



UNIVERSITY of WASHINGTON

APPROVAL OF NEW PROTOCOL SUBMISSION

September 28, 2020

Dear Dr. Abuzeid,

This email serves as written notice of animal use approval by the Institutional Animal Care and Use Committee (IACUC).

To help us better serve you, please take this [3 question survey](#) about your experience with the review process.

Type of Review:	Designated Member Review
Short Title of Protocol:	4503-01: Rabbit Sinusitis
Investigator:	Al Waleed Abuzeid
HoverBoard ID:	PROTO202000106

Please note the approval and expiration date listed. All animal use protocols that include USDA regulated species or receive support from the Department of Defense must be renewed annually from the date of IACUC approval, independent of project or funding dates. Please refer to the assigned protocol number for all animal orders and future correspondence with the IACUC.

Protocol Approval Dates: 9/28/2020 to 9/27/2023

Next Annual Expiration Date: 9/27/2021

Next Triennial Expiration Date: 9/27/2023

If you have any questions, contact OAWRSS at oawrss@uw.edu.

Sincerely,

Office of Animal Welfare



OFFICE of ANIMAL WELFARE

Research Support Services

From: Molly Lucas <mklucas@uw.edu>
Sent: Wednesday, November 18, 2020 1:38 PM
To: Bob Ennes
Subject: 4503-01 request
Attachments: RE: Notes from today's Zoom vet consult (Abuzeid protocol); Re: Notes from today's Zoom vet consult (Abuzeid protocol); Re: Notes from today's Zoom vet consult (Abuzeid protocol); Re: Abuzeid anesthesia; Re: Abuzeid anesthesia; Re: Abuzeid anesthesia; Abuzeid anesthesia

Hi Bob,

Here are some more attachments for this protocol.

Molly

From: wabuzeit <wabuzeit@uw.edu>
Sent: Monday, September 7, 2020 9:49 PM
To: Molly K. Lucas
Cc: Nicholas L. Reyes; Aubrey Schoenleben
Subject: RE: Notes from today's Zoom vet consult (Abuzeid protocol)

Thanks for the comprehensive notes, Molly. All discussed changes have been integrated into the protocol which has been resubmitted for pre-review.

Waleed

From: wabuzeit <wabuzeit@uw.edu>
Sent: Friday, September 4, 2020 8:16 AM
To: Aubrey Schoenleben <aubreys@uw.edu>; Molly K. Lucas <mkucas@uw.edu>
Cc: Nicholas L. Reyes <nireyes@uw.edu>
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

It was a very helpful conversation. I'll work on integrating the changes this weekend with a view of expediting review given your upcoming leave, Aubrey.

W

Get [Outlook for Android](#)

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Friday, September 4, 2020, 8:11 AM
To: Molly K. Lucas; wabuzeit
Cc: Nicholas L. Reyes
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

Thanks, Molly!

Waleed - Please let me know if you have questions or need help incorporating these revisions into the protocol.

Cheers,
Aubrey

From: Molly K. Lucas <mkucas@uw.edu>
Sent: Monday, August 31, 2020 3:56 PM
To: wabuzeit <wabuzeit@uw.edu>
Cc: Nicholas L. Reyes <nireyes@uw.edu>; Aubrey Schoenleben <aubreys@uw.edu>
Subject: Notes from today's Zoom vet consult (Abuzeid protocol)

Hi Dr. Abuzeit,

It was very nice to meet you today and I think it was a productive discussion about your protocol.

I've attached a new copy of the Word document we referred to today. The yellow highlights are my notes from today. If anything looks incorrect or you have additional thoughts or change your mind about something, just let me know. I've included Aubrey so she knows what changes to expect in Hoverboard, and she also may be able to assist you with some of them.

The protocol will come back to me for re-review once it has been revised.

Sincerely,
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From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Friday, September 4, 2020 8:05 AM
To: Molly K. Lucas; Waleed M Abuzeid
Cc: Nicholas L. Reyes
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

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Sincerely,
Molly Lucas

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Monday, August 31, 2020 3:30 PM
To: Molly K. Lucas
Subject: Re: Abuzeid anesthesia

Makes sense. We do monitor BP as a standard but maybe not for the inoculation if it will only be a few minutes.

Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nlreyes@uw.edu
206-543-0267

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Monday, August 31, 2020 3:27 PM
To: Nicholas L. Reyes <nlreyes@uw.edu>
Subject: Re: Abuzeid anesthesia

I was thinking maybe the ace would be a separate procedure since it wouldn't be part of induction, but I can ask him to include it there if you think that would be helpful.

Also - will VS monitor blood pressure? I'm thinking maybe not routinely?

Thanks! I'm writing up the notes now while it's fresh in my mind 😊

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Monday, August 31, 2020 3:22 PM
To: Molly K. Lucas <mklucas@uw.edu>
Subject: Re: Abuzeid anesthesia

That sounds good to me. We can work with that...and adding the acepromazine as an option as well?

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Thanks,
Molly

-Add K/X at ketamine 40-50 mg/kg IM and xylazine 3-5 mg/kg IM

-OK to leave the ket/dexmed option also

-Add atipamezole 0.25-1 mg/kg SC, IM, IV as reversal option for xylazine or dexmedetomidine (just an option)

-Add isoflurane/intubation/ventilation option

-Make it clear that buprenorphine would not be given until after animal intubated (because of risk of additive respiratory depression with induction drugs)

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Sent: Monday, August 31, 2020 3:06 PM
To: Nicholas L. Reyes
Subject: Abuzeid anesthesia

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From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/31/2020 3:05:46 PM
Subject: Abuzeid anesthesia

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Abuzeid Emails

Induction of anesthesia recommendations (PROTO202000106; 4502-01 rabbit sinusitis)

☐

wabuzeit <wabuzeit@uw.edu>
Mon 7/27/2020 11:08 PM

☐
Like
☐
☐
☐
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To:

- VET SERVICES PROTOCOL REVIEW

Thanks so much for the response, Megan. I appreciate your input.

There was an error in the dosing of dexmedetomidine that I provided – my colleagues used 0.25 mg/kg as you rightly suggested. I still wanted some leeway in dosing and so adjusted the dosing range accordingly as you can see below. As for the carprofen, I have modified the protocol to allow for only SQ delivery (for both anesthesia induction and for post-op pain control) at the recommended dose of 1-2 mg/kg. I have stuck with your recs for buprenorphine and ketamine.

So, in summary, we're looking at:

- 1) Buprenorphine HCl (0.02-0.03mg/kg IM)
- 2) Carprofen (1-2 mg/kg SQ)
- 3) Dexmedetomidine (0.15-0.3mg/kg IM)
- 4) Ketamine (15-20mg/kg IM)

Thanks again for your input!

Best,

Waleed

☐

From: VET SERVICES PROTOCOL REVIEW <vsreview@uw.edu>

Sent: Monday, July 27, 2020 4:44 PM

To: wabuzeit <wabuzeit@uw.edu>

Subject: RE: Induction of anesthesia recommendations (PROTO202000106; 4502-01 rabbit sinusitis)

Hello Waleed,

Thank you for reaching out! It sounds like, from what I understand from this and your protocol progress on Hoverboard, that your IACUC has wanted you to provide a range for anesthetic agents so that you can titrate them to effect and not essential be “locked in” to a single amount. Hopefully these ranges are not too wide! Typically for surgical anesthesia we give around 15mg/kg ketamine and 0.25mg/kg dexmedetomidine, but I wanted to leave more room to allow for the doses that your colleagues have used successfully on this procedure.

My suggestions would be as follows

- 1) Buprenorphine HCl (0.02-0.03mg/kg IM)
- 2) Consider excluding Carprofen—see below
- 3) Dexmedetomidine (0.05-0.2mg/kg IM)
- 4) Ketamine (15-20mg/kg IM)

I would not personally include carprofen in your IM anesthesia and analgesia cocktail. Carprofen has not been labeled for use IM, and therefore there is not a lot of efficacy, safety, or dosage data for it given IM. In rabbits, we typically give 1-2mg/kg carprofen SQ, or it can also be given orally at a dose of 1.5mg/kg. Since your reviewer has not noted it, it may be possible that they are okay with it being given IM with the previous success of your colleagues. It is just a bit surprising to see carprofen given IM, and it could be worth your consideration to give SQ.

Best,
Megan Ellis, DVM
Veterinary Resident

From: [wabuzeid](#)

Sent: Sunday, July 26, 2020 5:45 PM

To: [VET SERVICES PROTOCOL REVIEW](#)

Cc: [Aubrey Schoenleben](#)

Subject: Induction of anesthesia recommendations (PROTO202000106; 4502-01 rabbit sinusitis)

Hello to the veterinary team!

I am in the midst of editing a protocol for a series of rabbit experiments. This involves the induction of anesthesia and adequate intraop analgesia in the animals. Two procedures will be performed:

1. Placement of a tunneled indwelling surgical catheter into the maxillary sinus of the rabbit (duration 20-40 minutes per rabbit).
2. Brief nasal endoscopy for intranasal endoscopic grading and for endoscopic-guided culture swabs (duration 5-10 minutes per rabbit).

The proposed anesthesia/analgesia procedure is based on a protocol used safely and successfully by my colleagues which were approved by their respective IACUC (University of Alabama and University of Pennsylvania).

I am seeking your advice on reasonable dose ranges to submit to IACUC for review. The rabbits undergoing anesthesia would be female New Zealand white rabbits between 4-6 months of age (body weight 2-4 kg). Currently, the protocol consists of four medications administered intramuscularly as a cocktail:

1. Buprenorphine HCl (0.02 mg/kg IM)
2. Carprofen (5 mg/kg IM)
3. Dexmedetomidine (0.05 mg/kg IM)
4. Ketamine (20 mg/kg IM)

Thank you for your input!

Best,

Waleed M. Abuzeid, MD

Associate Professor

Rhinology and Endoscopic Skull Base Surgery

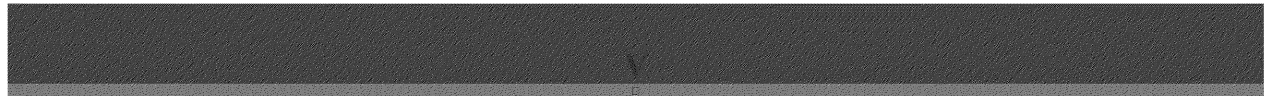
Department of Otolaryngology: Head and Neck Surgery

University of Washington

Email: wabuzeid@uw.edu

Reply

Forward



VET SERVICES PROTOCOL REVIEW

Mon 7/27/2020 4:44 PM



To:

- wabuzeid <wabuzeid@uw.edu>

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From: wabuzeid

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Cc: Aubrey Schoenleben

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Associate Professor

Rhinology and Endoscopic Skull Base Surgery

Department of Otolaryngology: Head and Neck Surgery

University of Washington

Email: wabuzeid@uw.edu

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Once I'm done with this doc I can share it with everyone and you may have things to add.

Molly

From: Molly K. Lucas <mkluucas@uw.edu>
Sent: Wednesday, August 19, 2020 1:59 PM
To: Nicholas L. Reyes
Subject: Abuzeid rabbit sinusitis

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Molly

Topics for discussion – Abuzeid new protocol

Anesthesia and analgesia:

-It sounds like vet services will run anesthesia. Are you open to considering additional anesthetic options (e.g., isoflurane)? Often when VS is hired to run anesthesia for large animals, we include a wide variety of anesthetic options, since the vet staff is comfortable with many options. Inhalational is less likely to lead to respiratory depression. Intubation would need to be done (rather than mask) in order for there to be access to the nasal cavity. This provides the advantage of more control over the airway and options to intervene if animals aren't breathing well on their own.

If the injectable procedure is retained:

-We should discuss whether the buprenorphine is being used as an analgesic or whether it's also a necessary component of the anesthetic procedure (this determines timing of administration), and potentially including a reversal agent (e.g., atipamezole) for the dexmedetomidine. Giving buprenorphine and dexmedetomidine at the same time increases the risk of respiratory depression.

-Review doses of ketamine/dexmedetomidine (impacted by timing of buprenorphine/whether it is part of the anesthetic plan).

-There is a slow release formulation of buprenorphine that would be a good option for the surgery. One injection at the time of surgery lasts for ~72 hr. The dose is 0.12 mg/kg SC. We should discuss the best timing for administration.

-Recommend removing the NSAID component (carprofen) from the anesthesia procedure since it's not an anesthetic, and is included in the analgesic procedure. The NSAID be in a separate analgesic procedure. This also means it can be removed from terminal anesthetic procedures, since it's not necessary for those. Buprenorphine may or may not be needed for terminal anesthetic events, depending on whether it's being used for anesthesia and analgesia or just analgesia.

-Would be a good idea to include oral meloxicam as an option, or replace carprofen (SC) with meloxicam (SC or oral). Rabbits tend to take the oral meloxicam well and it's less invasive than giving SC injections to post-op rabbits. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-May want to include option of sedation with acepromazine (0.8 mg/kg SC), for things like irrigation, nanoparticle administration? Also discuss rabbit snuggle (soft restraint) option in addition to restraint listed. Sedation may not be necessary but it can be helpful to have the option.

-Recommend reducing analgesic duration for endoscope procedures (minimum 12 hr with option to re-dose if needed).

-Recommend not specifying muscle for IM injections (epaxial are usually preferred over triceps in rabbits, but does not need to be specified).

Infection and antibiotics:

- We should talk about some of the issues we've been having at UW with NZW rabbits (dysbiosis).
- Are all antibiotics contraindicated in this study? We sometimes recommend prophylactic antibiotics to animals with implants that exit the skin, but this is an infection model (*Pseudomonas*), so antibiotic choice would need to be made carefully (if at all). Infections could be directly experimentally-related (e.g., catheter) or not (e.g., dysbiosis).
- I think it would be a good idea to create and include a Team Procedure of the type "Withhold medications" to describe which medications are contraindicated and which are not.

Q #7 of Experiments (monitoring and endpoints):

- Probably don't need to weigh daily unless this data is needed for experiments. OK to say will weigh 3x/wk (and can always increase frequency if there is a concern about a rabbit).
- Recommend a more narrow temperature range (currently 33-44C, recommend something more like 37-41C which is approx. 99-106F).
- Discuss possible additional euthanasia criteria such as respiratory distress, signs of infection (such as epiphora or conjunctivitis) not responsive to treatment. Harmonize euthanasia criteria listed here and in the surgery procedure.

Catheter surgery

- Discuss pros/cons of antibiotic ointment.
- Discuss potentially adding local anesthetics.
- Q #2: Please include sterile prep of the area between the ears where the catheter will exit.
- Q #3: Will the catheter end be covered for protection when not in use?
- Q #4: Recommend not including inappetance for 24 hr as a criterion for removal from study. Rabbits sometimes go off feed (e.g., will eat some hay/treats but not pellets), and while this is important clinically (and should be reported to vet services), I'm worried it might lead to a rabbit being prematurely removed from study. Harmonize euthanasia criteria in Q 4ii here and Q #7 of the Experiments.
- Q #5: Addition of more monitoring, e.g., oxygen saturation, heart rate, BP? (in addition to temperature and resp rate/pattern listed)

Sinus catheter

- Based on the implant procedure, it sounds like a repair would not be done. Let's discuss pros/cons (e.g., what if a relatively minor repair could be done under sedation?)

Endoscopy

- Discuss whether to add topical epinephrine/phenylephrine in case of significant bleeding.
- Approx. how long will this procedure last? May need to re-dose anesthesia if injectable.

-As mentioned in analgesia section above, probably don't need 72 hr analgesia for this.

Miscellaneous

-Please include approx. volume of nanoparticles to be administered to sinus (even though question indicates only for rodents/intracranial, it would be helpful to know for this route).

Topics for discussion – Abuzeid new protocol

Housing

-Looks like BSL-2 is required. Likely single housing of the female rabbits post-op?

Anesthesia and analgesia:

-It sounds like vet services will run anesthesia. Are you open to considering additional anesthetic options (e.g., isoflurane)? Often when VS is hired to run anesthesia for large animals, we include a wide variety of anesthetic options, since the vet staff is comfortable with many options. Inhalational is less likely to lead to respiratory depression. Intubation would need to be done (rather than mask) in order for there to be access to the nasal cavity. This provides the advantage of more control over the airway and options to intervene if animals aren't breathing well on their own.

If the injectable procedure is retained:

-We should discuss whether the buprenorphine is being used as an analgesic or whether it's also a necessary component of the anesthetic procedure (this determines timing of administration), and potentially including a reversal agent (e.g., atipamezole) for the dexmedetomidine. Giving buprenorphine and dexmedetomidine at the same time increases the risk of respiratory depression.

-Review doses of ketamine/dexmedetomidine (impacted by timing of buprenorphine/whether it is part of the anesthetic plan).

-There is a slow release formulation of buprenorphine that would be a good option for the surgery. One injection at the time of surgery lasts for ~72 hr. The dose is 0.12 mg/kg SC. We should discuss the best timing for administration.

-Recommend removing the NSAID component (carprofen) from the anesthesia procedure since it's not an anesthetic, and is included in the analgesic procedure. The NSAID be in a separate analgesic procedure. This also means it can be removed from terminal anesthetic procedures, since it's not necessary for those. Buprenorphine may or may not be needed for terminal anesthetic events, depending on whether it's being used for anesthesia and analgesia or just analgesia.

-Would be a good idea to include oral meloxicam as an option, or replace carprofen (SC) with meloxicam (SC or oral). Rabbits tend to take the oral meloxicam well and it's less invasive than giving SC injections to post-op rabbits. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-May want to include option of sedation with acepromazine (0.8 mg/kg SC), for things like irrigation, nanoparticle administration? Also discuss rabbit snuggle (soft restraint) option in addition to restraint listed. Sedation may not be necessary but it can be helpful to have the option.

-Recommend reducing analgesic duration for endoscope procedures (minimum 12 hr with option to re-dose if needed).

-Recommend not specifying muscle for IM injections (epaxial are usually preferred over triceps in rabbits, but does not need to be specified).

Infection and antibiotics:

-We should talk about some of the issues we've been having at UW with NZW rabbits (dysbiosis).

-Are all antibiotics contraindicated in this study? We sometimes recommend prophylactic antibiotics to animals with implants that exit the skin, but this is an infection model (*Pseudomonas*), so antibiotic choice would need to be made carefully (if at all). Infections could be directly experimentally-related (e.g., catheter) or not (e.g., dysbiosis).

-I think it would be a good idea to create and include a Team Procedure of the type "Withhold medications" to describe which medications are contraindicated and which are not.

Q #7 of Experiments (monitoring and endpoints):

-Probably don't need to weigh daily unless this data is needed for experiments. OK to say will weigh 3x/wk (and can always increase frequency if there is a concern about a rabbit).

-Recommend a more narrow temperature range (currently 33-44C, recommend something more like 37-41C which is approx. 99-106F).

-Discuss possible additional euthanasia criteria such as respiratory distress, signs of infection (such as epiphora or conjunctivitis) not responsive to treatment. Harmonize euthanasia criteria listed here and in the surgery procedure.

Catheter surgery

-Discuss pros/cons of antibiotic ointment.

-Discuss potentially adding local anesthetics.

-Q #2: Please include sterile prep of the area between the ears where the catheter will exit.

-Q #3: Will the catheter end be covered for protection when not in use?

-Q #4: Recommend not including inappetance for 24 hr as a criterion for removal from study. Rabbits sometimes go off feed (e.g., will eat some hay/treats but not pellets), and while this is important clinically (and should be reported to vet services), I'm worried it might lead to a rabbit being prematurely removed from study. Harmonize euthanasia criteria in Q 4ii here and Q #7 of the Experiments.

-Q #5: Addition of more monitoring, e.g., oxygen saturation, heart rate, BP? (in addition to temperature and resp rate/pattern listed)

Sinus catheter

-Based on the implant procedure, it sounds like a repair would not be done. Let's discuss pros/cons (e.g., what if a relatively minor repair could be done under sedation?)

Endoscopy

- Discuss whether to add topical epinephrine/phenylephrine in case of significant bleeding.
- Approx. how long will this procedure last? May need to re-dose anesthesia if injectable.
- As mentioned in analgesia section above, probably don't need 72 hr analgesia for this.

Miscellaneous

- Please include approx. volume of nanoparticles to be administered to sinus (even though question indicates only for rodents/intracranial, it would be helpful to know for this route).

Topics for discussion – Abuzeid new protocol

Housing

-Looks like BSL-2 is required. Include single housing of the female rabbits post-op (Q #12 Experiments, a husbandry exception)

Anesthesia and analgesia:

-It sounds like vet services will run anesthesia.

Yes. Gary Fye (gfyf@uw.edu), one of the vet techs in vet services can provide more information re: cost estimates for hiring VS for anesthesia; possibly some post-op care (optional)

Are you open to considering additional anesthetic options (e.g., isoflurane)? Often when VS is hired to run anesthesia for large animals, we include a wide variety of anesthetic options, since the vet staff is comfortable with many options. Inhalational is less likely to lead to respiratory depression. Intubation would need to be done (rather than mask) in order for there to be access to the nasal cavity. This provides the advantage of more control over the airway and options to intervene if animals aren't breathing well on their own.

-Edit to include xylazine in combination with ketamine (K 40-50 mg/kg IM, X 3-5 mg/kg IM)

-OK to leave ketamine/dexmedetomidine option

-Include atipamezole as an option in case reversal of xylazine/dexmed is indicated (0.25-1 mg/kg SC, IM, IV)

-Include isoflurane/intubation/ventilation

-Can leave buprenorphine here in anesthesia procedure, but indicate it would not be given until intubated, due to concerns re: additive respiratory depression with induction drugs

If the injectable procedure is retained:

-We should discuss whether the buprenorphine is being used as an analgesic or whether it's also a necessary component of the anesthetic procedure (this determines timing of administration), and potentially including a reversal agent (e.g., atipamezole) for the dexmedetomidine. Giving buprenorphine and dexmedetomidine at the same time increases the risk of respiratory depression. See above

-Review doses of ketamine/dexmedetomidine (impacted by timing of buprenorphine/whether it is part of the anesthetic plan). See above

-There is a slow release formulation of buprenorphine that would be a good option for the surgery. One injection at the time of surgery lasts for ~72 hr. The dose is 0.12 mg/kg SC. We should discuss the best timing for administration. Other option is Fentanyl patch. See below

Analgesia procedure summary

-Might be easiest to have 2 separate analgesia procedures, one for surgery (72 hr, opioid + NSAID) and one for endoscopy (12 hr either: NSAID + opioid, just NSAID or just opioid)

-Move NSAIDs here (take out of anesthesia protocol), include meloxicam in addition to carprofen. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-Include Fentanyl patch as an option for post-op analgesia for catheter surgery (can say that VS would determine proper patch size/dose).

-Include slow release buprenorphine option (0.12 mg/kg SC) as an option in addition to regular buprenorphine for post-op analgesia. Timing to be determined by VS (would be given after recovery if regular buprenorphine given during surgery).

-Recommend removing the NSAID component (carprofen) from the anesthesia procedure since it's not an anesthetic, and is included in the analgesic procedure. The NSAID be in a separate analgesic procedure. This also means it can be removed from terminal anesthetic procedures, since it's not necessary for those. Buprenorphine may or may not be needed for terminal anesthetic events, depending on whether it's being used for anesthesia and analgesia or just analgesia.

-Would be a good idea to include oral meloxicam as an option, or replace carprofen (SC) with meloxicam (SC or oral). Rabbits tend to take the oral meloxicam well and it's less invasive than giving SC injections to post-op rabbits. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-May want to include option of sedation with acepromazine (0.8 mg/kg SC), for things like irrigation, nanoparticle administration? Also discuss rabbit snuggle (soft restraint) option in addition to restraint listed. Sedation may not be necessary but it can be helpful to have the option. Agreed on this, best to make acepromazine as a stand-alone substance procedure since if it is used, it would be used for non-surgical. handling procedure such as irrigation

-Recommend reducing analgesic duration for endoscope procedures (minimum 12 hr with option to re-dose if needed). Agreed on this

-Recommend not specifying muscle for IM injections (epaxial are usually preferred over triceps in rabbits, but does not need to be specified). Agreed on this

Infection and antibiotics:

-We should talk about some of the issues we've been having at UW with NZW rabbits (dysbiosis).

Discussed

-Are all antibiotics contraindicated in this study? We sometimes recommend prophylactic antibiotics to animals with implants that exit the skin, but this is an infection model (*Pseudomonas*), so antibiotic choice would need to be made carefully (if at all). Infections could be directly experimentally-related (e.g., catheter) or not (e.g., dysbiosis). Topical betadine or antibiotic ointment generally OK (might as well include both as options), would like to avoid systemic abx whenever possible since would affect model, though would consider on a case-by-case basis with VS if a rabbit had an infection.

-I think it would be a good idea to create and include a Team Procedure of the type "Withhold medications" to describe which medications are contraindicated and which are not. Agreed to have this procedure included, with an explanation that PI should be contacted prior to admin of any systemic antibiotics, and include discussion that fluoroquinolones such as Baytril are particularly contraindicated due to activity against *Pseudomonas*.

Q #7 of Experiments (monitoring and endpoints):

-Probably don't need to weigh daily unless this data is needed for experiments. OK to say will weigh 3x/wk (and can always increase frequency if there is a concern about a rabbit). Agreed

-Recommend a more narrow temperature range (currently 33-44C, recommend something more like 37-41C which is approx. 99-106F). Agreed

-Discuss possible additional euthanasia criteria such as respiratory distress, signs of infection (such as epiphora or conjunctivitis) not responsive to treatment. Harmonize euthanasia criteria listed here and in the surgery procedure. Agreed. Include some language on level of infection expected in model (nasal discharge) vs. not expected (e.g., extension to orbit/CNS). OK to say that VS would be contacted in the case of infection beyond the sinuses and animal would either be treated or euthanized per VS recommendation and impact on experimental model.

Catheter surgery

-Discuss pros/cons of antibiotic ointment. OK to include it, will also include betadine topical

-Discuss potentially adding local anesthetics. Agreed – will include Standard Procedure for lidocaine/bupivacaine

-Q #2: Please include sterile prep of the area between the ears where the catheter will exit. Edit in Hoverboard

-Q #3: Will the catheter end be covered for protection when not in use? yes

-Q #4: Recommend not including inappetance for 24 hr as a criterion for removal from study. Rabbits sometimes go off feed (e.g., will eat some hay/treats but not pellets), and while this is important clinically (and should be reported to vet services), I'm worried it might lead to a rabbit being prematurely removed from study. Harmonize euthanasia criteria in Q 4ii here and Q #7 of the Experiments. Edit in Hoverboard

-Q #5: Addition of more monitoring, e.g., oxygen saturation, heart rate, BP (in addition to temperature and resp rate/pattern listed) Agreed/edit in Hoverboard

In post-op care, add that an e-collar may be used to reduce risk of rabbit disrupting catheter during the healing phase

Sinus catheter

-Based on the implant procedure, it sounds like a repair would not be done. Let's discuss pros/cons (e.g., what if a relatively minor repair could be done under sedation?) Significant repair (second full surgery) would not be done. Unlikely that damage would be minor/superficial enough to be repaired with sedation, but OK (and recommended) to include option of doing a minor external repair (e.g., suture repair) under sedation following consultation with VS.

Endoscopy

-Discuss whether to add topical epinephrine/phenylephrine in case of significant bleeding. Will be added

-Approx. how long will this procedure last? May need to re-dose anesthesia if injectable. Timing concerns should be covered by updates to anesthesia procedure/inclusion of isoflurane option.

-As mentioned in analgesia section above, probably don't need 72 hr analgesia for this. Probably best to create a separate 12 hr analgesia procedure for endoscopy, as mentioned above.

Miscellaneous

-Please include approx. volume of nanoparticles to be administered to sinus (even though question indicates only for rodents/intracranial, it would be helpful to know for this route). Edit in Hoverboard

From: "Molly K. Lucas" <mklucas@uw.edu>
To: Daniel Eldridge <deldrid@uw.edu>, "Jourdan E. Brune" <jourdi@uw.edu>, Kristin Zabrecky <zabrecky@uw.edu>, Leandra Mosca <lmosca@uw.edu>
CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, achris08 <achris08@uw.edu>, megellis <megellis@uw.edu>
Sent: 7/17/2020 12:05:26 PM
Subject: Assignment for 7/23/20 - Questions due by 8pm 7/22

Hi everyone,

For next week, and the last session with me, there are two items to review in Hoverboard (real time, not pdf's).

First, a new rabbit protocol, PI is Abuzeid (4502-01). Nick, I'm not sure if you are free next Thurs 10-11:30, but I thought you might want to take a look at this protocol as well, either along with us as part of seminar if you want, or another time (fyi it also looks like Zee is working on a new pig protocol, not in vet consult yet, and I thought I should also give you a heads-up about that).

Please write up your vet review questions for the Abuzeid protocol and send them to me as a Word doc (Alex and Meg, you're welcome to just listen in like you did this week or whatever works).

The other item is a Steinmetz amendment (second one) related to the citric acid water that we discussed in one of our earlier sessions. I haven't had a chance to look at it yet, but I thought we could all read it and discuss after we are done with the rabbit protocol. You don't have to write up questions for this amendment.

Let me know if you have any questions and hope you all have a good weekend,
Molly

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Friday, July 17, 2020 12:05 PM
To: Daniel Eldridge; Jourdan E. Brune; Kristin Zabrecky; Leandra Mosca
Cc: Nicholas L. Reyes; achris08; megellis
Subject: Assignment for 7/23/20 - Questions due by 8pm 7/22

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Molly



NOTIFICATION OF BIOLOGICAL USE AUTHORIZATION

Approval Date: 8/20/2020 **BUA #:** 0961-001
Expiration Date: Concurrent with IACUC 3 year renewal **Amendment #:** 1
To: Al Waleed Abuzeid, MD **IACUC #:** 4503-01
Dept: Otolaryngology **From:** Andrea Badger
Box: 356515 *Andrea Badger*
Project Title: Disruption of *Pseudomonas aeruginosa* biofilms in a rabbit sinusitis model using a nitric-oxide releasing nanoparticle

Your submitted request for Biological Use Authorization (BUA) has been reviewed and approved. Based on the information provided, approval is granted for work with the following biological agents, if conducted at the following biosafety levels in the location given as shown in the Biological Use Authorization table.

Provide documented training on the contents of this BUA letter and make it available to staff. Provide documented hazard awareness training to staff which includes the health hazards of each agent, appropriate work practices, personal protective equipment, emergency procedures in case of an exposure or spill, proper decontamination and disposal procedures, and signs and symptoms of exposure.

Inform staff that immunocompromised individuals may be at greater risk of illness should an exposure incident occur. Encourage employees to contact the UW Employee Health Center at 206-685-1026 if they have questions or concerns or if they develop signs or symptoms of exposure consistent with the agents in use.

(New)

Biological Use Authorization Table**Building/Room: Animal Research and Care Facility - Comparative Medicine ARCF ABSL-2 Vivarium**

<u>Agent</u>	<u>Uses</u>	<u>BSL/ABSL</u>	<u>NIH Section</u>
Pseudomonas aeruginosa	Rabbit	2	NA

Building/Room: HSB J-Wing - J280

<u>Agent</u>	<u>Uses</u>	<u>BSL/ABSL</u>	<u>NIH Section</u>
Pseudomonas aeruginosa	In Vitro	2	NA

Building/Room: HSB J-Wing - J281

<u>Agent</u>	<u>Uses</u>	<u>BSL/ABSL</u>	<u>NIH Section</u>
Pseudomonas aeruginosa	In Vitro	2	NA

Additional Requirements / Notes

- A** All aerosol-generating activities with risk group 2 or higher biological agents must be performed in a certified biosafety cabinet or other physical containment device. If this is not possible, contact EH&S Research and Occupational Safety at ehsbio@uw.edu or 206.221.7770 for risk assessment.
- B** You must receive approval from the IACUC prior to work with animals on the above IACUC protocol.
- C** Contact the vivarium supervisor for animal room assignments. Work with biohazardous agents in animals must be performed in the appropriate EH&S approved biohazard rooms. Refer to the tables in this letter for the specific biosafety containment and practices required for your biohazardous agents.
- D** Unless otherwise noted, the NIH section listed above denotes approval for both wild type and recombinant biological agents. NIH section "NA" designates approval for wild type agents only.
- E** Transportation of biohazardous material between facilities must be done in accordance with UW policies. See <https://www.ehs.washington.edu/system/files/resources/uw-biosafety-manual.pdf#page=98>

CC:

<u>Name</u>	<u>Role</u>	<u>E-mail</u>
Andrea Badger	Biosafety Officer	abadger@uw.edu
Robyn Kunsman	IBC Coordinator	rkunsman@uw.edu
UW Office of Animal Welfare		oawrss@uw.edu

Protocol Review: Abuzeid 7/23/20

- Exp 1, Q5, item 1 – Please reword the anesthetic cocktail via IM injection to not include carprofen and buprenorphine. Carprofen and buprenorphine are generally administered subcutaneously. They should be administered as a separate injection (not a 4 drug cocktail).
- Exp 1, Q7 – Is there a reasoning for daily weight taking? Daily weight of the rabbits seems unnecessary and may increase stressful handling, and as worded, will include weekends and holidays. Consider taking weight 2 or 3 times weekly. Appetite will be monitored daily by husbandry staff. Will you also be monitoring for the clinical signs daily, including holiday and weekend? How often will you be checking rectal temperature?

For euthanasia criteria, consider removing item 4: “excessive shaking”

- Procedure: induction of anesthesia: Please reword the anesthetic cocktail via IM injection to not include carprofen and buprenorphine. Carprofen and buprenorphine are generally administered subcutaneously. They should be administered as a separate injection (not a 4 drug cocktail).

Carprofen dose should be changed to 1-2 mg/kg, not 5. Consider removing “triceps” as site of IM injection, as this will limit your options. Additionally, epaxial muscles are generally preferred site in rabbits.

Who on your team will be monitoring and inducing anesthesia? They must be certified to do so before that can happen.

How long will the endoscopy procedure last? You may want to consider adding in the option for redosing the rabbits if it takes longer than 30 minutes.

Please consider adding in reversal agent for dexmedetomidine (atipamezole).

- Procedure: Abuzeid analgesia: Change wording of buprenorphine administration to say “every 6-12 hours for 24 hours, and may continue up 72 hours as needed”. Endoscopy alone or with inoculation may not require buprenorphine for a full 72 hours.

Carprofen dose is too high – typically 1-2 mg/kg every 24 hours. Consider similar duration of administration similar to buprenorphine recommendation – depending on procedure.

Change route of carprofen administration to subcutaneous or oral. Also, consider adding meloxicam as an option – compounded oral formulations are available.

- Exp 1 question 10: Please elaborate on why the experiment will need to be duplicated.
- Exp 2 - Survival surgery – sinus catheter – Q2: Please add in sterile skin prep of the area between the ears where the catheter will exit.

Q6 – consider reducing frequency of antibiotic ointment to once daily to reduce stressful handling.

From: Animal Use Training <auts@uw.edu>
Sent: Monday, September 14, 2020 2:54 PM
To: Aubrey Schoenleben
Cc: Waleed M Abuzeid
Subject: RE: Training Requirements for Visiting Scientist

Hi Aubrey and Dr. Abuzeid,

I checked with the Attending Vet, Dr. Stocking, to get clarifications. Dr. Cho would need to complete AUMS in any case.

If Dr. Cho would be performing some surgical procedures and not necessarily handling the rabbits, he would not need to do the hands-on training nor take the online trainings.

If he will actually do hands-on surgery (and not just observe the PI doing the surgical procedures), then Dr. Stocking will need to know Dr. Cho's training and experience. Most likely, we would exempt him from the surgery training requirement but that is depending on his background and Dr. Stocking's opinion before granting that exemption.

You can email auts@uw.edu with Dr. Cho's background and experience, and I will reach out to Dr. Stocking on your behalf so that AUTS can stay in the loop and take action when required. Thanks!

Sincerely,

Ashley Williams
Administrative Specialist
Office of Animal Welfare Research Support Services

Health Sciences Building Box 357160
1705 NE Pacific Street Seattle, WA 98195-7160
206.685.7363 fax 206.616.1297
auts@uw.edu / oaw.washington.edu

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From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, September 14, 2020 10:40 AM
To: Animal Use Training <auts@uw.edu>
Cc: wabuzeid <wabuzeid@uw.edu>
Subject: Training Requirements for Visiting Scientist

Hi AUTS,

How are you? I am working on a new protocol with Dr. Abuzeid (cc'd) that is developing a rabbit model of sinusitis. To help with model development, Dr. Abuzeid would like to have his colleague, Dr. Cho, from the University of Alabama, to provide pointers and technique suggestions.

If Dr. Cho comes to UW and provides observational (hands-off) advice while Dr. Abuzeid performs the hands-on work, am I correct in remember that Dr. Cho would just need to complete the occupational health screen?

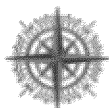
If Dr. Cho comes to UW and provides hands-on assistance with either nasal endoscopy and/or survival surgery to place a sinus catheter, what training would he need to complete? Dr. Cho has been working with rabbits (and this model in particular) for several years and has been involved in hands-on training for rabbit studies in centers in the U.S. and New Zealand. Dr. Cho would be at UW for only a short period of time to help with establishing this model.

Thanks so much,
Aubrey

AUBREY SCHOENLEBEN, PhD, CPIA

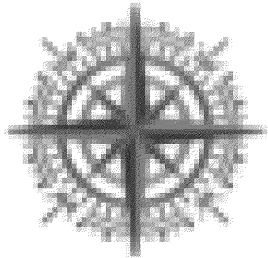
Scientific Liaison & Review Scientist
Office of Animal Welfare

Health Sciences Building, Box 357160
1705 NE Pacific Street, Seattle, WA 98195-7160
vm: 206.685.6923 / fax: 206.616.5664
aubreys@uw.edu / oaw.washington.edu



OFFICE OF ANIMAL WELFARE
Research Support Services





OFFICE OF ANIMAL WELFARE

Research Support Services

From: Nicholas L. Reyes <nreyes@uw.edu>
Sent: Wednesday, August 26, 2020 11:22 AM
To: Geena Gallardo; Molly K. Lucas
Cc: achris08; Leandra Mosca
Subject: Re: Zoom meeting with new PI

I'm not available in the morning but I should be flexible in the afternoon (except for the crisis team meeting at 4pm)

Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nreyes@uw.edu
206-543-0267

From: Geena Gallardo <gallardg@uw.edu>
Sent: Wednesday, August 26, 2020 10:04 AM
To: Molly K. Lucas <mklucas@uw.edu>; Nicholas L. Reyes <nreyes@uw.edu>
Cc: achris08 <achris08@uw.edu>; Leandra Mosca <lmosca@uw.edu>
Subject: RE: Zoom meeting with new PI

Hello everyone,

The doodle poll was a no-go in finding times that fit everyone and the PI. But, Dr. Abuzeid came forward and stated that on Monday 8/31 they could be available between 8-10am and any time after 1:30pm. Let me know if any of these time slots work for you, let's see if we can narrow this down for a meeting.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu

W UNIVERSITY of WASHINGTON

From: Molly K. Lucas [mailto:mklucas@uw.edu]
Sent: Thursday, August 20, 2020 12:02 PM
To: Nicholas L. Reyes <nreyes@uw.edu>; Geena Gallardo <gallardg@uw.edu>
Cc: achris08 <achris08@uw.edu>; Leandra Mosca <lmosca@uw.edu>
Subject: Re: Zoom meeting with new PI

Sounds good to me.

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Thursday, August 20, 2020 11:59 AM
To: Molly K. Lucas <mklucas@uw.edu>; Geena Gallardo <gallardg@uw.edu>
Cc: achris08 <achris08@uw.edu>; Leandra Mosca <lmosca@uw.edu>
Subject: Re: Zoom meeting with new PI

Can we include Leandra in this meeting as well. Sorry for the late addition Molly...after thinking more about the anesthesia component of this project I think she may benefit as well if she's available.
Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nlreyes@uw.edu
206-543-0267

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Thursday, August 20, 2020 11:54 AM
To: Geena Gallardo <gallardg@uw.edu>
Cc: Nicholas L. Reyes <nlreyes@uw.edu>; achris08 <achris08@uw.edu>
Subject: Zoom meeting with new PI

Hi Geena,

I'm hoping you (or Manny?) can help schedule a 1-1.5 hr Zoom meeting with a new PI to discuss his protocol, maybe starting with a Doodle poll?

The PI's name is Al Waleed Abuzeid, and email is wabuzeid@uw.edu. I'm not sure if they will want to include any other lab members in the meeting or not, but if so, that's OK. On our side it will be me, Nick and Alex (cc'ed).

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Sent: Wednesday, August 26, 2020 10:05 AM
To: Molly K. Lucas; Nicholas L. Reyes
Cc: achris08; Leandra Mosca
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Department of Comparative Medicine

Health Sciences Bldg Box 357340
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206-221-3396
gallardg@uw.edu



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To: Nicholas L. Reyes <nreyes@uw.edu>; Geena Gallardo <gallardg@uw.edu>
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From: Geena Gallardo <gallardg@uw.edu>
Sent: Wednesday, August 26, 2020 9:09 AM
To: Molly K. Lucas
Subject: RE: Zoom meeting with new PI

Hi Molly,

This did not drop off my radar, the PI just answered yesterday and no one has the same availability at the same time. There is a couple close days, but then we usually are missing 2 people or Nick.

Making a new doodle poll with dates and time additions to see if we can nail down something.

Best wishes,

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Cc: achris08; Leandra Mosca
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Great! I'll create the doodle poll and send it out to everyone and the PI.

Best wishes,

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Sent: Wednesday, August 26, 2020 11:28 AM
To: Molly K. Lucas
Cc: Nicholas L. Reyes; Geena Gallardo; achris08
Subject: Re: Zoom meeting with new PI

I'm available Monday afternoon before 3:30pm.

Thanks,
Leandra

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To: "Molly K. Lucas" <mklucas@uw.edu>
CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, Geena Gallardo <gallardg@uw.edu>, achris08 <achris08@uw.edu>
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Attach: [EMB4_image001.gif]

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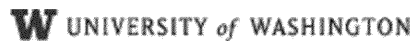
Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340

Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu



From: Molly K. Lucas [mailto:mklucas@uw.edu]
Sent: Thursday, August 20, 2020 12:02 PM
To: Nicholas L. Reyes <nlreyes@uw.edu>; Geena Gallardo <gallardg@uw.edu>
Cc: achris08 <achris08@uw.edu>; Leandra Mosca <lmosca@uw.edu>
Subject: Re: Zoom meeting with new PI

Sounds good to me.

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Thursday, August 20, 2020 11:59 AM
To: Molly K. Lucas <mklucas@uw.edu>; Geena Gallardo <gallardg@uw.edu>
Cc: achris08 <achris08@uw.edu>; Leandra Mosca <lmosca@uw.edu>
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Can we include Leandra in this meeting as well. Sorry for the late addition Molly...after thinking more about the anesthesia component of this project I think she may benefit as well if she's available.
Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nlreyes@uw.edu
206-543-0267

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CC: achris08 <achris08@uw.edu>, Leandra Mosca <lmosca@uw.edu>
Sent: 8/26/2020 10:04:38 AM
Subject: RE: Zoom meeting with new PI

Attach: [EMB4_image001.gif]

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W UNIVERSITY of WASHINGTON

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CC: achris08 <achris08@uw.edu>, Leandra Mosca <lmosca@uw.edu>
Sent: 8/20/2020 12:02:55 PM
Subject: RE: Zoom meeting with new PI

Attach: [EMB4_image001.gif]

Great! I'll create the doodle poll and send it out to everyone and the PI.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
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CC: achris08 <achris08@uw.edu>, Leandra Mosca <lmosca@uw.edu>
Sent: 8/20/2020 12:01:31 PM
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CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, achris08 <achris08@uw.edu>
Sent: 8/20/2020 11:54:16 AM
Subject: Zoom meeting with new PI

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From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nltreyes@uw.edu>
Sent: 8/19/2020 4:24:16 PM
Subject: Fw: Abuzeid rabbit sinusitis

Attach: [Abuzeid_8_19_20.docx]

Great, I will work on the meeting! Here's my current draft of topics. Let me know if anything looks weird. I've had a lot of distractions today - in addition to the usual interruptions (family) we're having a bathroom repaired (leaky shower replaced). Fun fun!

Some of them are really not strong suggestions on my part - like whether to add local anesthetics for the surgery - so I'm open to any opinions/improvements/additions.

Molly

From: Nicholas L. Reyes <nltreyes@uw.edu>
Sent: Wednesday, August 19, 2020 4:17 PM
To: Molly K. Lucas <mklucas@uw.edu>
Subject: Re: Abuzeid rabbit sinusitis

Hey Molly,
I like the idea of you me and Alex. We should be able to have a good discussion. Sounds like a plan.
Thanks,
Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nltreyes@uw.edu
206-543-0267

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From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, August 19, 2020 1:58:46 PM
To: Nicholas L. Reyes <nltreyes@uw.edu>
Subject: Abuzeid rabbit sinusitis

Hi Nick,

I'm finally getting back to this. Rather than working on the normal questions for Hoverboard, I'm working on a Word doc of "discussion topics" for the group that we can discuss at the meeting, and then they can work in HB after that.

I'm thinking I'll ask Geena to help us set up a Zoom meeting with the PI and anyone else from the group (though may just be the PI), and then you, me, and either the anesthesia resident or the resident on

ARCF (Alex). Sound OK? You/me/Alex plus the group? Anyone else?

Once I'm done with this doc I can share it with everyone and you may have things to add.

Molly



Topics for discussion – Abuzeid new protocol

Anesthesia and analgesia:

-It sounds like vet services will run anesthesia. Are you open to considering additional anesthetic options (e.g., isoflurane)? Often when VS is hired to run anesthesia for large animals, we include a wide variety of anesthetic options, since the vet staff is comfortable with many options. Inhalational is less likely to lead to respiratory depression. Intubation would need to be done (rather than mask) in order for there to be access to the nasal cavity. This provides the advantage of more control over the airway and options to intervene if animals aren't breathing well on their own.

If the injectable procedure is retained:

-We should discuss whether the buprenorphine is being used as an analgesic or whether it's also a necessary component of the anesthetic procedure (this determines timing of administration), and potentially including a reversal agent (e.g., atipamezole) for the dexmedetomidine. Giving buprenorphine and dexmedetomidine at the same time increases the risk of respiratory depression.

-Review doses of ketamine/dexmedetomidine (impacted by timing of buprenorphine/whether it is part of the anesthetic plan).

-There is a slow release formulation of buprenorphine that would be a good option for the surgery. One injection at the time of surgery lasts for ~72 hr. The dose is 0.12 mg/kg SC. We should discuss the best timing for administration.

-Recommend removing the NSAID component (carprofen) from the anesthesia procedure since it's not an anesthetic, and is included in the analgesic procedure. The NSAID be in a separate analgesic procedure. This also means it can be removed from terminal anesthetic procedures, since it's not necessary for those. Buprenorphine may or may not be needed for terminal anesthetic events, depending on whether it's being used for anesthesia and analgesia or just analgesia.

-Would be a good idea to include oral meloxicam as an option, or replace carprofen (SC) with meloxicam (SC or oral). Rabbits tend to take the oral meloxicam well and it's less invasive than giving SC injections to post-op rabbits. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-May want to include option of sedation with acepromazine (0.8 mg/kg SC), for things like irrigation, nanoparticle administration? Also discuss rabbit snuggle (soft restraint) option in addition to restraint listed. Sedation may not be necessary but it can be helpful to have the option.

-Recommend reducing analgesic duration for endoscope procedures (minimum 12 hr with option to re-dose if needed).

-Recommend not specifying muscle for IM injections (epaxial are usually preferred over triceps in rabbits, but does not need to be specified).

Infection and antibiotics:

- We should talk about some of the issues we've been having at UW with NZW rabbits (dysbiosis).
- Are all antibiotics contraindicated in this study? We sometimes recommend prophylactic antibiotics to animals with implants that exit the skin, but this is an infection model (*Pseudomonas*), so antibiotic choice would need to be made carefully (if at all). Infections could be directly experimentally-related (e.g., catheter) or not (e.g., dysbiosis).
- I think it would be a good idea to create and include a Team Procedure of the type "Withhold medications" to describe which medications are contraindicated and which are not.

Q #7 of Experiments (monitoring and endpoints):

- Probably don't need to weigh daily unless this data is needed for experiments. OK to say will weigh 3x/wk (and can always increase frequency if there is a concern about a rabbit).
- Recommend a more narrow temperature range (currently 33-44C, recommend something more like 37-41C which is approx. 99-106F).
- Discuss possible additional euthanasia criteria such as respiratory distress, signs of infection (such as epiphora or conjunctivitis) not responsive to treatment. Harmonize euthanasia criteria listed here and in the surgery procedure.

Catheter surgery

- Discuss pros/cons of antibiotic ointment.
- Discuss potentially adding local anesthetics.
- Q #2: Please include sterile prep of the area between the ears where the catheter will exit.
- Q #3: Will the catheter end be covered for protection when not in use?
- Q #4: Recommend not including inappetance for 24 hr as a criterion for removal from study. Rabbits sometimes go off feed (e.g., will eat some hay/treats but not pellets), and while this is important clinically (and should be reported to vet services), I'm worried it might lead to a rabbit being prematurely removed from study. Harmonize euthanasia criteria in Q 4ii here and Q #7 of the Experiments.
- Q #5: Addition of more monitoring, e.g., oxygen saturation, heart rate, BP? (in addition to temperature and resp rate/pattern listed)

Sinus catheter

- Based on the implant procedure, it sounds like a repair would not be done. Let's discuss pros/cons (e.g., what if a relatively minor repair could be done under sedation?)

Endoscopy

- Discuss whether to add topical epinephrine/phenylephrine in case of significant bleeding.
- Approx. how long will this procedure last? May need to re-dose anesthesia if injectable.

-As mentioned in analgesia section above, probably don't need 72 hr analgesia for this.

Miscellaneous

-Please include approx. volume of nanoparticles to be administered to sinus (even though question indicates only for rodents/intracranial, it would be helpful to know for this route).

From: Geena Gallardo <gallardg@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/20/2020 1:36:48 PM
Subject: FW: Doodle: "Protocol Discussion with PI Abuzeid" Update

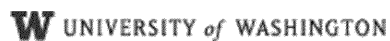
Attach: [EMB4_image001.gif]

Okay – over achiever. Lol!

Geena Lappin

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gallardg@uw.edu



From: Doodle [mailto:mailer@doodle.com]
Sent: Thursday, August 20, 2020 1:36 PM
To: Geena Gallardo <gallardg@uw.edu>
Subject: Doodle: "Protocol Discussion with PI Abuzeid" Update

Nick just participated.

Doodle

Hi Geena Lappin,

Nick just participated in the Doodle poll **Protocol Discussion with PI Abuzeid**.

| [Go to your poll](#) | [Close poll and send calendar invitation](#)

Best wishes,
The Doodle Team

See how easy it is to find a time to get people together when you use Doodle.























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











Doodle AG, Werdstrasse 21, 8021 Zürich









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Activity	Author	▼ Activity Date
 Congruence Letter Attached	Schoenleben, Aubrey	9/28/2020 11:29 AM
 Congruence Letter Attached	Schoenleben, Aubrey	9/28/2020 11:25 AM
 eGC1 A160477 (Abuzeid) - Approval.pdf		
 Letter Sent	Schoenleben, Aubrey	9/28/2020 11:10 AM
 Correspondence_for_PROTO202000106.doc		
 Letter Prepared	Schoenleben, Aubrey	9/28/2020 11:10 AM
 Correspondence_for_PROTO202000106.doc		
 Approval Period Edited	Schoenleben, Aubrey	9/28/2020 11:09 AM
 Designated Member Review Submitted	Haskell, Scott Raymond	9/25/2020 2:14 PM
No further questions, comments or concerns. SRR Haskell		
 Designated Reviewers Assigned	Huang, Stephanie W	9/25/2020 10:18 AM
 Assigned to Designated Review	Huang, Stephanie W	9/25/2020 10:17 AM
 Agenda Item Removed	Huang, Stephanie W	9/25/2020 10:16 AM
 Private Comment Added	Haskell, Scott Raymond	9/18/2020 11:49 AM
PROTO202000106 4503-01: Rabbit Sinusitis		
No further questions, comments or concerns. SRR Haskell		
 Meeting Assigned	Schoenleben, Aubrey	9/17/2020 11:35 AM
 Pre-Review Submitted	Schoenleben, Aubrey	9/17/2020 11:35 AM
 Vet Consult Submitted	Lucas, Molly K	9/17/2020 10:17 AM
Do you accept the submission? yes no further Qs/comments		
 Vet Consult Sent	Schoenleben, Aubrey	9/16/2020 8:08 AM
Vet review of revision		
 Response Submitted	Abuzeid, Al Waleed	9/14/2020 12:59 PM
Thanks, Aubrey, and team. I've made the requested changes and added clarification to the "Reviewer" questions.		
 Clarification by Pre-Reviewer Requested	Schoenleben, Aubrey	9/12/2020 9:34 AM
Hi Waleed, Nice work on the revision - we're getting close! A few last clarifying questions from the vets and me. Please see the new Reviewer Notes (6 total) and comment/edit as needed. Any questions, let me know. Thanks!		
 Vet Consult Submitted	Lucas, Molly K	9/11/2020 4:14 PM
Do you accept the submission? no I just made a few minor edits. I'd like to review responses to your comments/questions so I'll look at it one more time.		
 Private Comment Added	Schoenleben, Aubrey	9/10/2020 10:23 AM
Hi ML, How are you? I added a few clarifying questions, but overall the protocol is shaping up nicely. Let me know if you have additional follow up as well. Thanks!		
 Vet Consult Sent	Schoenleben, Aubrey	9/10/2020 10:23 AM
Vet review of revision		

Review or Revision	Response Submitted	Abuzeid, Al Waleed	9/7/2020 9:47 PM
Reviewer notes have been addressed in a point-by-point fashion. Furthermore, the comprehensive edits recommended by veterinary services have been integrated in this submission.			
	Ancillary Review Submitted	Cashman, Judy L	8/31/2020 12:52 PM
	Ancillary Reviews Managed	Cashman, Judy L	8/31/2020 12:52 PM
	Tags Managed	Cashman, Judy L	8/31/2020 12:52 PM
	OHRs attached	Cashman, Judy L	8/31/2020 12:52 PM
	Comment Added	Badger, Andrea	8/21/2020 3:09 PM
Hi, I've attached the BUA -- the housing on the IACUC protocol should reflect ABSL-2 rather than ABSL-1 for the Pseudomonas work. Thanks, Andrea			

Activity	Author	▼ Activity Date
 Ancillary Review Submitted	Badger, Andrea	8/21/2020 3:08 PM
 Tags Managed	Badger, Andrea	8/21/2020 3:07 PM
 BUA attached	Badger, Andrea	8/21/2020 3:06 PM
 Clarification by Pre-Reviewer Requested	Schoenleben, Aubrey	8/20/2020 12:02 PM
Hi Waleed, How are you? The vets and I looked over the revision and have some more follow up questions. Please see the new Reviewer Notes from me (5 total), as well as the comment below from the vets. Please let me know if you have questions or if I can help with the revision. Thanks!		
 Vet Consult Submitted	Lucas, Molly K	8/20/2020 11:44 AM
Do you accept the submission? no will re-review after edited, which will be after our meeting		
 Comment Added	Lucas, Molly K	8/20/2020 11:44 AM
Hello Dr. Abuzeid, I think it would be most efficient to have a meeting for the vet consult portion, since this is a new rabbit model. Attached are some notes for discussion at our meeting, and then after the meeting, the protocol can be modified per our agreements at the meeting (I think that will save some back-and-forth in Hoverboard). We will be in touch soon (email) regarding scheduling a time to have a Zoom meeting about these topics, in addition to any questions you may have for us. -Molly Lucas, DVM, Dept of Comparative Medicine read less ▲  vet review topics for discussion		
 Private Comment Added	Badger, Andrea	8/15/2020 10:27 PM
The Pseudomonas is going to require ABSL-2 (currently ABSL-1 housing is listed on the protocol).		
 Private Comment Added	Badger, Andrea	8/15/2020 10:25 PM
Hi, I will be the biosafety officer for this one. The lab still needs to be inspected. Please contact me with any questions, thanks, Andrea		
 Vet Consult Sent	Schoenleben, Aubrey	8/5/2020 2:04 PM
 Tags Managed	Williams, Ashley E	8/5/2020 9:28 AM
 Comment Added	Williams, Ashley E	8/5/2020 9:28 AM
Matthew Parsek has been removed from the protocol. Thanks!		
 Comment Added	Abuzeid, Al Waleed	8/4/2020 12:18 PM
Ashley, may we simply remove Dr. Parsek from the protocol as he will no longer be involved in administrative support etc? Thanks.		
 Comment Added	Williams, Ashley E	8/4/2020 11:43 AM
I am unable to approve Matthew Parsek on your protocol due to incomplete training. Please have Matthew complete all required training. If you believe this is an error, please send us verification of completed training.		
As administrative support and not handling the animals, the following needs to be completed: • UW Animal Use Laws & Regulations. Copy and paste the following link (https://depts.washington.edu/auts/courses/courses_online.html) to take the course.		
For additional information please visit the Animal Use Training website at: http://depts.washington.edu/auts/requirements.html read less ▲		
 Assigned Portfolio ID	Jimenez, Selesteen	7/31/2020 8:00 AM
 Comment Added	Jimenez, Selesteen	7/31/2020 7:56 AM
Administrative note: protocol number was updated due to duplicate number. Thanks Selesteen		
 Response Submitted	Abuzeid, Al Waleed	7/27/2020 11:07 PM
Requested edits have been made throughout the IACUC protocol. Additionally, the research plan document has been updated to reflect these changes.		
 Comment Added	Abuzeid, Al Waleed	7/27/2020 11:04 PM
Thanks, Aubrey, for all the input. I've made the requested changes and re-submitted for pre-review. The research plan document has also been updated to reflect the changes in the revised IACUC protocol.		

	Clarification by Pre-Reviewer Requested	Schoenleben, Aubrey	7/20/2020 4:14 PM
Hi Waleed, Nice work getting the protocol together! I've completed the initial pre-review, and there are a few questions to address before we send to the vets for review. Please see the Reviewer Notes (12 total) and edit the protocol/procedures as needed.			
	Ancillary Reviews Managed	Kunsman, Robyn	7/13/2020 8:22 AM
	Tags Managed	Kunsman, Robyn	7/13/2020 8:22 AM
	Comment Added	Williams, Ashley E	7/10/2020 2:49 PM
Dr. Abuzeid,			
Please note: Your rabbit hands-on training has been listed as "deferred". Therefore, you cannot touch any live rabbits until you complete the hands-on rabbit lab, of which you have submitted your registration form for, and have been placed on the waitlist. After completion, you will be approved to work with rabbits on your project per your listed duties.			
	Tags Managed	Williams, Ashley E	7/10/2020 2:46 PM
PI completed online training. Rabbit hands-on is deferred.			
	Coordinator Assigned	Schoenleben, Aubrey	7/7/2020 10:20 AM
Assigned to Aubrey Schoenleben			
	Coordinator Assigned	Jimenez, Selestee	7/7/2020 8:16 AM
Assigned to OAW Purple Team			
	Assigned Portfolio ID	Jimenez, Selestee	7/7/2020 8:15 AM

	Activity	Author	▼ Activity Date
	Tags Managed	Jimenez, Selesteen	7/7/2020 8:15 AM
Hold for PI aums, l&r, rabbit			
	Action Required for Training #4502-01.pdf		
	Submitted	Abuzeid, Al Waleed	7/6/2020 10:18 PM
I am in the process of scheduling and taking the institutionally required investigator training courses which will be completed ASAP.			
	Protocol Created	Abuzeid, Al Waleed	7/5/2020 11:10 AM

53 items

◀ page 3 of 3 ▶

25 / page

From: wabuzeid <wabuzeid@uw.edu>
Sent: Sunday, July 26, 2020 5:45 PM
To: VET SERVICES PROTOCOL REVIEW
Cc: Aubrey Schoenleben
Subject: Induction of anesthesia recommendations (PROTO202000106; 4502-01 rabbit sinusitis)

Hello to the veterinary team!

I am in the midst of editing a protocol for a series of rabbit experiments. This involves the induction of anesthesia and adequate intraop analgesia in the animals. Two procedures will be performed:

- 1) Placement of a tunneled indwelling surgical catheter into the maxillary sinus of the rabbit (duration 20-40 minutes per rabbit).
- 2) Brief nasal endoscopy for intranasal endoscopic grading and for endoscopic-guided culture swabs (duration 5-10 minutes per rabbit).

The proposed anesthesia/analgesia procedure is based on a protocol used safely and successfully by my colleagues which were approved by their respective IACUC (University of Alabama and University of Pennsylvania).

I am seeking your advice on reasonable dose ranges to submit to IACUC for review. The rabbits undergoing anesthesia would be female New Zealand white rabbits between 4-6 months of age (body weight 2-4 kg). Currently, the protocol consists of four medications administered intramuscularly as a cocktail:

- 1) Buprenorphine HCl (0.02 mg/kg IM)
- 2) Carprofen (5 mg/kg IM)
- 3) Dexmedetomidine (0.05 mg/kg IM)
- 4) Ketamine (20 mg/kg IM)

Thank you for your input!

Best,

Waleed M. Abuzeid, MD

Associate Professor

Rhinology and Endoscopic Skull Base Surgery

Department of Otolaryngology: Head and Neck Surgery

University of Washington

Email: wabuzeid@uw.edu

General comments:

- 1) Minor comment-under goals and significance, Q2 expense should not be used as a justification for selection of an animal model. Please remove this language and replace it explaining that rabbits are appropriate and the least sentient animal for the work proposed.
- 2) Under question 3 please briefly describe the monitoring of rabbit sinusitis symptoms and the establishment of humane endpoints for your model if significant morbidity is expected. If spontaneous mortality is expected, please address this here.
- 3) Approximately 20% of the animal numbers you requested are for training purposes. It may be best to organize and account for these animals in a separate experiment as their experience with regards to experimental manipulations are extensive and beyond the scope of Q3 which is typically used to described terminal non-survival surgeries. The 4 “practice” animals requested for experiment 1 and the proposed use for them read more like a pilot experiment than use for specific training of personnel.

Procedures:

- 1) Nanoparticle Administration
 - a. As you are intending to instill the particles into the sinus, please provide a volume that you intend to instill.
- 2) Induction of Anesthesia
 - a. Do you intend to perform the anesthesia in these rabbits yourself or hire vet services for these procedures?
 - b. Have you consulted with vet services staff regarding the administration of these drugs?
 - c. Please remove specific identification of the muscle that will be used for injection from the description.
 - d. Intramuscular injection is not absolutely required for buprenorphine and carprofen administration in this protocol. I recommend switching these to subcutaneous administration to reduce the volumes administered to the muscle. Addition of saline to increase the volume of these drugs is also contraindicated.
 - e. Emergency intubation is very difficult to perform in rabbits. Please describe that it will be attempted and that flow by oxygen will be available.
 - f. Please complete question 4 for all anesthetic agents being used by listing their known side effects and how monitoring of the rabbit will be performed to identify abnormalities early so they can be remedied. It is not appropriate to say “see question 4” and have question 4 read “NA” when there are well established side effects of anesthetic drugs listed.
- 3) Placement of Sinus Irrigating Catheter
 - a. Rabbits that are intermittently inappetant may not need to be removed from study. Often rabbits may avoid eating their pellet ration but still eat hay or preferred enrichment foods or nutritional support supplements. I would recommend not listing inappetence > 24 hours as a humane endpoint.

- b. Please describe the aseptic prep of the surgical site to include three alternating scrubs with isopropyl alcohol and povidine iodine solution.
 - c. Please complete the duration and expected deficits for this procedure. Rabbits are obligate nasal breathers, please comment if you expect any deficits based on your catheter's intended placement into the maxillary sinus.
 - d. Please describe how often rabbits will be monitored post-op.
- 4) Nasal Endoscopy
 - a. Please complete procedure preparation.
- 5) Sinus Catheter
 - a. Please describe if the catheter will be intermittently flushed to maintain patency under Q3.
 - b. Will sterile catheters be purchased and used a single time?
 - c. Please comment on your plan should catheters become inadvertently removed from the sinus while rabbits are on study. Do you anticipate performing a repair procedure if this were to happen? Would the rabbit be removed from study?

From: "Molly K. Lucas" <mklucas@uw.edu>
To: wabuzeid <wabuzeid@uw.edu>
CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, Aubrey Schoenleben <aubreys@uw.edu>
Sent: 8/31/2020 3:56:20 PM
Subject: Notes from today's Zoom vet consult (Abuzeid protocol)

Attach: [Abuzeid_8_31_20.docx]

Hi Dr. Abuzeid,

It was very nice to meet you today and I think it was a productive discussion about your protocol.

I've attached a new copy of the Word document we referred to today. The yellow highlights are my notes from today. If anything looks incorrect or you have additional thoughts or change your mind about something, just let me know. I've included Aubrey so she knows what changes to expect in Hoverboard, and she also may be able to assist you with some of them.

The protocol will come back to me for re-review once it has been revised.

Sincerely,
Molly Lucas



Topics for discussion – Abuzeid new protocol

Housing

-Looks like BSL-2 is required. Include single housing of the female rabbits post-op (Q #12 Experiments, a husbandry exception)

Anesthesia and analgesia:

-It sounds like vet services will run anesthesia.

Yes. one of the vet techs in vet services can provide more information re: cost estimates for hiring VS for anesthesia; possibly some post-op care (optional)

Are you open to considering additional anesthetic options (e.g., isoflurane)? Often when VS is hired to run anesthesia for large animals, we include a wide variety of anesthetic options, since the vet staff is comfortable with many options. Inhalational is less likely to lead to respiratory depression. Intubation would need to be done (rather than mask) in order for there to be access to the nasal cavity. This provides the advantage of more control over the airway and options to intervene if animals aren't breathing well on their own.

-Edit to include xylazine in combination with ketamine (K 40-50 mg/kg IM, X 3-5 mg/kg IM)

-OK to leave ketamine/dexmedetomidine option

-Include atipamezole as an option in case reversal of xylazine/dexmed is indicated (0.25-1 mg/kg SC, IM, IV)

-Include isoflurane/intubation/ventilation

-Can leave buprenorphine here in anesthesia procedure, but indicate it would not be given until intubated, due to concerns re: additive respiratory depression with induction drugs

If the injectable procedure is retained:

-We should discuss whether the buprenorphine is being used as an analgesic or whether it's also a necessary component of the anesthetic procedure (this determines timing of administration), and potentially including a reversal agent (e.g., atipamezole) for the dexmedetomidine. Giving buprenorphine and dexmedetomidine at the same time increases the risk of respiratory depression. See above

-Review doses of ketamine/dexmedetomidine (impacted by timing of buprenorphine/whether it is part of the anesthetic plan). See above

-There is a slow release formulation of buprenorphine that would be a good option for the surgery. One injection at the time of surgery lasts for ~72 hr. The dose is 0.12 mg/kg SC. We should discuss the best timing for administration. Other option is Fentanyl patch. See below

Analgesia procedure summary

-Might be easiest to have 2 separate analgesia procedures, one for surgery (72 hr, opioid + NSAID) and one for endoscopy (12 hr either: NSAID + opioid, just NSAID or just opioid)

-Move NSAIDs here (take out of anesthesia protocol), include meloxicam in addition to carprofen. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-Include Fentanyl patch as an option for post-op analgesia for catheter surgery (can say that VS would determine proper patch size/dose).

-Include slow release buprenorphine option (0.12 mg/kg SC) as an option in addition to regular buprenorphine for post-op analgesia. Timing to be determined by VS (would be given after recovery if regular buprenorphine given during surgery).

-Recommend removing the NSAID component (carprofen) from the anesthesia procedure since it's not an anesthetic, and is included in the analgesic procedure. The NSAID be in a separate analgesic procedure. This also means it can be removed from terminal anesthetic procedures, since it's not necessary for those. Buprenorphine may or may not be needed for terminal anesthetic events, depending on whether it's being used for anesthesia and analgesia or just analgesia.

-Would be a good idea to include oral meloxicam as an option, or replace carprofen (SC) with meloxicam (SC or oral). Rabbits tend to take the oral meloxicam well and it's less invasive than giving SC injections to post-op rabbits. Meloxicam doses for rabbits are 0.1-0.2 mg/kg PO q 24 hr and 0.2 mg/kg SC q 24 hr.

-May want to include option of sedation with acepromazine (0.8 mg/kg SC), for things like irrigation, nanoparticle administration? Also discuss rabbit snuggle (soft restraint) option in addition to restraint listed. Sedation may not be necessary but it can be helpful to have the option. Agreed on this, best to make acepromazine as a stand-alone substance procedure since if it is used, it would be used for non-surgical. handling procedure such as irrigation

-Recommend reducing analgesic duration for endoscope procedures (minimum 12 hr with option to re-dose if needed). Agreed on this

-Recommend not specifying muscle for IM injections (epaxial are usually preferred over triceps in rabbits, but does not need to be specified). Agreed on this

Infection and antibiotics:

-We should talk about some of the issues we've been having at UW with NZW rabbits (dysbiosis).

Discussed

-Are all antibiotics contraindicated in this study? We sometimes recommend prophylactic antibiotics to animals with implants that exit the skin, but this is an infection model (*Pseudomonas*), so antibiotic choice would need to be made carefully (if at all). Infections could be directly experimentally-related (e.g., catheter) or not (e.g., dysbiosis). Topical betadine or antibiotic ointment generally OK (might as well include both as options), would like to avoid systemic abx whenever possible since would affect model, though would consider on a case-by-case basis with VS if a rabbit had an infection.

-I think it would be a good idea to create and include a Team Procedure of the type "Withhold medications" to describe which medications are contraindicated and which are not. Agreed to have this procedure included, with an explanation that PI should be contacted prior to admin of any systemic antibiotics, and include discussion that fluoroquinolones such as Baytril are particularly contraindicated due to activity against *Pseudomonas*.

Q #7 of Experiments (monitoring and endpoints):

-Probably don't need to weigh daily unless this data is needed for experiments. OK to say will weigh 3x/wk (and can always increase frequency if there is a concern about a rabbit). Agreed

-Recommend a more narrow temperature range (currently 33-44C, recommend something more like 37-41C which is approx. 99-106F). Agreed

-Discuss possible additional euthanasia criteria such as respiratory distress, signs of infection (such as epiphora or conjunctivitis) not responsive to treatment. Harmonize euthanasia criteria listed here and in the surgery procedure. Agreed. Include some language on level of infection expected in model (nasal discharge) vs. not expected (e.g., extension to orbit/CNS). OK to say that VS would be contacted in the case of infection beyond the sinuses and animal would either be treated or euthanized per VS recommendation and impact on experimental model.

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-Discuss potentially adding local anesthetics. Agreed – will include Standard Procedure for lidocaine/bupivacaine

-Q #2: Please include sterile prep of the area between the ears where the catheter will exit. Edit in Hoverboard

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In post-op care, add that an e-collar may be used to reduce risk of rabbit disrupting catheter during the healing phase

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-Based on the implant procedure, it sounds like a repair would not be done. Let's discuss pros/cons (e.g., what if a relatively minor repair could be done under sedation?) Significant repair (second full surgery) would not be done. Unlikely that damage would be minor/superficial enough to be repaired with sedation, but OK (and recommended) to include option of doing a minor external repair (e.g., suture repair) under sedation following consultation with VS.

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-Discuss whether to add topical epinephrine/phenylephrine in case of significant bleeding. Will be added

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-As mentioned in analgesia section above, probably don't need 72 hr analgesia for this. Probably best to create a separate 12 hr analgesia procedure for endoscopy, as mentioned above.

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From: Molly K. Lucas <mklucas@uw.edu>
Sent: Monday, August 31, 2020 3:56 PM
To: Waleed M Abuzeid
Cc: Nicholas L. Reyes; Aubrey Schoenleben
Subject: Notes from today's Zoom vet consult (Abuzeid protocol)
Attachments: Abuzeid_8_31_20.docx

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Basic Information

1. * Select research team:

Abuzeid

2. * Title of protocol:

Rabbit Model of Sinusitis

3. * Short title:

4503-01: Rabbit Sinusitis

4. * Summary of research:

This projects aims to establish a rabbit-based model of acute and chronic bacterial sinusitis. The immunologic features of rabbit and human sinonasal epithelium are similar. The rabbit model provides an opportunity to disrupt, manipulate and study host-microbiome interplay in respiratory epithelium and could be of future benefit to researchers across UW and beyond.

Aim 1) Establish sinusitis in a rabbit model to allow longitudinal sampling of respiratory epithelium, assessment of mucociliary and ion transport function, and the evaluation of microbiome stability in the face of infection or therapeutic interventions.

Aim 2) Evaluate the efficacy and safety of various novel therapeutics in the eradication of biofilms associated with sinusitis.

5. * Principal investigator:

Al Waleed Abuzeid

6. * What is the intention of the animal protocol?

Experimental Research

Experimental Research Protocol Addition

1. * Will the protocol include breeding?

☐ Yes ☒ No

Protocol Team Members

1. Identify each additional person involved in the design, conduct, or reporting of the research:

Name	Role	Involved in Animal Handling	Authorized To Order Animals	E-mail	Phone
Vet Services	Other	yes	no	vsreview@uw.edu	(206) 583- 1853
Pradeep Singh	Co- Investigator	no	no	singhpr@uw.edu	+1 206 221- 7151

2. If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:

N/A

Funding Sources

1. Identify each organization supplying funding for the protocol:

Funding Organization		eGC1 Number(s)
View	Cystic Fibrosis Foundation	A160477

Scientific Aims

1. * Scientific aims of the research:

Specific Aim 1: Validate a preclinical rabbit model of *Pseudomonas aeruginosa* (PA) sinusitis using cystic fibrosis (CF)-associated strains. We will establish an existing rabbit model of frontal sinusitis to investigate the effect of CF-related PA strains on the sinonasal epithelial inflammation and physiology.

Specific Aim 2: Evaluation of nitric oxide (NO)-releasing nanoparticle (NO-NP) efficacy in a preclinical rabbit model of PA sinusitis. We will evaluate the efficacy of our NO-releasing platform in eradicating respiratory epithelial biofilms *in vivo*.

2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nose and sinuses that affects over 40 million people in the United States alone. This condition significantly impacts the quality-of-life of sufferers and, to date, there is no cure. Though CRS can affect anyone, those with cystic fibrosis (CF)--a genetic disorder that affects 1 in 3000 newborns resulting in a shortened life expectancy--are particularly susceptible. In fact, nearly all CF patients also have CRS.

As our understanding of CRS evolves, we are beginning to understand that bacterial infection with communities of bacteria called "biofilms" may play a role in the development of CRS. In the case of CF-associated CRS, biofilms containing the bacteria *Pseudomonas aeruginosa* (PA) play a major role. These biofilms are resistant to all known antibiotics and can travel to the lungs where they induce severe lung infections which are the primary cause of death in people with CF.

Though we have been able to test novel therapies against biofilms in the lab and have shown that they can disrupt biofilms to a much greater degree than standard antibiotics, the true test of effectiveness will be how these therapies work in an animal model. Specifically, we are using nanoparticles to deliver nitric oxide (NO), a molecule that is normally generated in small amounts by our innate immune system and that demonstrates anti-biofilm effects.

There are very few animal models that allow repeat sampling of the lining, or epithelium, of the sinuses. This is critical to allow for the measurement of biofilm growth, the effects of this growth on the natural bacteria or microbiome of the sinuses, and the effect of the biofilms on the function of the sinus epithelium. Rabbits are an ideal model for sinus-based experiments because they are an appropriate size to allow for safe surgical procedures that would be technically extremely challenging in a smaller animal like a mouse and would be far too expensive in larger animals. Rabbit sinuses, anatomically and from an immune function standpoint, are more like human sinuses than those of rodents.

In this protocol, we would plan on 1) Establishing sinusitis in the rabbit using PA strains associated with CF and evaluating the effect of the resulting biofilms on inflammation of the respiratory epithelium. This will increase our understanding of how biofilms produce disease in the sinuses. Then, we would 2) evaluate the ability of the NO-releasing nanoparticle in eliminating the biofilms and, potentially, curing the sinusitis by instilling this treatment into the diseased sinus of the rabbit.

Ultimately, we hope that the establishment of the rabbit model will have uses beyond our current goals as a means of studying infection and inflammation of the sinuses for researchers across UW and beyond. We aim to develop our nanoparticle therapeutics as a means of treating sinus disease and non-sinus infections in patients with and without CF -- a particularly critical goal in this era of increasing bacterial resistance to conventional antibiotics.

3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

Animals in this study will experience symptoms of sinusitis including nasal congestion, nasal drainage and facial pressure. Furthermore, rabbits may experience pain related to the surgical procedures -- namely placement of the indwelling irrigating sinus catheter which will likely cause discomfort for a period of 24-72 hours. This procedure will require intraoperative and postoperative analgesia as described. There should not be more than minimal transient discomfort related to the nasal endoscopy procedures performed at 3 time points (day 0, day 7 and day 21).

The results of this study will increase our understanding of biofilm-associated inflammation in the sinuses and will validate the use of a nitric oxide releasing nanoparticle therapy which has the potential to treat sinusitis and other respiratory illnesses, particularly in highly susceptible populations such as those with cystic fibrosis.

Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

1. * Define the experiments to be used in this protocol:

Name	Species	USDA Count	Pain Category	Count by Procedures	Husbandry Exception Types
00. Training & Procedure Optimization	Rabbits yes - New Zealand White	8	B: 0 C: 0 D: 8 E: 0	<ul style="list-style-type: none"> ■ Euthanasia: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution (Standard) ■ Implants: Abuzeid: Nasal Sponge (Team) ■ Implants: Abuzeid: Sinus Catheter (Team) ■ Other: Abuzeid: Nasal Endoscopy (Team) ■ Substance Administration: Abuzeid: Catheter Saline Flush and Nanoparticle Administration (Team) ■ Substance Administration: Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours) (Team) ■ Substance Administration: Abuzeid: Induction of Anesthesia (Team) ■ Substance Administration: Abuzeid: Inoculation of Sinus (Team) ■ Substance Administration: Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery) (Team) ■ Substance Administration: Abuzeid: Sedation for Non-Surgical Procedures (Team) ■ Substance Administration: Abuzeid: 	Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.

Name	Species	USDA Count	Count by Pain Category	Procedures	Husbandry Exception Types
				Phenylephrine Administration (Team) <ul style="list-style-type: none"> Substance Administration: Analgesia, Local, Bupivacaine and Lidocaine (Standard) Survival Surgery: Abuzaid: Placement of Sinus Irrigating Catheter (Team) Withholding Medications/Procedures: Abuzaid: Withhold Medications (Team) 	
01. Validate a preclinical rabbit model of sinusitis	Rabbits yes - New Zealand White	16	B: 0 C: 0 D: 16 E: 0	<ul style="list-style-type: none"> Euthanasia: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution (Standard) Implants: Abuzaid: Nasal Sponge (Team) Other: Abuzaid: Nasal Endoscopy (Team) Substance Administration: Abuzaid: Analgesia, Post-Nasal Endoscopy (not for survival surgery) (Team) Substance Administration: Abuzaid: Phenylephrine Administration (Team) Substance Administration: Abuzaid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours) (Team) Substance Administration: Abuzaid: Induction of Anesthesia (Team) Substance Administration: Abuzaid: Inoculation of Sinus (Team) Withholding Medications/Procedures: Abuzaid: Withhold Medications (Team) 	Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.
02. Evaluation of nitric oxide (NO)-releasing	Rabbits yes - New	12	B: 0 C: 0	<ul style="list-style-type: none"> Euthanasia: Anesthetic Overdose, Pentobarbital or 	Rabbits - Standard social

nanoparticle (NO- NP) efficacy in a preclinical rabbit model of sinusitis.	Zealand White Species	USDA Count	Count by Pain Category	Procedures	contact husbandry policy, is not acceptable for part or all of the study.
			D: 12 E: 0	Pentobarbital Solution (Standard) <ul style="list-style-type: none"> ■ Implants: Abuzeid: Sinus Catheter (Team) ■ Implants: Abuzeid: Nasal Sponge (Team) ■ Other: Abuzeid: Nasal Endoscopy (Team) ■ Substance Administration: Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery) (Team) ■ Substance Administration: Abuzeid: Sedation for Non- Surgical Procedures (Team) ■ Substance Administration: Abuzeid: Catheter Saline Flush and Nanoparticle Administration (Team) ■ Substance Administration: Abuzeid: Phenylephrine Administration (Team) ■ Substance Administration: Analgesia, Local, Bupivacaine and Lidocaine (Standard) ■ Substance Administration: Abuzeid: Analgesia, Post- Operative after Survival Surgery or Establishment of Sinusitis (72 hours) (Team) ■ Substance Administration: Abuzeid: Induction of Anesthesia (Team) ■ Substance Administration: Abuzeid: Inoculation of Sinus (Team) ■ Survival Surgery: Abuzeid: Placement of Sinus Irrigating Catheter (Team) ■ Withholding Medications/Procedures: Abuzeid: Withhold Medications (Team) 	

**2. Will any single animal undergo more than one survival surgery?
(include any animal that underwent surgery prior to use on this
protocol) ☐ Yes ☒ No**

Procedure Personnel Assignment

1. * Select the team members who will be performing each procedure:

Procedure	Species	Is USDA Species	Team Members
Euthanasia: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution, ver. 1 (Standard)	Rabbits - New Zealand White	yes	Vet Services
Implants: Abuzeid: Nasal Sponge, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Implants: Abuzeid: Sinus Catheter, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Other: Abuzeid: Nasal Endoscopy, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Substance Administration: Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery), ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid Vet Services
Substance Administration: Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours), ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid Vet Services
Substance Administration: Abuzeid: Catheter Saline Flush and Nanoparticle Administration, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Substance Administration: Abuzeid: Induction of Anesthesia , ver. 1 (Team)	Rabbits - New Zealand White	yes	Vet Services
Substance Administration: Abuzeid: Inoculation of Sinus, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Substance Administration: Abuzeid: Phenylephrine Administration, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid

Procedure	Species	Is USDA Species	Team Members
Substance Administration: Abuzeid: Sedation for Non-Surgical Procedures, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid Vet Services
Substance Administration: Analgesia, Local, Bupivacaine and Lidocaine, ver. 2 (Standard)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Survival Surgery: Abuzeid: Placement of Sinus Irrigating Catheter, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid
Withholding Medications/Procedures: Abuzeid: Withhold Medications, ver. 1 (Team)	Rabbits - New Zealand White	yes	AI Waleed Abuzeid Vet Services

2. Team member training:

First Name Last Name Training

AI Waleed	Abuzeid	Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
		Animal Facility Online Learning Course with ABSL-2	Orientation	Online	Basic Course	Stage 1	8/8/2020		
		Rabbit Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	9/1/2020		
		ARC Facility Orientation, ABSL-2 Users	Orientation	In Person	Basic Course	Stage 1	9/9/2020		
		Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	7/8/2020	7/8/2025	
		Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	9/9/2020		
		Animal Use Medical Screening	General	Online	Basic Course	Stage 1	7/9/2020	7/31/2023	

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
ARC Facility Orientation, Non-Rodent Users	Orientation	In Person	Basic Course	Stage 1	9/9/2020	
Foege Facility Orientation, ABSL-2 Users	Orientation	In Person	Basic Course	Stage 1	9/9/2020	
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	7/29/2020	
Annual DCM Facility Access Training (Non-Rodent)	General	Online	Basic Course	Stage 1	8/8/2020	8/31/2021

Vet Services No training data to display

No experience data to display

Pradeep Singh

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date	No experience data to display
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	1/14/2019	1/31/2022	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	12/23/2015	12/23/2020	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	12/13/2011		
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	1/12/2012		

Animal Details

1. * How are animals acquired?

Purchased

2. Describe the acquisition for:

a. Not purchasing through DCM or WaNPRC:

N/A

3. Identification of individual animals (other than cage cards):

a. Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.)

(Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):

Marked with a permanent marker for identification.

b. Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:

N/A

c. Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:

N/A

4. Identify strain/stock for rodents and genetically modified animals:

Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description
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There are no items to display

Animal Number Adjustments

"Animals Identified in Experiments" is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding "Adjusted Animal Count" column. ****Only input numeric values in this field; 0 is acceptable.****

If no adjustment is required, the values in the "Animals Identified in Experiments" and "Adjusted Animal Count" columns must match. Click Update in each Pain Category row to input the matching value.

For questions about adjusting animal numbers, contact OAW.

1. * Click Update to adjust the number of animals to be used or produced for this protocol:

	Species	USDA Covered Species	Pain Category	Animals Identified in Experiments	Adjusted Animal Count
View	Rabbits - New Zealand White	yes	Pain Category B	0	0
View	Rabbits - New Zealand White	yes	Pain Category C	0	0
View	Rabbits - New Zealand White	yes	Pain Category D	36	36
View	Rabbits - New Zealand White	yes	Pain Category E	0	0

2. If you adjusted the number of animals for this protocol, explain why:

N/A

3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

Yes - see Experiment 00 for details.

4. Supporting documents:

Document Name Date Modified

There are no items to display

Alternatives and Duplication Searches

Display Procedures that cause pain or distress:

- Substance Administration: Abuzeid: Inoculation of Sinus, ver. 1 (Team)
- Survival Surgery: Abuzeid: Placement of Sinus Irrigating Catheter, ver. 1 (Team)

1. Record all searches for any previous research that this protocol might duplicate:

	Search Date	Searched Databases	Other
View	7/6/2020	EMBASE (searches multiple databases) PubMed/Medline	N/A

2. Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:

We used the results of our search to refine the proposed experiments. Firstly, the reviewed literature justified the use of rabbits in these experiments as a much closer correlate of human sinus physiology than rodents. Secondly, we REFINED the methods for establishing sinusitis in the rabbit based on published techniques that used a sterile synthetic sponge to obstruct the sinus and then directly inoculating the sinus with bacteria endoscopically -- this represents a significantly less invasive and less painful method of inducing sinusitis than other, more invasive techniques described in the literature.

Our power calculation for animal number is based on the expected degree of change in bacterial counts between the control and infected sinuses as noted in the published literature. In this way, we believe we have REDUCED the number of animals to the minimum possible to allow for detection of a statistically significant result.

Lastly, we believe that we cannot REPLACE the use of rabbits in this study because the literature has yet to describe the characteristics of sinus disease induced by different strains of cystic fibrosis related bacteria (*Pseudomonas*) as we propose to in our study. Indeed, studies evaluating *Pseudomonas* sinusitis in the rabbit use either non-cystic fibrosis strains or only a single strain associated with cystic fibrosis compromising the ability to compare the sinusitis caused by different strains. Furthermore, there are no published studies evaluating any form of nitric oxide releasing therapeutic (nanoparticle based or otherwise) in the rabbit sinus model. Thus, the results of this study may prove to be highly novel and represent a significant contribution to the literature.

Additionally, an expert in rabbit studies in sinusitis that involve survival surgeries was consulted (Dr. Do-Yeon Cho, MD, MS Associate Professor, Dept. of Otolaryngology, University of Alabama).

3. Confirm that you have made every effort to ensure that this protocol is not unnecessary duplication of previous research: ☒

Housing and Use

Housing and use outside of the vivarium is not allowed without strong scientific justification.

1. Identify each location where animals will be housed:

	Facility	Species	Justification for Housing Outside Vivarium
View	ARCF ABSL1	Rabbits - New Zealand White	N/A
View	ARCF ABSL2	Rabbits - New Zealand White	N/A

2. Identify each location where animals will be used:

	Facility Use	Species	Justification for Use Outside Vivarium
View	ARCF Procedures: Anesthesia Analgesia (postoperative - 72 hrs), Analgesia (nasal endoscopy - 24h) Inoculation of sinuses with bacteria, Nasal endoscopy, Survival surgery - placement of sinus catheter, Restraint - irrigation of sinus catheter, Euthanasia ABSL2	Rabbits - New Zealand White	N/A
View	ARCF Practice/training surgeries ABSL1	Rabbits - New Zealand White	N/A

Disposition

1. Disposition plans for the animals when this research is complete:

(check all that apply)

Euthanasia

2. If other, provide an animal disposition description:

N/A

3. If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):

Maxillary sinus tissue will be fixed after euthanasia using formalin.

Refinement, Replacement and Reduction

1. Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:

*** Refinement (use of methods to decrease animals' sensitivity to pain)**

The anesthetic protocol was adapted from a published protocol by a well-established NIH-funded investigator (Do-Yeon Cho, MD, MS, Associate Professor, Dept. of Otolaryngology, University of Alabama) who was directly consulted in the design of this protocol. The protocol was further refined in direct consultation with UW veterinary services. The post-operative analgesic protocol is a combination of published protocols by Dr. Cho, the use of approved UW protocols and direct consultation with veterinary services. The animals will also be anesthetized for nasal endoscopic procedures to minimize discomfort associated with nasal endoscopy in the awake animal.

We considered intranasal administration of the nanoparticle rather than invasive catheter placement in the rabbits but, based on mammalian sinus anatomy, mucociliary clearance and the composition of the nanoparticles themselves, aerosolization or vaporization of the particles for intranasal use is not possible. Furthermore, the duration of action of the particles is reliably sustained for only 24-48 hours requiring a means of instilling the particles into the rabbit sinus regularly without resorting to overly frequent anesthesia hence the one-time placement of the indwelling sinus catheter.

*** Replacement (include in vitro tests, use of less sentient animals)**

We have performed several published studies evaluating the efficacy of our nitric oxide releasing nanoparticles in vitro. These have demonstrated a potent anti-biofilm effect. However, the next step on the road to eventual human clinical trials is evaluating the efficacy and safety of these therapeutic agents in a biologic model. As described in our protocol, rabbits have sinuses that are physiologically similar to humans and, because of their size and relative docility, allow for surgical procedures on the sinuses that are both technically feasible and safe.

*** Reduction (use of fewer animals to attain statistical significance)**

A power analysis was performed to justify the number of animals used and calculated based on the expected magnitude of change in bacterial counts between the infected and uninfected sinuses. We believe that the number of animals selected is the minimum that would allow an adequate number of treatment arms and detection of treatment effect.

2. Describe the rationale for using animals and the appropriateness of the species proposed:

Rabbits harbor considerable advantages over murine models. They are easier to handle due to their docile nature and allow for surgical procedures that would be technically challenging in rodents and resource-intensive in larger animals. Anatomically and immunologically, rabbit sinuses are more like human sinuses than those of rodents. The establishment of sinusitis in rabbits is well described. This is in contrast to the cystic fibrosis (CF) murine model where establishing sinus infection is problematic.

Though the proposed rabbit model does not harbor mutations in the CFTR gene, it does allow for establishing chronic infections in the sinuses and the experience gained will be critical for harnessing such models when they are developed in the future. Preliminary

CF rabbit models are in the process of being developed and demonstrate physiologic characteristics that, in many ways, mimics human CF.

In summary, the rabbit model provides an opportunity to disrupt, manipulate, and study host-microbiome interplay in respiratory epithelium, and leaves UW researchers well poised to exploit the inherent advantages of future CF rabbit models.

Supporting Documents

1. Attach supporting files:

Document Name	Date Modified
 Research Protocol	7/27/2020 11:06 PM

Procedures Appendix:



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Catheter Saline Flush and Nanoparticle Administration

1. * Name of the procedure or surgery:

Abuzeid: Catheter Saline Flush and Nanoparticle Administration

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View Nanoparticles	Standard	Other	N/A	10 mg/mL	Less than or equal to 5 mL	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Blank inactive nanoparticles or nitric oxide releasing active nanoparticles (suspended in saline) will be administered to rabbits via an indwelling sinus catheter. Just prior to nanoparticle administration, the catheter will be flushed with saline (5 mL) and fluid which emerges from the snout will be collected for longitudinal bacterial counts.

A rabbit restrainer (e.g., <https://conductscience.com/lab/stainless-steel-rabbit-restrainer/>) will be used to safely and humanely secure the rabbit in position for the saline flush and instillation of the nanoparticle therapeutic. Rabbits will be restrained for approximately 3-5 minutes.

Rabbits may be sedated during nanoparticle administration. See related substance administration procedure for details.

3. Describe the intended effects of administering the substance(s):

Treatment of sinusitis

4. Describe any potential adverse reactions to administering the substance(s):

None anticipated

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Nanoparticles

2. Route:

Other

If you indicated Other, specify the route:

Sinus via catheter

3. Dose:

N/A

4. Frequency and duration of dosages:

See experiment for timing and frequency

5. Volume (for rodents or intracranial injections):

Less than or equal to 5 mL

6. Concentration:

10 mg/mL

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Not available as a pharmaceutical grade agent. Solution will be prepared in a sterile hood and sterile filtered prior to administration.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Sedation for Non-Surgical Procedures

1. * Name of the procedure or surgery:

Abuzeid: Sedation for Non-Surgical Procedures

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Acepromazine maleate (Acetylpromazine, ACE, PromAce, Aceproject)	Standard	Subcutaneous	0.8 mg/kg	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Acepromazine will be used, if necessary, for non-surgical handling procedures. Specifically, this includes catheter irrigation and administration of nanoparticles.

3. Describe the intended effects of administering the substance(s):

Short-term sedation for efficient catheter irrigation/nanoparticle administration.

4. Describe any potential adverse reactions to administering the substance(s):

Eye trauma. To mitigate the risk of eye trauma, the eyes will be lubricated with ophthalmic ointment (e.g., Lacrilube) after induction of sedation to prevent corneal drying/trauma.

Hypothermia. If the rabbit becomes cool to the touch, demonstrates shivering or other indicators of hypothermia, then additional warming measures will be implemented including a circulating water blanket and/or air heating device.

Airway distress. Rabbits that demonstrate airway distress (dyspnea or cyanosis) will be endotracheally intubated and ventilated via bag-valve mask ventilation to supplement oxygenation.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA)

paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Acepromazine maleate (Acetylpromazine, ACE, PromAce, Aceproject)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.8 mg/kg

4. Frequency and duration of dosages:

Once at time of catheter irrigation

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery)

1. * Name of the procedure or surgery:

Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery)

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Fentanyl (Sublimaze)	Standard	Transdermal	TBD by veterinary services	N/A	N/A	N/A
View	Meloxicam (Metacam)	Standard	Subcutaneous	0.2 mg/kg	N/A	N/A	N/A
View	Meloxicam (Metacam)	Standard	Oral - Other	0.1-0.2 mg/kg	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The anticipated level of pain after nasal endoscopy is minimal.

Pain that does result will be controlled using NSAIDs. Either orally or subcutaneously administered meloxicam will be used. In the highly unlikely event that further analgesia is needed after nasal endoscopy, fentanyl patches will be administered.

If analgesia is deemed necessary, analgesia will be provided for a minimum of 24 hours following endoscopy. Animals may be re-dosed if needed.

3. Describe the intended effects of administering the substance(s):

Provide analgesia for 24 hours.

4. Describe any potential adverse reactions to administering the substance(s):

Overdose can result in sedation, bleeding, gastrointestinal stasis, inappetence, and respiratory depression.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Needles must not be recapped unless a recapping device is used.

Gloves must be worn when handling these agents.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Fentanyl (Sublimaze)

2. Route:

Transdermal

If you indicated Other, specify the route:

N/A

3. Dose:

TBD by veterinary services

4. Frequency and duration of dosages:

TBD by veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Meloxicam (Metacam)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.2 mg/kg

4. Frequency and duration of dosages:

q24h

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Meloxicam (Metacam)

2. Route:

Oral - Other

If you indicated Other, specify the route:
typically dosed via syringe

3. Dose:

0.1-0.2 mg/kg

4. Frequency and duration of dosages:

q24h

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Nasal Sponge

1. * Name of the procedure or surgery:

Abuzeid: Nasal Sponge

2. * Select procedure type:

Implants

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Implants

1. Type, including approximate size and weight:

Merocel nasal sponge (Medtronic, Jacksonville, FL). Size 2.5-5.5 cm. Weight: 2-3 g.

2. Site:

Sinus cavity (maxillary)

3. Maintenance and care of chronic implants:

Merocel nasal sponges do not require maintenance or care. These are specifically designed for placement in the nasal cavity.

4. Method used to sterilize implants:

Merocel sponges are single use and are contained in sealed, certified sterile packaging. This packaging will not be opened until the time of implant placement in the nasal cavity of the rabbit.

5. Describe implant procedure (if the implant is surgical, create a new Survival Surgery procedure and refer to it here):

Merocel sponge will be implanted with the aid of nasal endoscopy in the middle meatus so as to obstruct the maxillary sinus ostium. See related procedure for details.

6. Monitoring protocol:

See related endoscopy procedure and experiment.

7. Will it be necessary to surgically remove implants, or to re-implant or repair implants if they fail? If so, describe the circumstances and the maximum number of replacements if applicable:

Rabbits will be euthanized with the sponge in situ. There will be no removal of the implant prior to euthanasia.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Inoculation of Sinus

1. * Name of the procedure or surgery:

Abuzeid: Inoculation of Sinus

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

Rabbits may develop signs and symptoms of maxillary sinusitis. This includes serous to purulent nasal discharge, sneezing, crusting around the nostril, matted fur on medial metacarpi from rubbing of nose with the feet.

ii. Identify criteria under which animals will be removed from research:

Signs of deteriorating health leading to removal from research include: dyspnea, cyanosis, fevers, lack of normal grooming, avoidance behavior, bloody nasal discharge, conjunctivitis, cervical lymphadenopathy, rough coats, and circling or tilting of the head (torticollis).

Criteria for early euthanasia are:

1. >20% weight loss from time of bacterial inoculation
2. Body temperature below 37°C or above 41°C (normal body temperature is 38.3 – 39.4°C)
3. Inability to mobilize independently

4. Excessive shaking
5. Inability to drink or eat
6. Respiratory distress
7. Worsening of sinusitis beyond the expected symptoms of nasal discharge and congestion to symptoms associated with spread beyond the sinus into critical nearby structures such as the orbit and/or intracranial space (eye proptosis, restricted eye movement, eye closure, periorbital swelling, lethargy, confusion). In these circumstances, veterinary services will be contacted and the animal will either be treated or euthanized per veterinary recommendations.

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Pseudomonas aeruginosa	Standard	Intranasal	0.5 mL	Adjusted to an optical density of 0.6 at 600 nm in sterile saline (4.0 x 10 ⁸ colony forming units)	0.5 mL	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Nasal endoscopy will be used to insert a synthetic sponge in to the maxillary sinus. The sponge will then be inoculated with 0.5 mL of P aeruginosa. One of four strains will be used for inoculation. Specifically: Wild-type (PAO1) strain, PA14 strain, FRD1 strain or PAO1 delta-wspF strain. The concentration of inoculate is adjusted to an optical density of 0.6 at 600 nm in sterile saline (4.0 x 10⁸ colony forming units). See "Abuzeid: Nasal Endoscopy" for additional details.

3. Describe the intended effects of administering the substance(s):

Establishment of a unilateral (right sided) maxillary sinusitis.

4. Describe any potential adverse reactions to administering the substance(s):

Progression beyond a standard maxillary sinusitis to the orbit causing an orbital infection/cellulitis. Progression beyond a standard maxillary sinusitis to a systemic illness with associated fevers, loss of appetite, and malaise. Progression beyond a standard maxillary sinusitis to cause respiratory distress.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
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There are no items to display

1. * Substance:

Pseudomonas aeruginosa

2. Route:

Intranasal

If you indicated Other, specify the route:

N/A

3. Dose:

0.5 mL

4. Frequency and duration of dosages:

Once

5. Volume (for rodents or intracranial injections):

0.5 mL

6. Concentration:

Adjusted to an optical density of 0.6 at 600 nm in sterile saline (4.0×10^8 colony forming units)

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Only established reference strains of Pseudomonas aeruginosa will be used. These are appropriate for in vivo laboratory animal use.

8. Complication remediation:

See question 4.

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution

1. * Name of the procedure or surgery:

Anesthetic Overdose, Pentobarbital or Pentobarbital Solution

2. * Select procedure type:

Euthanasia

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Euthanasia

1. * Method of euthanasia:

Anesthetic Overdose

2. Describe procedure:

Rabbits will be injected IV with pentobarbital (Nembutal) or a pentobarbital solution at a dose of at least 87 mg/kg.

Examples of pentobarbital solutions include Beuthanasia, Euthasol and similar solutions containing a mixture of pentobarbital and phenytoin. Dosing is based on the pentobarbital component of the solution.

3. * Will anesthesia be used? Yes No

4. Describe how death will be confirmed:

Death will be confirmed by lack of respirations and heartbeat.

5. Is this method approved by the AVMA Guidelines on Euthanasia (2013)?

Yes No

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Induction of Anesthesia

1. * Name of the procedure or surgery:

Abuzeid: Induction of Anesthesia

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Atipamezole HCl (Antisedan)	Standard	Intramuscular	0.25 - 1.0 mg/kg	N/A	N/A	N/A
View	Atipamezole HCl (Antisedan)	Standard	Subcutaneous	0.25 - 1.0 mg/kg	N/A	N/A	N/A
View	Atipamezole HCl (Antisedan)	Standard	Intravenous - Various	0.25 - 1.0 mg/kg	N/A	N/A	N/A
View	Buprenorphine HCl (Buprenex, Simbadol)	Standard	Intramuscular	0.02 - 0.03 mg/kg	0.3 mg/mL	N/A	N/A
View	Dexmedetomidine hydrochloride (Dexdomitor)	Standard	Intramuscular	0.05 - 0.3 mg/kg	0.5 mg/mL	N/A	N/A
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A
View	Ketamine (Ketaset, Ketaflo, Vetalar)	Standard	Intramuscular	30 - 50 mg/kg	100 mg/mL	N/A	N/A
View	Xylazine	Standard	Intramuscular	3-5 mg/kg	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Anesthesia induction and maintenance will be managed by veterinary services. The choice of anesthetic drug(s) and route (intramuscular vs inhalational) will be decided in consultation with veterinary services. For irrigating catheter implantation, we will favor inhalational anesthesia with formal intubation.

Anesthetic sedation and induction will take place in a warm room. The rabbit will be placed on a blanket placed atop a heating pad.

Anesthetic induction will be performed using the combination of xylazine and ketamine or the combination of ketamine and dexmedetomidine. Depth of anesthesia will be maintained either through re-dosing of the above agents (xylazine, ketamine and/or dexmedetomidine) or through use of an inhalational agent (isoflurane).

Buprenorphine will not be administered unless the rabbit is intubated due to concern for respiratory depression when used in combination with the induction agents.

If necessary, atipamezole will be used to reverse xylazine and/or dexmedetomidine if indicated.

3. Describe the intended effects of administering the substance(s):

Induction of anesthesia to an adequate depth to allow for brief surgical procedures.
Anesthetic depth will be evaluated by toe pinch.

4. Describe any potential adverse reactions to administering the substance(s):

Eye trauma. To mitigate the risk of eye trauma, the eyes will be lubricated with ophthalmic ointment (e.g., Lacrilube) after induction of anesthesia to prevent corneal drying/trauma.

Hypothermia. If the rabbit becomes cool to the touch, demonstrates shivering or other indicators of hypothermia, then additional warming measures will be implemented including a circulating water blanket and/or air heating device.

Airway distress. Rabbits that demonstrate airway distress (dyspnea or cyanosis) will be endotracheally intubated (if not already intubated) and ventilated via bag-valve mask ventilation to supplement oxygenation.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
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There are no items to display

1. * Substance:

Atipamezole HCl (Antisedan)

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

0.25 - 1.0 mg/kg

4. Frequency and duration of dosages:

Once for reversal of xylazine or dexmedetomidine and then as needed per veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

Pharmaceutical grade agents will be used.

9. Substance order for the procedure:

N/A

1. * Substance:

Atipamezole HCl (Antisedan)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.25 - 1.0 mg/kg

4. Frequency and duration of dosages:

Once for reversal of xylazine or dexmedetomidine and then as needed per veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Atipamezole HCl (Antisedan)

2. Route:

Intravenous - Various

If you indicated Other, specify the route:

N/A

3. Dose:

0.25 - 1.0 mg/kg

4. Frequency and duration of dosages:

Once for reversal of xylazine or dexmedetomidine and then as needed per veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Buprenorphine HCl (Buprenex, Simbadol)

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

0.02 - 0.03 mg/kg

4. Frequency and duration of dosages:

Once at induction

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

0.3 mg/mL

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Dexmedetomidine hydrochloride (Dexdomitor)

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

0.05 - 0.3 mg/kg

4. Frequency and duration of dosages:

Once at induction; see experiment for timing and frequency of anesthetic events.

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

0.5 mg/mL

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Isoflurane

2. Route:

Inhalation

If you indicated Other, specify the route:

N/A

3. Dose:

1-5%

4. Frequency and duration of dosages:

See experiment for timing and frequency of anesthetic events.

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Ketamine (Ketaset, Ketaflo, Vetalar)

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

30 - 50 mg/kg

4. Frequency and duration of dosages:

Once at induction; see experiment for timing and frequency of anesthetic events.

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

100 mg/mL

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A

1. * Substance:

Xylazine

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

3-5 mg/kg

4. Frequency and duration of dosages:

Once at induction; see experiment for timing and frequency of anesthetic events.

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

See question 4 for monitoring information after administration of anesthesia.

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Placement of Sinus Irrigating Catheter

1. * Name of the procedure or surgery:

Abuzeid: Placement of Sinus Irrigating Catheter

2. * Select procedure type:

Survival Surgery

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

Post-operative pain, transient loss of appetite for <24 hours.

ii. Identify criteria under which animals will be removed from research:

Rabbits that develop dyspnea, cyanosis, wound infection, persistent bloody nasal discharge, limited mobility, and cervical lymphadenopathy will be removed from the research study.

Criteria for early euthanasia are:

1. >20% weight loss from time of bacterial inoculation
2. Body temperature below 37°C or above 41°C (normal body temperature is 38.3 – 39.4°C)
3. Inability to mobilize independently
4. Excessive shaking
5. Inability to drink or eat
6. Respiratory distress
7. Worsening of sinusitis beyond the expected symptoms of nasal discharge and congestion to symptoms associated with spread beyond the sinus into critical nearby structures such as the orbit and/or intracranial space (eye proptosis, restricted eye movement, eye closure, periorbital swelling, lethargy, confusion). In these circumstances, veterinary services will be contacted and the animal will either be treated or euthanized per veterinary recommendations.

Survival Surgery

1. * Surgery Type:

Major

2. * Describe how the animal, surgeon, and instruments will be prepared for surgery:

All surgical instruments will be sterilized in an autoclave. Anesthesia is induced as described. The hair overlying the dorsum of the nose and between the ears will be clipped and the areas prepped with three alternating scrubs of povidine iodine solution and 70% ethanol. The surgical site will then be draped for surgery using sterile technique.

The surgeon will perform a surgical scrub and wear a sterile gown, surgical cap, mask and sterile gloves before starting the surgery.

3. * Describe the surgical procedure, including any deficits expected as a result of the surgery:

Animals will be prepped as described above. The skin overlying the dorsum of the nose is then incised with a scalpel blade in a vertical line drawn down the midline after injection of this area with subcutaneous local anesthetic (lidocaine or bupivacaine). A laterally based flap of periosteum is elevated from the midline to expose the underlying bone of the maxilla.

The right maxillary sinus is entered by creating a small, approximately 4 x 4 mm dorsal hole using a trocar. Sterile single lumen tubing (approximately 0.32 cm diameter x 30 cm length) is passed into the sinus thru the antrostomy. The other end of the tubing is then tunneled under the skin of the nasal dorsum and brought out through an incision at the vertex of the cranium between the ears. Absorbable sutures (typically 4-0 size and Vicryl material) are used to drape the periosteum over the catheter. The hub of the tubing is capped and secured to the skin with a purse string non-absorbable suture (typically nylon material). The periosteum, subcutaneous tissue, and skin incision are closed with buried absorbable suture (typically 4-0 size and Vicryl material).

Of note, the catheter tubing will be capped when not in use during the post-procedure period.

Duration: 20-40 minutes per rabbit.

Expected deficits: Potential for limited ear movement secondary to tissue tugging and associated discomfort at the catheter exit site at the vertex of the cranium for a period of 24-72 hours.

4. * Select associated substance administration procedures, including anesthesia and analgesia procedures to be used:

Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)	Substance Administration	1 Team
Abuzeid: Induction of Anesthesia	Substance Administration	1 Team

5. Describe how animals will be monitored during the procedure:

Rabbits will be monitored to avoid excessive depression of cardiorespiratory function and to evaluate for inadequate anesthesia.

Anesthetic depth: toe pinch

Respiratory rate and pattern: observation of 20-30 breaths per minute under anesthesia

Heart rate and blood pressure

Mucous membrane color: observation of intraoral mucosa for pallor or cyanosis (normal is pink coloration)

Temperature: rectal thermometer (normal 38-40C). Temperature will be maintained using heating pad. Hypothermia (<37C) will be addressed with circulating water blanket or air heating devices.

Oxygen saturation in real-time

6. Describe the routine for postoperative care: (including removal of sutures, if applicable)

During the immediate post-anesthetic period, the animal will not be returned to its cage until it is fully awake and ambulatory. Rabbits will be encouraged to eat as soon as possible after the operation to avoid ileus.

Rabbit respiration will be monitored for a return to the normal range of 30-60 breaths/minute.

Temperature will be maintained >37C using heating pad in a warm room.

For the first 72 hours following catheter placement, topical betadine or antibiotic ointment (e.g., bacitracin) will be applied daily to the catheter exit site at the skin. Thereafter, Vaseline will be applied to the catheter site daily throughout the duration of the experiment. An Elizabethan collar may be used to reduce the risk of the rabbit disrupting the catheter during the healing phase.

We would like to avoid the use of systemic antibiotics whenever possible as this could impact the sinus infection model by inadvertently treating the established sinusitis. However, systemic antibiotics will be considered on a case-by-case basis with veterinary services if a rabbit develops a clinical infection.

7. Describe how postoperative pain and distress will be assessed:

(including need for further care)

Postoperative pain will be evaluated by monitoring for: restlessness, decreased appetite, dullness, inactivity, increased aggression, immobility, hunched posture, tooth grinding, salivation, persistent scratching/licking of surgical site, social isolation, vocalization or elevated respiratory rate. Rabbits will be monitored a minimum of twice in the first 24 hours after the surgery and then daily for a further 72 hours. Post-operative analgesia will be provided as described in the related substance administration procedure. Failure of this regimen to control pain will prompt veterinary consultation.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Nasal Endoscopy

1. * Name of the procedure or surgery:

Abuzeid: Nasal Endoscopy

2. * Select procedure type:

Other

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Other

1. Description of Procedure:

Procedure Preparation:

As this is a clean contaminated natural orifice procedure, akin to nasal endoscopy in humans, the rabbit will not need to be prepped using sterile technique prior to nasal endoscopy. The surgeon will don sterile gloves, a mask, a surgical cap and a sterile gown for nasal endoscopy procedures. The rigid nasal endoscope is a re-usable instrument and will be sterilized in a steam autoclave before the planned nasal endoscopy procedures.

Procedure description:

To induce sinusitis, animals will be anesthetized (see related substance administration procedure for details). Topical phenylephrine will be applied intranasally to facilitate mucosal decongestion and optimize the endoscopic view (see related substance administration procedure for details). Nasal endoscopy will then be performed using a 1.7 - 2.7 mm 0 or 30 degree endoscope bilaterally to exclude pre-existing infection. Under endoscopic vision, a synthetic sponge is inserted into the right middle meatus to occlude the maxillary sinus. Then, under endoscopic vision, a spinal needle (typically 22G) is used to directly inoculate the right maxillary sinus with *P aeruginosa* (see related substance administration procedure for details). Topical phenylephrine will be used to control any bleeding related to intranasal mucosal trauma which is of low likelihood and self-limited when it does occur. This procedure will take approximately 5-10 minutes per animal.

Animals will also undergo nasal endoscopy to collect culture swabs and evaluate the extent of edema and related discharge in the sinus cavity (generally performed as separate anesthetic events; see experiment for timing). In these instances, animals will be anesthetized and nasal endoscopy will be performed bilaterally as described above. Culture swabs will be used to sample both the occluded and non-occluded sinus cavity. Both sinus cavities may also be visually examined and graded under endoscopic vision for edema (0 = no edema, 1 = mild to moderate edema, 2 = polypoid degeneration), and type of discharge (0 = absent, 1 = hyaline, 2 = thick/purulent). Nasal endoscopy with endoscopic grading and culture will take approximately 5-10 minutes per animal.

Analgesic and Post-Procedure Monitoring Plan:

Multi-modal analgesia will be administered for 72 hours on occasions where nasal endoscopy is performed in concert with a survival surgery (e.g., after sinus irrigating catheter placement, see procedure for details) and after establishment of sinusitis. In these cases, animals will be monitored 1-3 times/day from infection to the time of euthanasia. Animals will be monitored for signs of respiratory distress including dyspnea and cyanosis, bloody nasal discharge, and body temperature, as well as general signs of deteriorating health.

Routine nasal endoscopy performed for endoscopic grading and sinus culture will typically not require post-procedural analgesia as the procedure would be expected to induce zero to minimal discomfort. If necessary, analgesia will be administered for a period of 24 hours after routine nasal endoscopy (see related substance administration procedure for details).

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Sinus Catheter

1. * Name of the procedure or surgery:

Abuzeid: Sinus Catheter

2. * Select procedure type:

Implants

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Implants

1. Type, including approximate size and weight:

Single lumen tubing (approximately 0.32 cm diameter x 30 cm length; weight 10 grams)

2. Site:

Sinus cavity (maxillary)

3. Maintenance and care of chronic implants:

The exposed portion of the catheter will emerge from beneath the skin between the ears of the rabbit. This portion will be cleaned before and after every use (treatment irrigation and saline flushes every 48 hours) with isopropyl alcohol wipes (minimum 70% concentration). For the first 72 hours following catheter placement, topical betadine or antibiotic ointment (e.g., bacitracin) will be applied daily to the catheter exit site at the skin. Thereafter, Vaseline will be applied to the catheter site daily throughout the duration of the experiment.

We would like to avoid the use of systemic antibiotics whenever possible as this could impact the sinus infection model by inadvertently treating the established sinusitis. However, systemic antibiotics will be considered on a case-by-case basis with veterinary services if a rabbit develops a clinical infection.

4. Method used to sterilize implants:

Single-use sterile packed catheters will be used and implanted using sterile surgical technique. There will be no re-use of catheters between cases and any compromise of sterility will result in discarding of the catheter and use of a new, sterile packed replacement.

5. Describe implant procedure (if the implant is surgical, create a new Survival Surgery procedure and refer to it here):

See related survival surgery for implant procedure.

6. Monitoring protocol:

See related survival surgery and experiment.

7. Will it be necessary to surgically remove implants, or to re-implant or repair implants if they fail? If so, describe the circumstances and the maximum number of replacements if applicable:

Significant repair (i.e., a second full surgery with placement of a new catheter in the event of dislodgement or damage to the initial catheter placed) will not be performed. If there is minor damage to the catheter post-operatively (e.g., anchoring sutures break), we will consider performing minor external repair under sedation following consultation with veterinary services.

It will not be necessary to surgically remove implants. Rabbits will be euthanized with the catheter implant in situ.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Withhold Medications

1. * Name of the procedure or surgery:

Abuzeid: Withhold Medications

2. * Select procedure type:

Withholding Medications/Procedures

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Withhold Medications/Procedures

- 1. List any medications or procedures that should not be administered by veterinary personnel because they would render the results of the study invalid and why:**

Systemic antibiotics (in particular fluoroquinolones such as Baytril).

- 2. Provide rationale for withholding the above:**

Systemic antibiotics have a high potential of not only altering the microbial flora of the established sinusitis but may inadvertently eradicate the established sinusitis rendering the results of the experiment invalid.

Fluoroquinolones such as Baytril are contraindicated due to their specific activity against *Pseudomonas* species which are a focus of this study.

- 3. In the event that veterinary staff considers treatment essential for animal welfare, list steps that should be taken (e.g., If can't contact PI group, then either treat or euthanize):**

Contact PI if administration of systemic antibiotics is considered medically necessary for the well-being of the animal. If the PI cannot be reached, proceed with treatment per veterinary services recommendation.

Procedure Documents

1. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Procedure Identification

Procedure Identification: Analgesia, Local, Bupivacaine and Lidocaine

1. * Name of the procedure or surgery:

Analgesia, Local, Bupivacaine and Lidocaine

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Bupivacaine HCl	Standard	Intramuscular	1-2 mg/kg	N/A	N/A	N/A
View	Bupivacaine HCl	Standard	Subcutaneous	1-2 mg/kg	N/A	N/A	N/A
View	Lidocaine	Standard	Subcutaneous	1-2 mg/kg	N/A	N/A	N/A
View	Lidocaine	Standard	Intramuscular	1-2 mg/kg	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

A mixture of bupivacaine and lidocaine will be injected subcutaneously (SC) and/or intramuscularly (IM) along the incision site just prior to incision.

3. Describe the intended effects of administering the substance(s):

Local analgesia.

4. Describe any potential adverse reactions to administering the substance(s):

None anticipated.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Needles must not be recapped unless a recapping device is used.

Gloves must be worn when handling these agents.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Bupivacaine HCl

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

1-2 mg/kg

4. Frequency and duration of dosages:

Once, just prior to surgery

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Bupivacaine will be pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Bupivacaine HCl

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

1-2 mg/kg

4. Frequency and duration of dosages:

Once, just prior to surgery

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Bupivacaine will be pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Lidocaine

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

1-2 mg/kg

4. Frequency and duration of dosages:

Once, just prior to surgery

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Lidocaine will be pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Lidocaine

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

1-2 mg/kg

4. Frequency and duration of dosages:

Once, just prior to surgery

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Lidocaine will be pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)

1. * Name of the procedure or surgery:

Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Buprenorphine HCl (Buprenex, Simbadol)	Standard	Intramuscular	0.02-0.05 mg/kg	N/A	N/A	N/A
View	Buprenorphine HCl (Buprenex, Simbadol)	Standard	Subcutaneous	0.02-0.05 mg/kg	N/A	N/A	N/A
View	Buprenorphine SR (Zoopharm)	Standard	Subcutaneous	0.12 mg/kg	N/A	N/A	N/A
View	Carprofen (Rimadyl)	Standard	Subcutaneous	1 - 2 mg/kg	50 mg/mL	N/A	N/A
View	Fentanyl (Sublimaze)	Standard	Transdermal	Patch size and dose will be determined by veterinary services	N/A	N/A	N/A
View	Meloxicam (Metacam)	Standard	Subcutaneous	0.2 mg/kg	N/A	N/A	N/A
View	Meloxicam (Metacam)	Standard	Oral - Other	0.1-0.2 mg/kg	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Post-operative analgesia (after irrigating catheter placement survival surgery) will be achieved by using a combination of an opioid and an NSAID. Similarly, post-procedural anesthesia will also be provided after placement of the MeroCel sponge and inoculation of the maxillary sinus with bacterial strains to induce sinusitis. In both of these scenarios (irrigating catheter placement survival surgery and establishment of sinusitis), analgesia will be provided for 72 hours.

Opioid options include fentanyl transdermal patch (1st line) and regular buprenorphine or slow-release buprenorphine (2nd line). The timing and dose size will be determined by veterinary services.

NSAID options include meloxicam (1st line) and carprofen (2nd line). The timing and dose size will be determined by veterinary services.

Note: Most Category 3 procedures require multimodal analgesia and more than one type of analgesic is ideally administered. Please consult with Veterinary Services if questions.

Additional information can also be found in the IACUC policy on “Analgesia in Research Animals.”

3. Describe the intended effects of administering the substance(s):

Provide analgesia for 72 hours.

4. Describe any potential adverse reactions to administering the substance(s):

Overdose can result in sedation, bleeding, gastrointestinal stasis, inappetence, and respiratory depression.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Needles must not be recapped unless a recapping device is used.

Gloves must be worn when handling these agents.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Buprenorphine HCl (Buprenex, Simbadol)

2. Route:

Intramuscular

If you indicated Other, specify the route:

N/A

3. Dose:

0.02-0.05 mg/kg

4. Frequency and duration of dosages:

Once at the time of procedure, then every 6-12 hours for 72 hours

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Buprenorphine HCl is pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Buprenorphine HCl (Buprenex, Simbadol)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.02-0.05 mg/kg

4. Frequency and duration of dosages:

Once at the time of procedure, then every 6-12 hours for 72 hours

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Buprenorphine HCl is pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Buprenorphine SR (Zoopharm)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.12 mg/kg

4. Frequency and duration of dosages:

TBD by veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Carprofen (Rimadyl)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

1 - 2 mg/kg

4. Frequency and duration of dosages:

SID (Daily) for 24-72 hours post-procedure

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

50 mg/mL

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agent will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Fentanyl (Sublimaze)

2. Route:

Transdermal

If you indicated Other, specify the route:

3. Dose:

Patch size and dose will be determined by veterinary services

4. Frequency and duration of dosages:

TBD by veterinary services

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Meloxicam (Metacam)

2. Route:

Subcutaneous

If you indicated Other, specify the route:

N/A

3. Dose:

0.2 mg/kg

4. Frequency and duration of dosages:

SID (daily) for 24-72 hours post-procedure

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

1. * Substance:

Meloxicam (Metacam)

2. Route:

Oral - Other

If you indicated Other, specify the route:

typically dosed via syringe

3. Dose:

0.1-0.2 mg/kg

4. Frequency and duration of dosages:

SID (daily) for 24-72 hours post-procedure

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Pharmaceutical grade agents will be used.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A



View: Custom SF: Procedure Identification

Procedure Identification: Abuzeid: Phenylephrine Administration

1. * Name of the procedure or surgery:

Abuzeid: Phenylephrine Administration

2. * Select procedure type:

Substance Administration

3. * Species:

Rabbits - New Zealand White

4. * Will administering this procedure cause any more than momentary pain or distress? Yes No

If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Phenylephrine (Metaoxedrin, Metasymptol, Mezatol, Neo-Synephrine)	Standard	Topical	0.5% (0.1-0.5 mL per nostril via syringe)	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Topical phenylephrine will be applied intranasally during endoscopy procedure (see related procedure for details).

3. Describe the intended effects of administering the substance(s):

To facilitate mucosal decongestion and optimize the endoscopic view. To control any bleeding related to intranasal mucosal trauma (which is of low likelihood and self-limited when it does occurs).

4. Describe any potential adverse reactions to administering the substance(s):

None expected.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Substance:

Phenylephrine (Metaoxedrin, Metasympatol, Mezaton, Neo-Synephrine)

2. Route:

Topical

If you indicated Other, specify the route:

N/A

3. Dose:

0.5% (0.1-0.5 mL per nostril via syringe)

4. Frequency and duration of dosages:

1-2 times per endoscopic session

5. Volume (for rodents or intracranial injections):

N/A

6. Concentration:

N/A

7. Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:

Agents will be pharmaceutical grade.

8. Complication remediation:

N/A

9. Substance order for the procedure:

N/A

Substances Appendix:



View: Custom SF: Substance Information

Substance Information: Nanoparticles

1. * Name:

Nanoparticles

2. * Substance types: (select all that apply)

Nanoparticle

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Fentanyl (Sublimaze)

1. * Name:

Fentanyl (Sublimaze)

2. * Substance types: (select all that apply)

Analgesic

Anesthetic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Pseudomonas aeruginosa

1. * Name:

Pseudomonas aeruginosa

2. * Substance types: (select all that apply)

Bacteria

Biological Agent

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Buprenorphine SR (Zoopharm)

1. * Name:

Buprenorphine SR (Zoopharm)

2. * Substance types: (select all that apply)

Analgesic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Ketamine (Ketaset, Ketaflo, Vetalar)

1. * Name:

Ketamine (Ketaset, Ketaflo, Vetalar)

2. * Substance types: (select all that apply)

Anesthetic
Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified
There are no items to display



View: Custom SF: Substance Information

Substance Information: Xylazine

1. * Name:

Xylazine

2. * Substance types: (select all that apply)

Anesthetic

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified
There are no items to display



View: Custom SF: Substance Information

Substance Information: Meloxicam (Metacam)

1. * Name:

Meloxicam (Metacam)

2. * Substance types: (select all that apply)

Analgesic
Reproductive Hazard/Teratogen
Other

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Acepromazine maleate (Acetylpromazine, ACE, PromAce, Aceproject)

1. * Name:

Acepromazine maleate (Acetylpromazine, ACE, PromAce, Aceproject)

2. * Substance types: (select all that apply)

Antiemetic
Chemical Agent

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Atipamezole HCl (Antisedan)

1. * Name:

Atipamezole HCl (Antisedan)

2. * Substance types: (select all that apply)

Other

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Carprofen (Rimadyl)

1. * Name:

Carprofen (Rimadyl)

2. * Substance types: (select all that apply)

Analgesic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Buprenorphine HCl (Buprenex, Simbadol)

1. * Name:

Buprenorphine HCl (Buprenex, Simbadol)

2. * Substance types: (select all that apply)

Analgesic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Dexmedetomidine hydrochloride (Dexdomitor)

1. * Name:

Dexmedetomidine hydrochloride (Dexdomitor)

2. * Substance types: (select all that apply)

Analgesic

Anesthetic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Isoflurane

1. * Name:

Isoflurane

2. * Substance types: (select all that apply)

Anesthetic

Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Lidocaine

1. * Name:

Lidocaine

2. * Substance types: (select all that apply)

Analgesic

Anesthetic

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name

Date Modified

There are no items to display



View: Custom SF: Substance Information

Substance Information: Phenylephrine (Metaoxedrin, Metasymptol, Mezaton, Neo-Synephrine)

1. * Name:

Phenylephrine (Metaoxedrin, Metasymptol, Mezaton, Neo-Synephrine)

2. * Substance types: (select all that apply)

Reproductive Hazard/Teratogen
Other

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display



View: Custom SF: Substance Information

Substance Information: Bupivacaine HCl

1. * Name:

Bupivacaine HCl

2. * Substance types: (select all that apply)

Analgesic
Anesthetic
Particularly Hazardous Chemical

3. * Is this a hazardous agent: Yes No

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization (BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu.

4. Supporting documents:

Document Name	Date Modified
---------------	---------------

There are no items to display

1. * Select the funding organization:

Cystic Fibrosis Foundation

If Other was selected in question 1, provide Funding Organization:

2. * All animal use projects must be reviewed for scientific merit prior to initiating animal use. Choose the required reviews for this project:

Has already been conducted and approved by a funding agency

3. Provide name of the committee or the department reviewer (Required if

“Has been conducted by my department or school and has been found to be scientifically meritorious” was selected):

N/A

4. eGC1 Number(s):(assigned internally)

A160477

Experiments Appendix:

00. Training & Procedure Optimization

1. * Experiment name:

00. Training & Procedure Optimization

2. * Species:

Rabbits - New Zealand White

3. If other was selected, provide a species:

N/A

4. What is the scientific goal of this experiment:

We will practice and refine procedures to be used in Experiments 1 and 2.

5. * Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:

Inoculation Practice & Optimization

4 rabbits will be purchased to practice Experiment 1 ("Validate a preclinical rabbit model of sinusitis"). Each of the 4 animals will be inoculated with a different strain of *Pseudomonas aeruginosa* to ensure that each of the selected strains reliably establishes a sinusitis in the rabbits that persists for the duration of the planned experiment. The experimental timeline for these animals is as follows:

Day 0: Animals receive bilateral nasal endoscopic examination (with sinus culture) and are infected with bacteria under anesthesia.

Day 7: Animals undergo bilateral nasal endoscopic examination and sinus culture.

Day 21: Animals undergo bilateral nasal endoscopic examination and sinus culture. Animals are euthanized and tissue evaluated histologically.

Survival Surgery Practice & Refinement

4 rabbits will be purchased to practice the survival surgery described in Experiment 2 ("Evaluate the efficacy of nitric oxide-releasing nanoparticles in a preclinical rabbit model of sinusitis"). These animals will only experience placement of the sinus catheter, and will not be inoculated with bacteria. We will test the functionality of the irrigating catheter 48 hours after the catheter placement by flushing the catheter with saline and optimizing collection of resulting nasal drainage from the rabbit nostril. The animals will not undergo administration of nanoparticles. The animals will be sacrificed 72 hours after performance of the survival surgery.

Note: Female rabbits will be used for all studies on this protocol. The rationale for use of female rabbits is because the widely accepted rabbit model of sinusitis on which this work is based and on which this work builds was performed exclusively in female New Zealand white rabbits. Our background information on the expected immune and inflammatory response to sinusitis is also based on this published literature which, again, was performed in female rabbits. Use of female rabbits only

will aid in comparing our novel data to the pre-existing literature and will help mitigate any unknown gender-related differences in immune or inflammatory responses.

Animal Sex:
Female

Animal Ages:
4-6 months

Animal Size:
2-4 kg

6. Select experimental procedures:

Name	Type	Version	Scope
Anesthetic Overdose, Pentobarbital or Pentobarbital Solution	Euthanasia	1	Standard
Abuzeid: Nasal Sponge	Implants	1	Team
Abuzeid: Sinus Catheter	Implants	1	Team
Abuzeid: Nasal Endoscopy	Other	1	Team
Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery)	Substance Administration	1	Team
Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)	Substance Administration	1	Team
Abuzeid: Catheter Saline Flush and Nanoparticle Administration	Substance Administration	1	Team
Abuzeid: Induction of Anesthesia	Substance Administration	1	Team
Abuzeid: Inoculation of Sinus	Substance Administration	1	Team
Abuzeid: Phenylephrine Administration	Substance Administration	1	Team

Name	Type	Version	Scope
Abuzeid: Sedation for Non-Surgical Procedures	Substance Administration	1	Team
Analgesia, Local, Bupivacaine and Lidocaine	Substance Administration	2	Standard
Abuzeid: Placement of Sinus Irrigating Catheter	Survival Surgery	1	Team
Abuzeid: Withhold Medications	Withholding Medications/Procedures	1	Team

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):

Animals will be weighed prior to infection and every 72 hours post-infection until the time of euthanasia. Monitoring will take place one to three times daily (including weekends and holidays) from infection up to the time of euthanasia.

Specifically, animals will be monitored for signs of respiratory distress including dyspnea and cyanosis, bloody nasal discharge, and body temperature. Clinical signs of deteriorating health status that will be monitored daily include hunched stature, inactivity, salivation, rough coats, unformed feces, cervical lymphadenopathy, decreased appetite, circling or tilting of head (torticollis) and conjunctivitis.

See nasal endoscopy and survival surgery procedures for additional monitoring and analgesic plans.

Criteria for early euthanasia are:

1. >20% weight loss from time of bacterial inoculation
2. Body temperature below 37°C or above 41°C (normal body temperature is 38.3 – 39.4°C)
3. Inability to mobilize independently
4. Excessive shaking
5. Inability to drink or eat
6. Respiratory distress
7. Worsening of sinusitis beyond the expected symptoms of nasal discharge and congestion to symptoms associated with spread beyond the sinus into critical nearby structures such as the orbit and/or intracranial space (eye proptosis, restricted eye movement, eye closure, periorbital swelling, lethargy, confusion). In these circumstances, veterinary services will be contacted and the animal will either be treated or euthanized per veterinary recommendations.

8. If there is expected mortality (spontaneous death) in this experiment:

- a. Procedure/condition associated with mortality:

N/A

- b.** Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):

N/A

- c.** Explain why euthanasia is not possible or appropriate:

N/A

- 9.** Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.

N/A

- 10.** * Total number of animals used in this experiment:(including all the animals to be produced)

8

- a.** Justify total number of animals used in this experiment:

Inoculation Practice & Optimization

The use of 4 animals is justified to confirm that each of the four strains of *Pseudomonas aeruginosa* used reliably establishes a sinusitis in the rabbits that persist for the duration of the planned experiment. This will also provide the opportunity for our group to become proficient in the nasal endoscopy and inoculation procedure.

Survival Surgery Practice & Refinement

The use of 4 animals is justified to allow refinement of the surgical technique and, importantly, more efficient progress through the surgery so that the animals used in Experiment 2 undergo the most efficient and expeditious surgery possible. The stages of the surgery that will be refined are: i) creation of the periosteal flap that allows for exposure of the face of the maxillary sinus, ii) creation of the maxillotomy opening with the trocar for catheter insertion, iii) insertion of the catheter into the sinus and tunneling of the catheter tubing subcutaneously along the nasal dorsum and to the vertex of the cranium, and iv) securing the catheter tubing between the ears in the least intrusive and most comfortable way possible and wound closure.

- 11.** Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0

C: 0

D: 8

E: 0

- a.** Justify the need for any animals in pain category E:

N/A

- 12.** * Identify husbandry exceptions:

Exception Type	Description and Justification
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	Exception Type	Description and Justification
View	Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.	Rabbits will need to be singly housed for the duration of the experiment. This is justified in order to prevent cage-mate disturbance of the indwelling catheter after implantation. Furthermore, in the time period prior to and after inoculation of bacteria into the sinuses, the rabbits will need to be singly housed to prevent cross-contamination of different strains of <i>Pseudomonas aeruginosa</i> .

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

1. * Exception type:

Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.

2. Description and justification:

Rabbits will need to be singly housed for the duration of the experiment. This is justified in order to prevent cage-mate disturbance of the indwelling catheter after implantation. Furthermore, in the time period prior to and after inoculation of bacteria into the sinuses, the rabbits will need to be singly housed to prevent cross-contamination of different strains of *Pseudomonas aeruginosa*.

01. Validate a preclinical rabbit model of sinusitis

1. * Experiment name:

01. Validate a preclinical rabbit model of sinusitis

2. * Species:

Rabbits - New Zealand White

3. If other was selected, provide a species:

4. What is the scientific goal of this experiment:

We will establish a rabbit model of maxillary sinusitis to investigate the effect of bacterial strains on the sinonasal epithelial inflammation and physiology.

5. * Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:

Experiment Procedural Steps

1. Rabbits will be anesthetized by using induction agents (xylazine combined with ketamine or ketamine combined with dexmedetomidine). Anesthetic depth will either be maintained by re-dosing intramuscular agents or via inhaled isoflurane after intubation. This decision will be made in consultation with veterinary services who will manage and maintain anesthesia.

2. Nasal endoscopy will be performed to occlude and inoculate the right maxillary sinus with one of four bacterial strains of *Pseudomonas aeruginosa*.

3. One week after inoculation (Day 7), nasal endoscopy will be repeated under anesthesia to culture purulence in the right middle meatus to confirm establishment of sinusitis. The contralateral non-occluded sinus will serve as a negative control and will also undergo endoscopically-guided culture.

4. On day 21, three weeks after inoculation, nasal endoscopy will be repeated using the same anesthetic induction and maintenance protocol described above and in the "Anesthesia" procedure. The bilateral middle meatus will, again, undergo endoscopically-guided culture. Extent of edema and type of discharge from the middle meatus will be graded bilaterally using a validated scale.

5. Immediately after the final endoscopy and culture on day 21, animals will be euthanized and tissues harvested for histologic and microbiologic analysis.

General timeline:

Day 0: "pre-sinusitis" Animals receive nasal endoscopic examination and are infected with bacteria under anesthesia

Day 7: "post-sinusitis" Animals undergo nasal endoscopic examination and endoscopic culture.

Day 21: "post-treatment" Animals undergo nasal endoscopic examination and endoscopic culture. Animals are euthanized and tissue evaluated histologically.

Animal Sex:
Female

Animal Ages:
4-6 months

Animal Size:
2-4 kg

6. Select experimental procedures:

Name	Type	Version	Scope
Anesthetic Overdose, Pentobarbital or Pentobarbital Solution	Euthanasia	1	Standard
Abuzeid: Nasal Sponge	Implants	1	Team
Abuzeid: Nasal Endoscopy	Other	1	Team
Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery)	Substance Administration	1	Team
Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)	Substance Administration	1	Team
Abuzeid: Induction of Anesthesia	Substance Administration	1	Team
Abuzeid: Inoculation of Sinus	Substance Administration	1	Team
Abuzeid: Phenylephrine Administration	Substance Administration	1	Team
Abuzeid: Withhold Medications	Withholding Medications/Procedures	1	Team

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):

Animals will be weighed prior to infection and every 72 hours post-infection until the time of euthanasia. Monitoring will take place one to three times daily (including weekends and holidays) from infection up to the time of euthanasia.

Specifically, animals will be monitored for signs of respiratory distress including dyspnea and cyanosis, bloody nasal discharge, and body temperature. Clinical signs of deteriorating health status that will be monitored daily included hunched

stature, inactivity, salivation, rough coats, unformed feces, cervical lymphadenopathy, decreased appetite, circling or tilting of head (torticollis) and conjunctivitis.

See nasal endoscopy procedure for additional monitoring and analgesic plan.

Criteria for early euthanasia are:

1. >20% weight loss from time of bacterial inoculation
2. Body temperature below 37°C or above 41°C (normal body temperature is 38.3 – 39.4°C)
3. Inability to mobilize independently
4. Excessive shaking
5. Inability to drink or eat
6. Respiratory distress
7. Worsening of sinusitis beyond the expected symptoms of nasal discharge and congestion to symptoms associated with spread beyond the sinus into critical nearby structures such as the orbit and/or intracranial space (eye proptosis, restricted eye movement, eye closure, periorbital swelling, lethargy, confusion). In these circumstances, veterinary services will be contacted and the animal will either be treated or euthanized per veterinary recommendations.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:
N/A

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):
N/A

c. Explain why euthanasia is not possible or appropriate:
N/A

9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.

N/A

10. * Total number of animals used in this experiment:(including all the animals to be produced)

16

a. Justify total number of animals used in this experiment:

Estimates for the number of experimental animals were based on detecting a 0.15 log (30%) effect and a standard deviation of 1 log in outcome measure at an alpha value of 0.05 and a power value of 0.8 (beta value of 0.2). These parameters result in an “n” of 2 animals per group. We will use four different bacterial strains and, given the need to duplicate the experiment, this equates to a total of 16 animals.

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0

C: 0

D: 16

E: 0

a. Justify the need for any animals in pain category E:

N/A

12. * Identify husbandry exceptions:

Exception Type Description and Justification

View	Rabbits - Standard housing, as outlined in the policy, is not acceptable for part or all of the study.	The rabbits will need to be singly housed after inoculation of the sinus with the bacterial strain. This is to prevent cross-contamination of bacterial species across rabbits. Consequently, single housing will need to be continued from the time of inoculation of the sinuses to the time of animal sacrifice.
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13. Supporting documents:

Document Name

Date Modified

There are no items to display

1. * Exception type:

Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.

2. Description and justification:

The rabbits will need to be singly housed after inoculation of the sinus with the bacterial strain. This is to prevent cross-contamination of bacterial species across rabbits.

Consequently, single housing will need to be continued from the time of inoculation of the sinuses to the time of animal sacrifice.

02. Evaluation of nitric oxide (NO)-releasing nanoparticle (NO-NP) efficacy in a preclinical rabbit model of sinusitis.

1. * Experiment name:

02. Evaluation of nitric oxide (NO)-releasing nanoparticle (NO-NP) efficacy in a preclinical rabbit model of sinusitis.

2. * Species:

Rabbits - New Zealand White

3. If other was selected, provide a species:

4. What is the scientific goal of this experiment:

We will evaluate the efficacy of our NO-releasing platform in eradicating respiratory epithelial biofilms in vivo.

5. * Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:

Experiment Procedural Steps

1. Rabbits will be anesthetized by using induction agents (xylazine combined with ketamine or ketamine combined with dexmedetomidine). Anesthetic depth will either be maintained by re-dosing intramuscular agents or via inhaled isoflurane after intubation. This decision will be made in consultation with veterinary services who will manage and maintain anesthesia.

2. Nasal endoscopy will be performed to occlude and inoculate the right maxillary sinus with one of four bacterial strains of *Pseudomonas aeruginosa*. We will use the same anesthetic induction and maintenance protocol described above and in the "Anesthesia" procedure.

3. One week after inoculation (Day 7), nasal endoscopy will be repeated under anesthesia to culture purulence in the right middle meatus to confirm establishment of sinusitis. The contralateral non-occluded middle meatus will serve as a negative control and will also undergo endoscopically-guided culture.

4. While under anesthesia, the rabbits will also undergo survival surgery to implant a sinus irrigating catheter.

5. Between Day 7 and Day 21, the indwelling catheter will be irrigated every 48 hours with one of the following depending on treatment group assignment: i) saline, ii) blank inactive nanoparticle, iii) nitric oxide releasing active nanoparticle (NO-NP). Just prior to instillation of either i) saline, ii) blank inactive nanoparticle or iii) active nanoparticle, as described in the prior statement, the catheter will be flushed with saline and the fluid which emerges from the snout and collected for longitudinal bacterial counts. These irrigations will take place with the rabbit restrained in a rabbit holder or soft restraint (rabbit snuggle) and, if necessary, under sedation using acepromazine (see sedation procedure).

6. On Day 21, rabbits will undergo a final nasal endoscopy under anesthetic. This will be performed bilaterally and endoscopically-guided cultures of the middle meatus obtained.

7. The rabbits will then be euthanized on Day 21 immediately after nasal endoscopy and sinus tissue harvested for histologic and microbiologic analysis.

General timeline:

Day 0: "pre-sinusitis" Animals receive nasal endoscopic examination and are infected with bacteria under anesthesia.

Day 7: "post-sinusitis, pre-treatment" Animals undergo nasal endoscopic examination and culture. Irrigating catheter is placed under anesthesia.

Day 7-21: "Treatment" irrigations are performed every 48 hours and will be immediately preceded by saline irrigations through the catheter with collection of resulting nasal fluid for CFU quantification.

Day 21: "post-treatment, euthanasia" Animals undergo nasal endoscopic examination and culture. Animals are euthanized and tissue evaluated histologically.

Animal Sex:
Female

Animal Ages:
4-6 months

Animal Size:
2-4 kg

6. Select experimental procedures:

Name	Type	Version	Scope
Anesthetic Overdose, Pentobarbital or Pentobarbital Solution	Euthanasia	1	Standard
Abuzeid: Nasal Sponge	Implants	1	Team
Abuzeid: Sinus Catheter	Implants	1	Team
Abuzeid: Nasal Endoscopy	Other	1	Team
Abuzeid: Analgesia, Post-Nasal Endoscopy (not for survival surgery)	Substance Administration	1	Team

Name	Type	Version	Scope
Abuzeid: Analgesia, Post-Operative after Survival Surgery or Establishment of Sinusitis (72 hours)	Substance Administration	1	Team
Abuzeid: Catheter Saline Flush and Nanoparticle Administration	Substance Administration	1	Team
Abuzeid: Induction of Anesthesia	Substance Administration	1	Team
Abuzeid: Inoculation of Sinus	Substance Administration	1	Team
Abuzeid: Phenylephrine Administration	Substance Administration	1	Team
Abuzeid: Sedation for Non-Surgical Procedures	Substance Administration	1	Team
Analgesia, Local, Bupivacaine and Lidocaine	Substance Administration	2	Standard
Abuzeid: Placement of Sinus Irrigating Catheter	Survival Surgery	1	Team
Abuzeid: Withhold Medications	Withholding Medications/Procedures	1	Team

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):

Animals will be weighed prior to infection and every 72 hours post-infection until the time of euthanasia. Monitoring will take place one to three times daily (including weekends and holidays) from infection up to the time of euthanasia.

Specifically, animals will be monitored for signs of respiratory distress including dyspnea and cyanosis, bloody nasal discharge, and body temperature. Clinical signs of deteriorating health status that will be monitored daily included hunched stature, inactivity, salivation, rough coats, unformed feces, cervical lymphadenopathy, decreased appetite, circling or tilting of head (torticollis) and conjunctivitis.

See nasal endoscopy and survival surgery procedures for additional monitoring and analgesic plans.

Criteria for early euthanasia are:

1. >20% weight loss from time of bacterial inoculation
2. Body temperature below 37°C or above 41°C (normal body temperature is 38.3 –

39.4°C)

3. Inability to mobilize independently

4. Excessive shaking

5. Inability to drink or eat

6. Respiratory distress

7. Worsening of sinusitis beyond the expected symptoms of nasal discharge and congestion to symptoms associated with spread beyond the sinus into critical nearby structures such as the orbit and/or intracranial space (eye proptosis, restricted eye movement, eye closure, periorbital swelling, lethargy, confusion). In these circumstances, veterinary services will be contacted and the animal will either be treated or euthanized per veterinary recommendations.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:

N/A

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):

N/A

c. Explain why euthanasia is not possible or appropriate:

N/A

9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.

N/A

10. * Total number of animals used in this experiment:(including all the animals to be produced)

12

a. Justify total number of animals used in this experiment:

Estimates for the number of experimental animals were based on detecting a 0.15 log (30%) effect and a standard deviation of 1 log in outcome measure at an alpha value of 0.05 and a power value of 0.8 (beta value of 0.2). These parameters result in an "n" of 2 animals per group. As there are three different treatment groups (saline, blank nanoparticle, NO-NP) and we anticipate duplication of the experiment, this equates to a total of 12 animals.

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0

C: 0

D: 12

E: 0

a. Justify the need for any animals in pain category E:

N/A

12. * Identify husbandry exceptions:

	Exception Type	Description and Justification
View	Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.	Rabbits will need to be singly housed for the duration of the experiment. This is justified in order to prevent cage-mate disturbance of the indwelling catheter after implantation. Furthermore, in the time period prior to and after inoculation of bacteria into the sinuses, the rabbits will need to be singly housed to prevent cross-contamination of different strains of <i>Pseudomonas aeruginosa</i> .

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

1. * Exception type:

Rabbits - Standard social contact housing, as outlined in the policy, is not acceptable for part or all of the study.

2. Description and justification:

Rabbits will need to be singly housed for the duration of the experiment. This is justified in order to prevent cage-mate disturbance of the indwelling catheter after implantation. Furthermore, in the time period prior to and after inoculation of bacteria into the sinuses, the rabbits will need to be singly housed to prevent cross-contamination of different strains of *Pseudomonas aeruginosa*.

1. * Identify the location where animals will be used:

ARCF ABSL1

- a. For locations that are lab managed, provide justification for housing outside of the vivarium:**

N/A

2. * What species will be housed in this location?

Common Name	Scientific Name
Rabbits - New Zealand White	Leporidae

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

1. * Identify the location where animals will be used:

ARCF ABSL2

- a. For locations that are lab managed, provide justification for housing outside of the vivarium:**

N/A

2. * What species will be housed in this location?

Common Name	Scientific Name
Rabbits - New Zealand White	Leporidae

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL2

1. * Identify the location where animals will be used:

ARCF ABSL2

- a. For locations that are outside of the vivarium, provide justification for the use of this space:**

N/A

2. * What species will be used in this location?

Common Name	Scientific Name
Rabbits - New Zealand White	Leporidae

3. Describe how this location will be used:

Procedures:

Anesthesia

Analgesia (postoperative - 72 hrs), Analgesia (nasal endoscopy - 24h)

Inoculation of sinuses with bacteria,

Nasal endoscopy,

Survival surgery - placement of sinus catheter,

Restraint - irrigation of sinus catheter,

Euthanasia

4. * If animals are left unattended in this location, provide an explanation and include maximum duration:

Animals will not be left unattended during procedures.

5. Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):

Animals will be housed and undergo procedures in the ARC facility.

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL2

1. * Identify the location where animals will be used:

ARCF ABSL1

- a. For locations that are outside of the vivarium, provide justification for the use of this space:**

N/A

2. * What species will be used in this location?

Common Name	Scientific Name
Rabbits - New Zealand White	Leporidae

3. Describe how this location will be used:

Practice/training surgeries

4. * If animals are left unattended in this location, provide an explanation and include maximum duration:

Animals will not be left unattended during procedures.

5. Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):

N/A

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

From: Geena Gallardo <gallardg@uw.edu>

To: "Molly K. Lucas" <mklucas@uw.edu>, "Nicholas L. Reyes" <nlreyes@uw.edu>, achris08 <achris08@uw.edu>, Leandra Mosca <lmosca@uw.edu>, wabuzeit <wabuzeit@uw.edu>

Sent: 8/20/2020 1:29:04 PM

Subject: Protocol Discussion Meeting

Attach: [EMB4_image001.gif]

Hello Dr. Abuzeit,

Molly Lucas, whom I believe you have been in contact with here in the Department of Comparative Medicine, has asked me to set up a meeting to discuss your protocol with several of our veterinarians. Please see this Doodle Poll linked here with availability for next week: <https://doodle.com/poll/g5gmhefxy8pcpee5>. You are welcome to share this doodle poll with any lab members you would like to be present in the meeting, you would just need to notify me of their contact so I can include them on the invite with the zoom details.

Molly and team, if you could also fill out your availability so I can pick the best time, that would be wonderful. Thank you everyone.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu

W UNIVERSITY of WASHINGTON

From: Geena Gallardo <gallardg@uw.edu>
Sent: Thursday, August 20, 2020 1:29 PM
To: Molly K. Lucas; Nicholas L. Reyes; achris08; Leandra Mosca; Waleed M Abuzeid
Subject: Protocol Discussion Meeting

Importance: High

Hello Dr. Abuzeid,

Molly Lucas, whom I believe you have been in contact with here in the Department of Comparative Medicine, has asked me to set up a meeting to discuss your protocol with several of our veterinarians. Please see this Doodle Poll linked here with availability for next week: <https://doodle.com/poll/g5gmhefxy8pcpee5>. You are welcome to share this doodle poll with any lab members you would like to be present in the meeting, you would just need to notify me of their contact so I can include them on the invite with the zoom details.

Molly and team, if you could also fill out your availability so I can pick the best time, that would be wonderful. Thank you everyone.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu

W UNIVERSITY of WASHINGTON

Protocol Team Members: Is there a member that will be handling the rabbits? Currently, there is no one listed to handle animals on the protocol.

Needed: surgery certification, all group members having rabbit hands on laboratory.

Scientific Aims: Question 3

What are the potential harms associated with the surgery and indwelling catheter?

Experiment 1

Question 7: Do you expect weight loss with sinusitis infection in rabbits? When do you expect to see increases in temperature? Daily weighing and temperatures can be stressful for rabbits, and if not needed, can be reduced to 2 or 3 times per week to reduce stress.

Question 10: Please check your power calculations, based on an effect size of 0.15log 10, I calculated the need for 8 rabbits per group. (the same calculation is used in experiment 2)

Removal criteria: the temperature ranges listed through the experiments and procedures for euthanasia criteria are too wide (33-44C). The normal temperature range for a rabbit is 38-40C or 100.4-104F. You should give yourself some room for variation among rabbits, but I would recommend lower than 99F or higher than 106F.

Procedure: Analgesia, Buprenorphine and Carprofen

Based on the description of the endoscope procedures, I would classify it as a category 1. To help reduce the stress of handling, I would suggest reducing the opioid and NSAID administration for the endoscope procedures for 12hr with the option to redo the NSAID if signs of pain observed. Also, for Carprofen injections in rabbits, we recommend a dose of 1-2mg/kg SQ every 12 hours.

Procedure: Induction of Anesthesia:

Question 2: How will the rabbits be monitored under anesthesia? You say you will inject IM into the triceps – I would recommend the lumbar epaxial muscles for ease of access and administration; however to make the protocol more flexible, you do not need to state what muscle you will be injecting into.

Question 4: How will you be performing rabbit intubation as this is a difficult procedure?

Dosing for the drugs: [I would like to discuss this further with everyone in class. The Carprofen dose is too high. The Dex dose seems low, and the Ketamine dose seems low when looking at other Ketamine anesthetic combos for rabbits. I did find a reference that uses Ketamine (35mg/kg), medetomidine (0.5mg/kg) and Buprenorphine (0.3mg/kg). This paper compares the use of this to Ketamine (35mg/kg) and Xylazine (5mg/kg). I also found another paper that looked at ketamine (35 mg/kg) and dexmedetomidine (0.25 mg/kg) without or with buprenorphine (0.03 mg/kg). Both the KX and KDB provided over an hour of anesthesia.]

Experiment 2

Substance Administration, Nanoparticles How long is the flushing and restraint? [with rabbits being obligate nasal breathers, is there time of rest between flushings?]

Survival Surgery:

Analgesia recommendations as described above – recommend at least 24 to 48 hours of analgesia. Carprofen dose is too high. SQ recommended.

Question 3: Will the catheter end be covered for protection while not in use?

Question 4: “inappetence >24 hours will be removed from the research study.” I don’t think this is necessary, rabbits can go off feed for stress related reasons. I would make this statement more broad indicating in consultation with Veterinary Services.

Question 5: Describe in further detail anesthetic monitoring. Will you have continuous monitoring through machines or manually monitoring? (ei heart rate measurements). Other anesthetic depth measurements: respiratory rate, heart rate.

Question 6: Is the antibiotic ointment necessary?

Husbandry Exceptions: Will the rabbits need to be singly housed following catheter placement? If so, this is a husbandry exception since our standard is social housing.

Animal Details:

Question 3. A. – Please be specific on method of identification.

KZ musings:

Refinement, Replacement and Reduction

“We considered the use of inhalational anesthetics but, given the expected duration of the catheter placement surgical procedure and to minimize the risk of respiratory depression and potential death, non-inhalational agents were instead selected.” – interesting... I assumed they chose injectable due to the need to assess the nasal cavity.

“Though the proposed rabbit model does not harbor mutations in the CFTR gene, it does allow for establishing chronic infections in the sinuses and the experience gained will be critical for harnessing such models when they are developed in the future. Preliminary CF rabbit models are in the process of being developed and demonstrate physiologic characteristics that, in many ways, mimics human CF.” – the beginning of the experiment made it seem like they were going to use a CF rabbit model.

From: Molly K. Lucas <mkluucas@uw.edu>
Sent: Thursday, July 16, 2020 1:57 PM
To: Kim Stocking
Subject: protocol review seminar

Hi Kim,

I need to pick a protocol to assign the residents for seminar next week (7/23), 10-11:30. We haven't done a USDA spp yet, so they asked if we could do one of those if possible. I was just looking at the choices that are either in vet consult or pre-review, and I came up with either Abuzeid (a new rabbit protocol in pre-review with Aubrey) or the Neil King protocol we've talked about previously (rabbits, Jenny) that is back in vet consult after a long time in review.

I sent Aubrey a note asking if Abuzeid would be an OK choice and she said yes, but I also thought I'd check in with you to see if you'd be interested in joining us next week to talk about King? There is a bit of a benefit in that sometimes the residents catch things we might miss, so it can make writing questions easier.

I haven't looked at either one of those protocols in much detail and I'm fine with either plan. I'll probably go with Abuzeid if you are unable to join us for the King discussion, but if you are interested, it might be a good discussion...

Molly

Leandra Mosca
4502-01: Rabbit Sinus
Review Comments

Scientific Aims

1. Statement to address potential harm doesn't encompass the potential detrimental effects of the surgeries (that is bacterial inoculation, endoscopy, culture). There is potential harm in repeated anesthetic events and manipulation of the nasal sinuses beyond sinusitis.

Refinement, Replacement, and Reduction

1. Placement of an indwelling sinus catheter. Cetin et al., 2002 demonstrated nasal catheterization as a predisposing factor in the development of sinusitis and the increase of sinusitis development risk in relation with the catheterization period and the catheters' thickness was shown.

Experiments

Sinus Catheter/Endoscopy on Day 7:

1. How long do you expect this procedure to take? Depending on the length of time of this procedure, it may actually be most prudent to use inhalant anesthesia. The airway can be secured (e.g. intubation). The anesthetic plane can be more tightly controlled for a longer period of time. There will be challenges associated with the animal breathing for an extended period of time during manipulation of the nasal cavity. It's probably a superior option to intubate with the potential for ventilating the animal (e.g. providing oxygen even if apneic). Furthermore, this also allows for adjunct medications like buprenorphine to be given during the procedure, which I otherwise wouldn't recommend in an animal without a secured airway due to respiratory depressant side effects.
2. I would remove social isolation from how post-op pain will be assessed since these rabbits will be singly-housed.

Inoculation of Sinus and Administration of Substances to Nasal Passages

1. I would recommend including euthanasia criteria for severe respiratory distress, nasal passages blocked by mucous and pus or so severely swollen they cannot breathe, epiphora and conjunctivitis (or other signs that there is extension of disease into the tear ducts).
2. Please describe the rhinoscopy procedure in further detail. Position of animal, antegrade or retrograde approach, and how the animal will be positioned after the procedure (sternal with head support?). How will you prevent aspiration of nasal exudates?
3. How will you facilitate outflow of fluids used to flush nasal cavities (not just during procedure, but also during sample collection)?
4. Many nasal endoscopic procedures come with the risk of profuse bleeding and blood loss which may be countered with topical phenylephrine or epinephrine. I would recommend adding this anticipated challenge to the protocol as well as one of these substances.
5. Finally, I would recommend specifying that during endoscopy and culture, the "control" sinus will be explored first to avoid contaminating/inoculating the "clean" side.

Administration of Substances

1. Please change the routes of Buprenorphine and Carprofen to S.C. Repeated IM injections are stressful and painful, especially with the Buprenorphine being up to three times daily. The doses can remain as is. They should be mixed in the same syringe (OK to do) whenever possible to minimize injections.
2. If the dose route remains as is potential adverse reactions should include injection site reactions, muscle inflammation, and pain secondary to IM injections.

Anesthesia:

1. I recommend waiting until recovery to administer buprenorphine due to the potential respiratory depressant effects. While premedication with buprenorphine has been shown to significantly prolong the time of anesthesia induced by ketamine-medetomidine in rabbits (Gonzalez-Gil et al., 2015) I would be concerned about the rabbit going apneic during the study. Dexmedetomidine alone causes bradycardia and bradyapnea (due to decreased sympathetic tone) and this would amplify those effects,
2. Anesthetic reversal is advantageous by speeding up recovery to reduce the risk of hypothermia, which is a considerable problem associated with anesthesia in these species. I recommend adding an atipamezole dose S.C. (NOT IM like the anesthesia dose—S.C.) at the same dose volume as the preceding dose volume of dexmedetomidine (dose ranges 0.1 – 1.0 mg/kg, manufacturer recommends volume of atipamezole same volume as dex).

Survival Surgery:

1. Please specify more specifically what will be performed for the surgical scrub (ethanol and povidone-iodine x 3 scrubs). You may also need to specify that this scrub is performed before the surgeon is in sterile attire.

From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/31/2020 3:27:12 PM
Subject: Re: Abuzeid anesthesia

I was thinking maybe the ace would be a separate procedure since it wouldn't be part of induction, but I can ask him to include it there if you think that would be helpful.

Also - will VS monitor blood pressure? I'm thinking maybe not routinely?

Thanks! I'm writing up the notes now while it's fresh in my mind 😊

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Monday, August 31, 2020 3:22 PM
To: Molly K. Lucas <mklucas@uw.edu>
Subject: Re: Abuzeid anesthesia

That sounds good to me. We can work with that...and adding the acepromazine as an option as well?
Nick

Nicholas Reyes DVM, MS, DACLAM
Sr. Staff Veterinarian
Co-Director of Animal Facility Operations
Dept. of Comparative Medicine
University of Washington, Seattle
nlreyes@uw.edu
206-543-0267

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Monday, August 31, 2020 3:05 PM
To: Nicholas L. Reyes <nlreyes@uw.edu>
Subject: Abuzeid anesthesia

Hi Nick,

I think the meeting was really productive. I was just looking at some other rabbit protocols for K/X doses (Dichek, Lukehart, Giacani)... I'm thinking of suggesting the following for anesthesia. Let me know if this looks OK/feel free to edit anything, esp. please look at the K/X and make sure it's what you want.

Thanks,
Molly

-Add K/X at ketamine 40-50 mg/kg IM and xylazine 3-5 mg/kg IM

-OK to leave the ket/dexmed option also

-Add atipamezole 0.25-1 mg/kg SC, IM, IV as reversal option for xylazine or dexmedetomidine (just an option)

-Add isoflurane/intubation/ventilation option

-Make it clear that buprenorphine would not be given until after animal intubated (because of risk of additive respiratory depression with induction drugs)

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Thursday, July 16, 2020 1:53 PM
To: Molly K. Lucas
Subject: Re: Abuzeid new protocol

Yeah, I think it would be a good one for the residents - it centers around creating a new model of sinusitis and then evaluating therapeutics. In general, I think the protocol is pretty well written and most of my comments thus far are minor.

Aubrey

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Thursday, July 16, 2020 1:47 PM
To: Aubrey Schoenleben <aubreys@uw.edu>
Subject: Abuzeid new protocol

Hi Aubrey,

I am looking for a protocol to assign to the residents to do a practice review on for a session a week from today, 7/23. I'm specifically hoping for a USDA spp, as up until now we've focused on rodents and fish. Do you think this protocol would be a reasonable choice? I know it's a work in progress and isn't in vet consult yet, but I thought that might be OK (at least it's not being held up).

If it's not a good choice for any reason, it looks like there are some other choices in there.

What do you think?

Molly

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, July 27, 2020 9:08 AM
To: Molly K. Lucas
Subject: Re: Abuzeid protocol

Sounds good! If Tuesday works better for you, I am free any time but 12-2:30pm.
Hope you had a good weekend,
Aubrey

From: Molly K. Lucas <mkucas@uw.edu>
Sent: Friday, July 24, 2020 6:50 PM
To: Aubrey Schoenleben <aubreys@uw.edu>
Subject: Re: Abuzeid protocol

Hi Aubrey,

I'll have to get back to you re: timing for either Monday or Tuesday. I'm going to work one day, and go for a day trip one day, and we haven't quite settled on which one is which yet.

Hope you have a nice weekend!

Molly

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Friday, July 24, 2020 2:32 PM
To: Molly K. Lucas <mkucas@uw.edu>
Subject: Re: Abuzeid protocol

Hi Molly,

Awesome - glad this protocol was good pick for the residents! It seems like a chat on the phone might be easiest. Do you have any availability on Monday? My schedule is wide open, so any time would be okay with me.

Thanks,
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To: Aubrey Schoenleben <aubreys@uw.edu>
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you rather I bullet out some ideas/questions in an email, or chat on the phone? I'm OK with either, although I won't be available today until ~3:30. I'm also around next week.

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Sent: Friday, July 24, 2020 6:51 PM
To: Aubrey Schoenleben
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From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, July 27, 2020 1:01 PM
To: Molly K. Lucas
Subject: Re: Abuzeid protocol

That sounds good - I'll give you a call around 2pm!
Aubrey

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Monday, July 27, 2020 12:09 PM
To: Aubrey Schoenleben <aubreys@uw.edu>
Subject: Re: Abuzeid protocol

RCW 42.56.250(4)

Hi Aubrey,

Actually this afternoon works great for me. How does ~2pm sound? My cell is 

Thanks,
Molly

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, July 27, 2020 9:07 AM
To: Molly K. Lucas <mklucas@uw.edu>
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From: Nicholas L. Reyes <nreyes@uw.edu>
Sent: Wednesday, August 19, 2020 4:17 PM
To: Molly K. Lucas
Subject: Re: Abuzeid rabbit sinusitis

Hey Molly,
I like the idea of you me and Alex. We should be able to have a good discussion. Sounds like a plan.
Thanks,
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University of Washington, Seattle
nreyes@uw.edu
206-543-0267

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From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, August 19, 2020 1:58:46 PM
To: Nicholas L. Reyes <nreyes@uw.edu>
Subject: Abuzeid rabbit sinusitis

Hi Nick,

I'm finally getting back to this. Rather than working on the normal questions for Hoverboard, I'm working on a Word doc of "discussion topics" for the group that we can discuss at the meeting, and then they can work in HB after that.

I'm thinking I'll ask Geena to help us set up a Zoom meeting with the PI and anyone else from the group (though may just be the PI), and then you, me, and either the anesthesia resident or the resident on ARCF (Alex). Sound OK? You/me/Alex plus the group? Anyone else?

Once I'm done with this doc I can share it with everyone and you may have things to add.

Molly

From: Molly K. Lucas <mklucas@uw.edu>
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To: Nicholas L. Reyes
Subject: Re: Abuzeid rabbit sinusitis

PS Unfortunately Alex wasn't here yet when we did this for protocol class (that was back in June), but that's OK.

From: Molly K. Lucas
Sent: Wednesday, August 19, 2020 1:58 PM
To: Nicholas L. Reyes <nlreyes@uw.edu>
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There are 36 total rabbits on the protocol, 8 for initial experiments, then 12 & 16 for the other two.

Molly

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, August 19, 2020 4:24 PM
To: Nicholas L. Reyes <nltreyes@uw.edu>
Subject: Fw: Abuzeid rabbit sinusitis

Great, I will work on the meeting! Here's my current draft of topics. Let me know if anything looks weird. I've had a lot of distractions today - in addition to the usual interruptions (family) we're having a bathroom repaired (leaky shower replaced). Fun fun!

Some of them are really not strong suggestions on my part - like whether to add local anesthetics for the surgery - so I'm open to any opinions/improvements/additions.

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To: Nicholas L. Reyes <nlreyes@uw.edu>
Subject: Abuzeid rabbit sinusitis

Hi Nick,

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I'm thinking I'll ask Geena to help us set up a Zoom meeting with the PI and anyone else from the group (though may just be the PI), and then you, me, and either the anesthesia resident or the resident on ARCF (Alex). Sound OK? You/me/Alex plus the group? Anyone else?

Once I'm done with this doc I can share it with everyone and you may have things to add.

Molly

From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/19/2020 2:47:25 PM
Subject: Re: Abuzeid rabbit sinusitis

PS Unfortunately Alex wasn't here yet when we did this for protocol class (that was back in June), but that's OK.

From: Molly K. Lucas
Sent: Wednesday, August 19, 2020 1:58 PM
To: Nicholas L. Reyes <nlreyes@uw.edu>
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From: "Molly K. Lucas" <mklucas@uw.edu>
To: Daniel Eldridge <deldrid@uw.edu>, "Jourdan E. Brune" <jourdi@uw.edu>, Kristin Zabrecky <zabrecky@uw.edu>, Leandra Mosca <lmosca@uw.edu>
CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, achris08 <achris08@uw.edu>, megellis <megellis@uw.edu>
Sent: 7/23/2020 1:13:19 PM
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

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Molly

From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 7/21/2020 8:41:55 PM
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

Sounds great!

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Sunday, July 19, 2020 9:14 AM
To: Molly K. Lucas <mklucas@uw.edu>
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

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From: Nicholas L. Reyes <nreyes@uw.edu>
Sent: Thursday, July 23, 2020 10:07 AM
To: Molly K. Lucas
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

Hey Molly,
I can't find the zoom link. can you resend it to me?
Nick

From: Molly K. Lucas <mkucas@uw.edu>
Sent: Tuesday, July 21, 2020 8:41 PM
To: Nicholas L. Reyes <nreyes@uw.edu>
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

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Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

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Molly

From: Leandra Mosca <lmosca@uw.edu>
Sent: Wednesday, July 22, 2020 1:53 PM
To: Molly K. Lucas
Subject: RE: Assignment for 7/23/20 - Questions due by 8pm 7/22
Attachments: Rabbits Sinuses Comments_LMosca.docx

Hi Molly,

My comments for this protocol are attached.

Thank you,
Leandra

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Friday, July 17, 2020 12:05 PM
To: Daniel Eldridge <deldrid@uw.edu>; Jourdan E. Brune <jourdi@uw.edu>; Kristin Zabrecky <zabrecky@uw.edu>; Leandra Mosca <lmosca@uw.edu>
Cc: Nicholas L. Reyes <nltreyes@uw.edu>; achris08 <achris08@uw.edu>; megellis <megellis@uw.edu>
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Let me know if you have any questions and hope you all have a good weekend,
Molly

Leandra Mosca
4502-01: Rabbit Sinus
Review Comments

Scientific Aims

1. Statement to address potential harm doesn't encompass the potential detrimental effects of the surgeries (that is bacterial inoculation, endoscopy, culture). There is potential harm in repeated anesthetic events and manipulation of the nasal sinuses beyond sinusitis.

Refinement, Replacement, and Reduction

1. Placement of an indwelling sinus catheter. Cetin et al., 2002 demonstrated nasal catheterization as a predisposing factor in the development of sinusitis and the increase of sinusitis development risk in relation with the catheterization period and the catheters' thickness was shown.

Experiments

Sinus Catheter/Endoscopy on Day 7:

1. How long do you expect this procedure to take? Depending on the length of time of this procedure, it may actually be most prudent to use inhalant anesthesia. The airway can be secured (e.g. intubation). The anesthetic plane can be more tightly controlled for a longer period of time. There will be challenges associated with the animal breathing for an extended period of time during manipulation of the nasal cavity. It's probably a superior option to intubate with the potential for ventilating the animal (e.g. providing oxygen even if apneic). Furthermore, this also allows for adjunct medications like buprenorphine to be given during the procedure, which I otherwise wouldn't recommend in an animal without a secured airway due to respiratory depressant side effects.
2. I would remove social isolation from how post-op pain will be assessed since these rabbits will be singly-housed.

Inoculation of Sinus and Administration of Substances to Nasal Passages

1. I would recommend including euthanasia criteria for severe respiratory distress, nasal passages blocked by mucous and pus or so severely swollen they cannot breathe, epiphora and conjunctivitis (or other signs that there is extension of disease into the tear ducts).
2. Please describe the rhinoscopy procedure in further detail. Position of animal, anterograde or retrograde approach, and how the animal will be positioned after the procedure (sternal with head support?). How will you prevent aspiration of nasal exudates?
3. How will you facilitate outflow of fluids used to flush nasal cavities (not just during procedure, but also during sample collection)?
4. Many nasal endoscopic procedures come with the risk of profuse bleeding and blood loss which may be countered with topical phenylephrine or epinephrine. I would recommend adding this anticipated challenge to the protocol as well as one of these substances.
5. Finally, I would recommend specifying that during endoscopy and culture, the "control" sinus will be explored first to avoid contaminating/inoculating the "clean" side.

Administration of Substances

1. Please change the routes of Buprenorphine and Carprofen to S.C. Repeated IM injections are stressful and painful, especially with the Buprenorphine being up to three times daily. The doses can remain as is. They should be mixed in the same syringe (OK to do) whenever possible to minimize injections.
2. If the dose route remains as is potential adverse reactions should include injection site reactions, muscle inflammation, and pain secondary to IM injections.

Anesthesia:

1. I recommend waiting until recovery to administer buprenorphine due to the potential respiratory depressant effects. While premedication with buprenorphine has been shown to significantly prolong the time of anesthesia induced by ketamine-medetomidine in rabbits (Gonzalez-Gil et al., 2015) I would be concerned about the rabbit going apneic during the study. Dexmedetomidine alone causes bradycardia and bradyapnea (due to decreased sympathetic tone) and this would amplify those effects,
2. Anesthetic reversal is advantageous by speeding up recovery to reduce the risk of hypothermia, which is a considerable problem associated with anesthesia in these species. I recommend adding an atipamezole dose S.C. (NOT IM like the anesthesia dose—S.C.) at the same dose volume as the preceding dose volume of dexmedetomidine (dose ranges 0.1 – 1.0 mg/kg, manufacturer recommends volume of atipamezole same volume as dex).

Survival Surgery:

1. Please specify more specifically what will be performed for the surgical scrub (ethanol and povidone-iodine x 3 scrubs). You may also need to specify that this scrub is performed before the surgeon is in sterile attire.

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Tuesday, July 21, 2020 8:42 PM
To: Nicholas L. Reyes
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

Sounds great!

Molly

From: Nicholas L. Reyes <nlreyes@uw.edu>
Sent: Sunday, July 19, 2020 9:14 AM
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Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

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Cc: Nicholas L. Reyes <nlreyes@uw.edu>; achris08 <achris08@uw.edu>; megellis <megellis@uw.edu>
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Let me know if you have any questions and hope you all have a good weekend,
Molly

From: Daniel Eldridge <deldrid@uw.edu>
Sent: Tuesday, July 21, 2020 2:56 PM
To: Molly K. Lucas
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22
Attachments: DE_Abuzeid protocol review 7:23:20.docx

Hi Molly,

See attached for class. See you Thursday.

-Daniel

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Friday, July 17, 2020 12:05 PM
To: Daniel Eldridge <deldrid@uw.edu>; Jourdan E. Brune <jourdi@uw.edu>; Kristin Zabrecky <zabrecky@uw.edu>; Leandra Mosca <lmosca@uw.edu>
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Protocol Review: Abuzeid 7/23/20

- Exp 1, Q5, item 1 – Please reword the anesthetic cocktail via IM injection to not include carprofen and buprenorphine. Carprofen and buprenorphine are generally administered subcutaneously. They should be administered as a separate injection (not a 4 drug cocktail).
- Exp 1, Q7 – Is there a reasoning for daily weight taking? Daily weight of the rabbits seems unnecessary and may increase stressful handling, and as worded, will include weekends and holidays. Consider taking weight 2 or 3 times weekly. Appetite will be monitored daily by husbandry staff. Will you also be monitoring for the clinical signs daily, including holiday and weekend? How often will you be checking rectal temperature?

For euthanasia criteria, consider removing item 4: “excessive shaking”

- Procedure: induction of anesthesia: Please reword the anesthetic cocktail via IM injection to not include carprofen and buprenorphine. Carprofen and buprenorphine are generally administered subcutaneously. They should be administered as a separate injection (not a 4 drug cocktail).

Carprofen dose should be changed to 1-2 mg/kg, not 5. Consider removing “triceps” as site of IM injection, as this will limit your options. Additionally, epaxial muscles are generally preferred site in rabbits.

Who on your team will be monitoring and inducing anesthesia? They must be certified to do so before that can happen.

How long will the endoscopy procedure last? You may want to consider adding in the option for redosing the rabbits if it takes longer than 30 minutes.

Please consider adding in reversal agent for dexmedetomidine (atipamezole).

- Procedure: Abuzeid analgesia: Change wording of buprenorphine administration to say “every 6-12 hours for 24 hours, and may continue up 72 hours as needed”. Endoscopy alone or with inoculation may not require buprenorphine for a full 72 hours.

Carprofen dose is too high – typically 1-2 mg/kg every 24 hours. Consider similar duration of administration similar to buprenorphine recommendation – depending on procedure.

Change route of carprofen administration to subcutaneous or oral. Also, consider adding meloxicam as an option – compounded oral formulations are available.

- Exp 1 question 10: Please elaborate on why the experiment will need to be duplicated.
- Exp 2 - Survival surgery – sinus catheter – Q2: Please add in sterile skin prep of the area between the ears where the catheter will exit.

Q6 – consider reducing frequency of antibiotic ointment to once daily to reduce stressful handling.

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Sent: Thursday, July 23, 2020 2:20 PM
To: Molly K. Lucas
Subject: Re: Assignment for 7/23/20 - Questions due by 8pm 7/22

The soft tissues over the bone are minimal but they currently propose doing quite a bit of tunneling and they will need to undermine the soft tissue to create a bone flap-so I think a local is a good idea.
Jourdan

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Sent: Thursday, July 23, 2020 1:13 PM
To: Daniel Eldridge <deldrid@uw.edu>; Jourdan E. Brune <jourdi@uw.edu>; Kristin Zabrecky <zabrecky@uw.edu>; Leandra Mosca <lmosca@uw.edu>
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From: Geena Gallardo <gallardg@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/20/2020 2:01:30 PM
Subject: RE: Doodle: "Protocol Discussion with PI Abuzeid" Update

Attach: [EMB4_image001.gif]

Nevermind! Thea says stay in offices. :P

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu

W UNIVERSITY of WASHINGTON

From: Geena Gallardo
Sent: Thursday, August 20, 2020 1:42 PM
To: Nick Reyes <nlreyes@uw.edu>
Subject: RE: Doodle: "Protocol Discussion with PI Abuzeid" Update

Also, I set up the conference room for the faculty meeting, feel free to join us in there if you'd like.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
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W UNIVERSITY of WASHINGTON

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Sent: Thursday, August 20, 2020 1:37 PM
To: Nick Reyes <nlreyes@uw.edu>
Subject: FW: Doodle: "Protocol Discussion with PI Abuzeid" Update

Okay – over achiever. Lol!

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb

Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
gallardg@uw.edu

W UNIVERSITY of WASHINGTON

From: Doodle [<mailto:mailer@doodle.com>]
Sent: Thursday, August 20, 2020 1:36 PM
To: Geena Gallardo <gallardg@uw.edu>
Subject: Doodle: "Protocol Discussion with PI Abuzeid" Update

Nick just participated.

Doodle

Hi Geena Lappin,

Nick just participated in the Doodle poll **Protocol Discussion with PI Abuzeid**.

| [Go to your poll](#) | [Close poll and send calendar invitation](#)

Best wishes,
The Doodle Team

See how easy it is to find a time to get people together when you use Doodle.

Create a Doodle now

Doodle

Doodle AG, Werdstrasse 21, 8021 Zürich

[Why did I receive this email?](#)

[Unsubscribe](#)

From: Geena Gallardo <gallardg@uw.edu>
To: "Nicholas L. Reyes" <nireyes@uw.edu>
Sent: 8/20/2020 1:42:07 PM
Subject: RE: Doodle: "Protocol Discussion with PI Abuzeid" Update

Attach: [EMB4_image001.gif]

Also, I set up the conference room for the faculty meeting, feel free to join us in there if you'd like.

Best wishes,

Geena Lappin

Assistant to the Chair, Dr. Thea Brabb
Department of Comparative Medicine

Health Sciences Bldg Box 357340
Seattle, WA 98195-7340
206-221-3396
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Doodle AG, Werdstrasse 21, 8021 Zürich

[Why did I receive this email?](#)

[Unsubscribe](#)

From: wabuzeid <wabuzeid@uw.edu>
Sent: Friday, May 29, 2020 5:23 PM
To: Aubrey Schoenleben
Subject: RE: New animal protocol submission

Great. I'll await your Zoom invite for 11:30 am on Monday. Thank you and have an enjoyable weekend.

Best,

Waleed

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Friday, May 29, 2020 5:22 PM
To: wabuzeid <wabuzeid@uw.edu>
Subject: Re: New animal protocol submission

Hi Waleed,

11:30am works fine. Everyone in our office is working remotely these days. I will send you a calendar invite with a link for a zoom meeting so that we can screen share.

For future reference, once we return to a more normal pace, our office is located in the Health Sciences Building, room T254.

Take care,
Aubrey

Sent from my iPhone

On May 29, 2020, at 3:31 PM, wabuzeid <wabuzeid@uw.edu> wrote:

That would be perfect. Any chance we can push to 11:30 am on Monday? If not, I can make 11 am work. Where is your office located?

Best,

Waleed Abuzeid

Sent from [Outlook Mobile](#)

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Friday, May 29, 2020 2:18:31 PM
To: wabuzeid <wabuzeid@uw.edu>
Subject: Re: New animal protocol submission

Hi Waleed,

My Monday is also quite open. Would 11am work well?

Cheers,
Aubrey

From: wabuzeit <wabuzeit@uw.edu>
Sent: Thursday, May 28, 2020 5:20 PM
To: Ashley E. Williams <aew33@uw.edu>; Aubrey Schoenleben <aubreys@uw.edu>
Cc: Kailee McGeer <kmcgeer@uw.edu>; Matthew R. Parsek <parsem@uw.edu>
Subject: Re: New animal protocol submission

Hi Aubrey and Ashley,

Thank you for the thoughtful response.

Aubrey: My availability is fairly reasonable at the moment but dwindling everyday as my clinic volume picks up. As it happens, I do have availability all day tomorrow as well as on Monday. I, generally, have availability every Monday. Please let me know what works for you.

Ashley: thanks for setting up the account. I confirmed that I can access Hoverboard and will sign up for training including the hands on portion as this project does involve direct work with the rabbits.

Thanks again,

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Sent from [Outlook Mobile](#)

From: Ashley E. Williams <aew33@uw.edu>
Sent: Thursday, May 28, 2020, 10:46 AM
To: wabuzeit
Cc: Kailee McGeer; Matthew R. Parsek; Pradeep Singh; Aubrey Schoenleben
Subject: RE: New animal protocol submission

Hi Dr. Abuzeit,

Welcome to UW! I have set up an active account for you in [HoverBoard](#). You should now be able to access the site. Please let me know if you still receive the internal server error.

We do have resources located in the [HoverBoard Help Center](#). I would also strongly recommend an orientation with Aubrey, since having a guide through HoverBoard makes the process much easier.

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- [Animal Use Laws and Regulations Training course and exam](#)
- [Rabbit Hands-on Laboratory](#) (if you are working directly with rabbits) – email auts@uw.edu to enroll

Please feel free to let me know if you have any questions at all.

Sincerely,

Ashley Williams

Administrative Specialist

Office of Animal Welfare Research Support Services

Health Sciences Building Box 357160

1705 NE Pacific Street Seattle, WA 98195-7160

206.685.7363 fax 206.616.1297

auts@uw.edu / oaw.washington.edu

<image001.jpg>

<image002.jpg>

Dare 2 Care... | explore [UW's Compassion Fatigue Program](#)

From: Aubrey Schoenleben <aubreys@uw.edu>

Sent: Thursday, May 28, 2020 8:41 AM

To: wabuzeid <wabuzeid@uw.edu>; Ashley E. Williams <aew33@uw.edu>

Cc: Kailee McGeer <kmcgeer@uw.edu>; Matthew R. Parsek <parsem@uw.edu>; Pradeep Singh <singhpr@uw.edu>

Subject: Re: New animal protocol submission

Hi Dr. Abuzeid,

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Ashley - Dr. Abuzeid is a new faculty member in the Department of Otolaryngology. Could you please help him get an account set up in HoverBoard?

Many thanks,
Aubrey

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Cc: Kailee McGeer <kmcgeer@uw.edu>; Matthew R. Parsek <parsem@uw.edu>; Pradeep Singh <singhpr@uw.edu>
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Would you mind helping me clarify the protocol submission process so that we may facilitate appropriate review?

Thank you,

Waleed
Waleed M. Abuzeid, MD
Associate Professor
Rhinology and Endoscopic Skull Base Surgery
Department of Otolaryngology: Head and Neck Surgery
University of Washington
Email: wabuzeid@uw.edu

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, June 8, 2020 6:12 PM
To: Waleed M Abuzeid
Cc: Michelle Brot
Subject: Re: New animal protocol submission

Hi Waleed,

It really nice talking with you last week! Just following up on a couple of questions that come up during our conversation:

- In addition to an IACUC protocol, you will need to get a Biological Use Authorization (BUA) in place since your work includes the use of *Pseudomonas*. The BUA review process is overseen by our partner department, Environmental Health & Safety. You can find the BUA application and more about the BUA review process [here](#).
- As I mentioned, your OAW team will be the Purple Team, which consists of myself and Michelle Brot (cc'd above so that you have her contact information as well). We can help you with all things animal related, including protocol review, grant/protocol congruence and periodic post-approval monitoring meetings.

Please don't hesitate to get in touch with any questions or if you need help getting your protocol together.

Take care,
Aubrey

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<image001.jpg>

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Waleed

Waleed M. Abuzeid, MD

Associate Professor

Rhinology and Endoscopic Skull Base Surgery

Department of Otolaryngology: Head and Neck Surgery

University of Washington

Email: wabuzeid@uw.edu

From: wabuzeit <wabuzeit@uw.edu>
Sent: Monday, September 7, 2020 9:49 PM
To: Molly K. Lucas
Cc: Nicholas L. Reyes; Aubrey Schoenleben
Subject: RE: Notes from today's Zoom vet consult (Abuzeid protocol)

Thanks for the comprehensive notes, Molly. All discussed changes have been integrated into the protocol which has been resubmitted for pre-review.

Waleed

From: wabuzeit <wabuzeit@uw.edu>
Sent: Friday, September 4, 2020 8:16 AM
To: Aubrey Schoenleben <aubreys@uw.edu>; Molly K. Lucas <mkucas@uw.edu>
Cc: Nicholas L. Reyes <nireyes@uw.edu>
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

It was a very helpful conversation. I'll work on integrating the changes this weekend with a view of expediting review given your upcoming leave, Aubrey.

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From: Aubrey Schoenleben <aubreys@uw.edu>
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To: Molly K. Lucas; wabuzeit
Cc: Nicholas L. Reyes
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

Thanks, Molly!

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Cheers,
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From: Molly K. Lucas <mkucas@uw.edu>
Sent: Monday, August 31, 2020 3:56 PM
To: wabuzeit <wabuzeit@uw.edu>
Cc: Nicholas L. Reyes <nireyes@uw.edu>; Aubrey Schoenleben <aubreys@uw.edu>
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Sincerely,
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From: wabuzeit <wabuzeit@uw.edu>
To: "Molly K. Lucas" <mklucas@uw.edu>
CC: "Nicholas L. Reyes" <nlreyes@uw.edu>, Aubrey Schoenleben <aubreys@uw.edu>
Sent: 9/7/2020 9:49:02 PM
Subject: RE: Notes from today's Zoom vet consult (Abuzeid protocol)

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CC: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 9/4/2020 8:15:35 AM
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

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To: "Molly K. Lucas" <mklucas@uw.edu>, wabuzeit <wabuzeit@uw.edu>
CC: "Nicholas L. Reyes" <nltreys@uw.edu>
Sent: 9/4/2020 8:04:50 AM
Subject: Re: Notes from today's Zoom vet consult (Abuzeid protocol)

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From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Tuesday, September 29, 2020 6:59 AM
To: Waleed M Abuzeid
Cc: Kim Stocking
Subject: Re: Protocol Update

You're welcome, Waleed!

Happy to hear that you're up for presenting at one of our IACUC meetings. Meetings are always on the third Thursday of the month. Would presenting at the November (11/19) or December (12/17) IACUC meeting be a possibility?

Cheers,
Aubrey

On 9/23/20, 10:14 AM, "wabuzeid" <wabuzeid@uw.edu> wrote:

Hi Aubrey,

Thanks for all your help getting the protocol ready for IACUC review. There's no way this would have been do-able without your input!

As far as the presentation, I would be happy to present the sinusitis model but am already committed to a full clinic on 10/15. I can see two potential options: 1) Present earlier that day between 12:30 pm – 1:00 pm (during a break in the clinic session) or 2) Present on a different day if possible. Typically, I am available every Monday as this is my designated non-clinical research time!

Do either of those options work?

Thanks,

Waleed

From: Aubrey Schoenleben <aubreys@uw.edu>
Sent: Monday, September 21, 2020 11:59 AM
To: wabuzeid <wabuzeid@uw.edu>
Cc: Kim Stocking <kstock@uw.edu>
Subject: Protocol Update

Hi Waleed,

How are you? I wanted to pass along an update on your protocol. The last round of revisions looked good, so we included your protocol in the committee packet that was sent out on Friday. The IACUC will have one week to review the protocol and pose any questions. The review period closes on 9/25. We will send you any questions at that time.

I also wanted to reach out to see if you would be available/interested in presenting on the rabbit sinusitis model at our next IACUC meeting. To help keep the committee informed about the wide range of research taking place at UW, we often like to have researchers present on new models or projects.

The presentation wouldn't need to be long, typically ~20 minutes. The October IACUC meeting will be held virtually and is scheduled for 10/15 at 2:30pm.

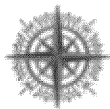
Since my due date is fast approaching, I'm also including the OAW director/AV, Kim Stocking, on this email. If needed, Kim can help with IACUC meeting arrangements.

Let me know your thoughts, and thank you again for all your hard work on this protocol!

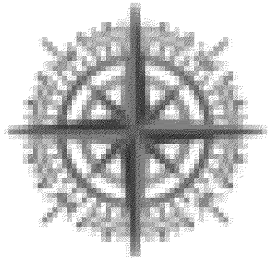
Cheers,
Aubrey

AUBREY SCHOENLEBEN, PhD, CPIA
Scientific Liaison & Review Scientist
Office of Animal Welfare

Health Sciences Building, Box 357160
1705 NE Pacific Street, Seattle, WA 98195-7160
vm: 206.685.6923 / fax: 206.616.5664
aubreys@uw.edu / oaw.washington.edu



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Research Support Services



OFFICE OF ANIMAL WELFARE

Research Support Services

From: Jourdan E. Brune <jourdi@uw.edu>
Sent: Wednesday, July 22, 2020 7:46 PM
To: Molly K. Lucas
Subject: Re: reminder - assignment due by 8pm
Attachments: Jourdan Brune_Abuzeid_Protocol Review.docx

Attached.
Jourdan

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, July 22, 2020 2:27 PM
To: Molly K. Lucas <mklucas@uw.edu>
Cc: Jourdan E. Brune <jourdi@uw.edu>; Kristin Zabrecky <zabrecky@uw.edu>
Subject: reminder - assignment due by 8pm

Thanks,
Molly

General comments:

- 1) Minor comment-under goals and significance, Q2 expense should not be used as a justification for selection of an animal model. Please remove this language and replace it explaining that rabbits are appropriate and the least sentient animal for the work proposed.
- 2) Under question 3 please briefly describe the monitoring of rabbit sinusitis symptoms and the establishment of humane endpoints for your model if significant morbidity is expected. If spontaneous mortality is expected, please address this here.
- 3) Approximately 20% of the animal numbers you requested are for training purposes. It may be best to organize and account for these animals in a separate experiment as their experience with regards to experimental manipulations are extensive and beyond the scope of Q3 which is typically used to described terminal non-survival surgeries. The 4 “practice” animals requested for experiment 1 and the proposed use for them read more like a pilot experiment than use for specific training of personnel.

Procedures:

- 1) Nanoparticle Administration
 - a. As you are intending to instill the particles into the sinus, please provide a volume that you intend to instill.
- 2) Induction of Anesthesia
 - a. Do you intend to perform the anesthesia in these rabbits yourself or hire vet services for these procedures?
 - b. Have you consulted with vet services staff regarding the administration of these drugs?
 - c. Please remove specific identification of the muscle that will be used for injection from the description.
 - d. Intramuscular injection is not absolutely required for buprenorphine and carprofen administration in this protocol. I recommend switching these to subcutaneous administration to reduce the volumes administered to the muscle. Addition of saline to increase the volume of these drugs is also contraindicated.
 - e. Emergency intubation is very difficult to perform in rabbits. Please describe that it will be attempted and that flow by oxygen will be available.
 - f. Please complete question 4 for all anesthetic agents being used by listing their known side effects and how monitoring of the rabbit will be performed to identify abnormalities early so they can be remedied. It is not appropriate to say “see question 4” and have question 4 read “NA” when there are well established side effects of anesthetic drugs listed.
- 3) Placement of Sinus Irrigating Catheter
 - a. Rabbits that are intermittently inappetant may not need to be removed from study. Often rabbits may avoid eating their pellet ration but still eat hay or preferred enrichment foods or nutritional support supplements. I would recommend not listing inappetence > 24 hours as a humane endpoint.

- b. Please describe the aseptic prep of the surgical site to include three alternating scrubs with isopropyl alcohol and povidine iodine solution.
 - c. Please complete the duration and expected deficits for this procedure. Rabbits are obligate nasal breathers, please comment if you expect any deficits based on your catheter's intended placement into the maxillary sinus.
 - d. Please describe how often rabbits will be monitored post-op.
- 4) Nasal Endoscopy
 - a. Please complete procedure preparation.
- 5) Sinus Catheter
 - a. Please describe if the catheter will be intermittently flushed to maintain patency under Q3.
 - b. Will sterile catheters be purchased and used a single time?
 - c. Please comment on your plan should catheters become inadvertently removed from the sinus while rabbits are on study. Do you anticipate performing a repair procedure if this were to happen? Would the rabbit be removed from study?

From: Kristin Zabrecky <zabrecky@uw.edu>
Sent: Wednesday, July 22, 2020 2:40 PM
To: Molly K. Lucas
Subject: Re: reminder - assignment due by 8pm
Attachments: protocol questions 7.23.20.docx

Just finished it 😊

Kristin

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, July 22, 2020 2:27 PM
To: Molly K. Lucas <mklucas@uw.edu>
Cc: Jourdan E. Brune <jourdi@uw.edu>; Kristin Zabrecky <zabrecky@uw.edu>
Subject: reminder - assignment due by 8pm

Thanks,
Molly

Protocol Team Members: Is there a member that will be handling the rabbits? Currently, there is no one listed to handle animals on the protocol.

Needed: surgery certification, all group members having rabbit hands on laboratory.

Scientific Aims: Question 3

What are the potential harms associated with the surgery and indwelling catheter?

Experiment 1

Question 7: Do you expect weight loss with sinusitis infection in rabbits? When do you expect to see increases in temperature? Daily weighing and temperatures can be stressful for rabbits, and if not needed, can be reduced to 2 or 3 times per week to reduce stress.

Question 10: Please check your power calculations, based on an effect size of 0.15log 10, I calculated the need for 8 rabbits per group. (the same calculation is used in experiment 2)

Removal criteria: the temperature ranges listed through the experiments and procedures for euthanasia criteria are too wide (33-44C). The normal temperature range for a rabbit is 38-40C or 100.4-104F. You should give yourself some room for variation among rabbits, but I would recommend lower than 99F or higher than 106F.

Procedure: Analgesia, Buprenorphine and Carprofen

Based on the description of the endoscope procedures, I would classify it as a category 1. To help reduce the stress of handling, I would suggest reducing the opioid and NSAID administration for the endoscope procedures for 12hr with the option to redo the NSAID if signs of pain observed. Also, for Carprofen injections in rabbits, we recommend a dose of 1-2mg/kg SQ every 12 hours.

Procedure: Induction of Anesthesia:

Question 2: How will the rabbits be monitored under anesthesia? You say you will inject IM into the triceps – I would recommend the lumbar epaxial muscles for ease of access and administration; however to make the protocol more flexible, you do not need to state what muscle you will be injecting into.

Question 4: How will you be performing rabbit intubation as this is a difficult procedure?

Dosing for the drugs: [I would like to discuss this further with everyone in class. The Carprofen dose is too high. The Dex dose seems low, and the Ketamine dose seems low when looking at other Ketamine anesthetic combos for rabbits. I did find a reference that uses Ketamine (35mg/kg), medetomidine (0.5mg/kg) and Buprenorphine (0.3mg/kg). This paper compares the use of this to Ketamine (35mg/kg) and Xylazine (5mg/kg). I also found another paper that looked at ketamine (35 mg/kg) and dexmedetomidine (0.25 mg/kg) without or with buprenorphine (0.03 mg/kg). Both the KX and KDB provided over an hour of anesthesia.]

Experiment 2

Substance Administration, Nanoparticles How long is the flushing and restraint? [with rabbits being obligate nasal breathers, is there time of rest between flushings?]

Survival Surgery:

Analgesia recommendations as described above – recommend at least 24 to 48 hours of analgesia. Carprofen dose is too high. SQ recommended.

Question 3: Will the catheter end be covered for protection while not in use?

Question 4: “inappetence >24 hours will be removed from the research study.” I don’t think this is necessary, rabbits can go off feed for stress related reasons. I would make this statement more broad indicating in consultation with Veterinary Services.

Question 5: Describe in further detail anesthetic monitoring. Will you have continuous monitoring through machines or manually monitoring? (ei heart rate measurements). Other anesthetic depth measurements: respiratory rate, heart rate.

Question 6: Is the antibiotic ointment necessary?

Husbandry Exceptions: Will the rabbits need to be singly housed following catheter placement? If so, this is a husbandry exception since our standard is social housing.

Animal Details:

Question 3. A. – Please be specific on method of identification.

KZ musings:

Refinement, Replacement and Reduction

“We considered the use of inhalational anesthetics but, given the expected duration of the catheter placement surgical procedure and to minimize the risk of respiratory depression and potential death, non-inhalational agents were instead selected.” – interesting... I assumed they chose injectable due to the need to assess the nasal cavity.

“Though the proposed rabbit model does not harbor mutations in the CFTR gene, it does allow for establishing chronic infections in the sinuses and the experience gained will be critical for harnessing such models when they are developed in the future. Preliminary CF rabbit models are in the process of being developed and demonstrate physiologic characteristics that, in many ways, mimics human CF.” – the beginning of the experiment made it seem like they were going to use a CF rabbit model.