA Policy stipulates that for each search performed, you must provide the information requested in the table below. For additional information regarding performing such searches, see the **IACUC Information Sheet**. A representative in the Kornhauser Library is also available to assist you: **j0chen05@exchange.louisville.edu**

Keywords must include the procedure itself (e.g., abdominal surgery, nephrectomy, thoracotomy, craniotomy, etc. Keywords should include terms for refinement as well as replacement for the painful procedure, such as analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare.

Database Name / Search Date	Keywords Used / Results
<div>Database Name</div> <div>PubMed</div> <div>Date Search Performed</div> <div>02/06/2024</div> <div>Dates seached, if not ALL</div>	<div>List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).</div> <div>abscess, bacteria, analges*, distress*</div> <div>Results:</div> <div>No viable alternatives</div>
<div>Database Name</div> <div>Google Scholar</div> <div>Date Search Performed</div> <div>02/06/2024</div> <div>Dates seached, if not ALL</div>	<div>List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).</div> <div>abscess, bacteria, analges*, distress*</div> <div>Results:</div> <div>No viable alternatives</div>
<div>Database Name</div> <div>PubMed</div> <div>Date Search Performed</div> <div>02/06/2024</div> <div>Dates seached, if not ALL</div>	<div>List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).</div> <div>oral, bacteria, analges*, distress*</div> <div>Results:</div> <div>No viable alternatives</div>
<div>Database Name</div> <div>Google Scholar</div> <div>Date Search Performed</div> <div>02/06/2024</div> <div>Dates seached, if not ALL</div>	<div>List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).</div> <div>oral, bacteria, analges*, distress*</div> <div>Results:</div> <div>No viable alternatives</div>
<div>Database Name</div> <div>PubMed</div> <div>Date Search Performed</div> <div>04/14/2025</div> <div>Dates seached, if not ALL</div>	<div>List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).</div> <div>gingivalis, Alzheimer's, murine</div> <div>Results:</div>

	No viable alternatives
Database Name	List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).
Google Scholar	
Date Search Performed	gingivalis, Alzheimer's, murine
04/14/2025	Results:
Dates seached, if not ALL	No viable alternatives

29.5 Humane Endpoints For some Class I and *all* Class II and III procedures, there is a potential for adverse effects. Humane endpoints are objective signs indicating a pain/distress level that warrants intervention (usually euthanasia), regardless of experimental timelines. These may be specific for each procedure or may be general for an experimental group or the entire *Proposal*. Often, basic “sick animal” signs such as inappetance or lethargy lasting over 24-48 hours or weight loss exceeding 10% are used. Other signs/criteria may be more appropriate for this study. [\[IACUC Policy and Pain Scoring Sheet Templates\]](#) - Make sure that your response

1. Precisely defines the humane endpoint, including assessment criteria
2. Describes the frequency of animal observation
3. Describes the response required upon reaching the humane endpoint

Experimental Group or Procedure	Endpoint and Assessment Criteria	Frequency of Observation	Response
Abscess	One or more of these criteria: Loss of > 10% of body weight, Poor body condition score <2/5 Inability to obtain food or water Hypo /Unresponsive Dehydration Organ dysfunction/failure	Weighed every three days. Monitored daily	euthanasia
Oral	One or more of these criteria: Loss of > 10% of body weight, Poor body condition score <2/5 Inability to obtain food or water Hypo /Unresponsive Dehydration Organ dysfunction/failure	Weighed every three days. Monitored daily	euthanasia
AD	Animals with the 5XFAD background will be monitored closely for signs of frailty such as kyphosis starting at 210 days of age. Animals showing signs of frailty will be given supportive care such as wet food, napa nectar or other supplemental hydration. Mice will be supported to	Monitored daily at 210 days of age	supplemental hydration, euthanasia if necessary

reach the endpoint of 240 days. We will follow BCS scoring to determine timing of euthanasia with a BCS of 2 or less requiring euthanasia.		
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30.0 Other Information for IACUC Review

30.1 Is there any additional information that may assist the IACUC in their review, *e.g.*, request for exemptions to IACUC policies not described elsewhere in this Proposal?

☐ Yes ☒ No

31.0 End of Form

31.1 **STOP** To Submit Proposal click "Save & Continue," and complete the Initial Review Submission Packet Otherwise - Log Out or return to the sections you wish to revise.