

**Marquette University
Institutional Biosafety Committee**

MINUTES

IBC Member Roster: Mr. Austin Fritsch (Voting Contact), Mr. Dennis Daye (Member), Dr. Michael Schlappi (Plant Expert), Dr. Murray Blackmore (Chair, Animal Expert), Dr. Krassimira R. Hristova (Member), Dr. Edward M. Blumenthal (Member, Vice Chair), Dr. M. Behnam Ghasemzadeh (Animal Expert), Mr. Jason M. Keaton (Biosafety Officer), Dr. Allison E. Reeme (Member), Mr. Jerome Donohoe (Non-Affiliated), Mr. Eli Colina (Non-Affiliated), Ms. Rebecca A. Seevers (Non-Affiliated)

Present: Mr. Austin Fritsch (Voting Contact), Mr. Dennis Daye (Member), Dr. Michael Schlappi (Plant Expert), Dr. Murray Blackmore (Chair, Animal Expert), Dr. M. Behnam Ghasemzadeh (Animal Expert), Mr. Jason M. Keaton (Biosafety Officer), Dr. Allison E. Reeme (Member), Mr. Jerome Donohoe (Non-Affiliated), Mr. Eli Colina (Non-Affiliated)

Absent: Dr. Edward M. Blumenthal (Member, Vice Chair), Dr. Krassimira R. Hristova (Member), Ms. Rebecca A. Seevers (Non-Affiliated)

Guests: Dr. Kristi Streeter, Mr. Nicholas (Nick) Raddatz, Dr. Anthony (Tony) Gamble

- I. MEETING DATE:** Friday, August 15th, 2025
- II. MEETING TYPE:** In-Person
- III. MEETING STATUS:** Open
- IV. QUORUM:** Present
- V. CALL TO ORDER:** 10:04 AM
- VI. CONFLICTS OF INTEREST:** No conflicts of interest were observed.
- VII. ANNOUNCEMENTS:** N/A
- VIII. REVIEW MEETING MINUTES**
 - A.** July 2025 Meeting Minutes
 - i.** Motion to approve the meeting minutes as written:
 - Approve: 6, Deny: 0, Abstain: 3
 - Conflicts of interest: N/A
- IX. NEW PROTOCOL(S)**
 - A.** N/A
- X. THREE YEAR RENEWAL(S)**
 - A.** Kristi Streeter; PT; #5124: "Phrenic afferents and diaphragm pacing-induced recovery of breathing following spinal cord injury"
 - i.** Project overview: The project involves the use of adeno associated viruses (AAVs), non-toxic cholera toxin B (CTB) fragments, and small

interfering RNA (siRNA) in rats for the purpose of labeling and manipulating activity within respiratory neurons. The utilized AAVs contain synthetic components as well nucleic acid sequences sourced from coral, jellyfish, green algae, and muscarinic receptors. These AAVs serve to transduce fluorescent proteins as well as chemogenetic and optogenetic actuators in cells of interest. The nucleic acid sequences present in the siRNA are synthetic in nature, but based on rat sequences for brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TRKB). The outlined materials pose minimal risk to humans, animals, and the environment due to their non-pathogenic and non-hazardous nature, as well as replication incompetence in the absence of a helper virus. For this reason, they can be handled under BSL-1 conditions.

ii. Discussion:

- **Training verification:** The training of the listed personnel was verified. All listed individuals completed the initial required Biosafety 101 Training which includes the Animal Biosafety CITI training course, as well as Animal Care and Use Training.
- **Applicable section(s) of the NIH Guidelines:** The committee determined that sections III-D-4, III-E-1, III-E-3, and III-F-8 Appendix C-VII are applicable.
- **Collaborator Involvement:** The committee inquired about the collaborator's role in the project. They were informed that the collaborator's role is limited to providing equipment and expertise.
- **PPE Disposal:** The PPE disposal procedures were reviewed and determined as appropriate by the committee.
- **Decontamination Procedures:** The decontamination procedures were reviewed by the committee. It was noted that the procedures outlined in the attachment do not match the procedures outlined in the registration, and must be modified to exclude the autoclaving of bleach-exposed items.

iii. Required changes:

- **"Personnel" Section**
 - Ensure that all listed personnel complete any expired CITI modules required for Biosafety 101 Training.
- **"Project Details" Section**
 - Specify that the collaborator's role is limited to providing equipment and expertise, and will not involve any direct contact with animals.
 - Clarify the biohazard bin disposal procedure, including the location of disposal.
- **"Attachments" Section**
 - Modify the decontamination procedures outlined in the attachment to ensure that they match with the procedures described in the registration.
- **"Health Surveillance" Section**

- Choose “yes” in answer to “do you monitor the health of personnel listed on this protocol?” and answer the subsequent questions, indicating that all individuals working under this registration complete the Occupational Health and Safety risk assessment as well as the yearly follow-up.
- iv. Motion to provisionally approve the protocol subject to the provisions discussed following the review of the designated reviewer:
 - Approve: 9 , Deny: 0, Abstain: 0
 - Conflicts of interest: None
- B. Nicholas Raddatz; BISC; #5129: “The Role of Extrasynaptic Glutamate Transmission in Neuron-Astrocyte Communications”
 - i. Project overview: The project seeks to manipulate the activity of specific cells of interest in order to gain a better understanding of the glutamate system. Breeding of transgenic rats and the use of AAVs in rats will take place as part of the project. AAVs expressing biosensors, fluorophores, peptides, and short hairpin RNA (shRNA) sequences designed to modulate protein expressions will be administered to anesthetized rats via intracranial infusion. The utilized materials pose minimal risk to humans, animals, and the environment due to their non-pathogenic and non-hazardous nature, and replication incompetence in the absence of a helper virus. For this reason, they can be handled under BSL-1 conditions.
 - ii. Discussion:
 - **Training verification:** The training of the listed personnel was verified. All listed individuals completed the initial required Biosafety 101 Training which includes the Animal Biosafety CITI training course, as well as Animal Care and Use Training.
 - **Applicable section(s) of the NIH Guidelines:** The committee determined that sections III-D-4 and III-E-1 are applicable.
 - **shRNA Homology:** The committee inquired about the degree of homology between the shRNA sequences and the human genome. It was noted that the utilized shRNAs exhibit approximately 90% homology with the human genome for the Pituitary Adenylate Cyclase-activating Polypeptide Type I Receptor (PAC1) receptor.
 - **PI Listing Change:** The committee discussed whether the lab manager can assume responsibility for the registration and be listed as the PI. It was determined that this arrangement is permissible. To ensure clarity, the fiscal and regulatory obligations associated with serving as PI were communicated to the lab manager.
 - **Decontamination Procedures:** The committee reviewed and discussed the decontamination procedures. The location of the

autoclave and the sterility validation methods for the autoclave were shared with the committee upon request. It was determined that the decontamination procedures subsection should be revised to ensure a 1:1 match with current lab practices.

- **Waste Disposal Procedures:** The committee reviewed the waste disposal procedures. It was recommended the section be revised to include additional details on the disposal of animal carcasses.
- **Surgical Space:** The committee inquired about the equipment of the surgical space. It was noted that the space is equipped with stainless steel downdraft tables, eyewash stations, biohazardous waste receptacles, and sharps containers.
- **Surgical PPE:** The committee reviewed the PPE utilized during the surgical procedures and determined that it is appropriate.
- **Centrifuging Procedures:** The committee reviewed the centrifuging procedures. They were informed that the centrifuge safety cups are opened within the biosafety cabinet to ensure that personnel exposure to aerosols is minimized.

iii.

Required changes:

- **“Personnel” Section**
 - Remove the current PI from the “People” section and list the lab manager as the new PI.
 - Updated “ARC/CITI Biosafety training” to “IBC Biosafety 101 Training.”
 - Include additional details on how procedural proficiency is assessed for new trainees.
- **“Viral Vectors” Section**
 - Update all references to the NIH Guidelines to reflect the use of the revised version published in 2024.
 - Change “low immunogenicity” to “low pathogenicity” in reference to the utilized AAVs.
- **“Project Details” Section**
 - Specify the location of the autoclave used for decontamination.
 - Provide additional information about the surgical space, including the presence of a stainless-steel downdraft workstation, eyewash station, sharps containers, and biohazardous waste receptacles.
 - Clearly outline the PPE requirements specific to surgical procedures and distinguish them from the PPE required for all other listed activities.
 - Familiarize yourself with the updated autoclaving policy and align text with its content.
 - Revise waste disposal procedures to indicate that animal carcasses are to be transported to the ARC for collection and storage, include the room number of the collection

site, and state that final disposal is conducted by incineration through a contracted company.

- Revise the decontamination procedures for surgical instruments to specify that bleach will not be used on instruments that will be decontaminated by autoclaving.
- Include a statement conveying that spills and accidental exposures will be reported to the Marquette University IBC.
- Specify that centrifuge cups are opened in the lab's biosafety cabinet.

- **“Health Surveillance” Section**

- Choose “yes” in answer to “do you monitor the health of personnel listed on this protocol?” and answer the subsequent questions, indicating that all individuals working under this registration complete the Occupational Health and Safety risk assessment as well as the yearly follow-up.
- Motion to provisionally approve the protocol subject to the provisions discussed following the review of the designated member reviewer:
 - Approve: 9 , Deny: 0, Abstain: 0
 - Conflicts of interest: None

C. Anthony Gamble, BIOL; #5116: “Collaborative Research: EDGE FGT: DEVELOPMENT OF FUNCTIONAL GENETIC TOOLS IN GECKOS

- i. Project overview: The project is funded by an NIH grant, and aims to develop functional genetic tools for geckos. CRISPR/Cas reagents consisting of the combined guide RNA (gRNA) and Cas9 nuclease complex will be injected into the oocytes of female geckos via microinjection to generate targeted gene knockouts in the offspring. Although based on naturally occurring sequences, the utilized gRNA are synthetic in nature. The target gene regions relate to color and pattern, limb development, or sexual development. The utilized materials are not virulent or pathogenic. Furthermore, the utilized RNAs lack exact matches with the human genome. For this reason, the materials pose no dangers to humans, animals, or the environment, and can be handled under BSL-1 conditions.

ii. Discussion:

- **Training verification:** The training of the listed personnel was reviewed. It was noted that two of the added individuals did not complete the initial required biosafety 101 training. The researcher was notified that all listed individuals must complete biosafety 101 training and as well as any expired training modules before approval can be secured.
- **Applicable section(s) of the NIH Guidelines:** The committee determined that section III-D-4 is applicable.

- **Human Genome Mismatches:** The committee discussed the mismatch between the utilized gRNA and the human genome. It was noted that the utilized gRNAs have a 3 base pair difference from potential human targets as determined by a Basic Local Alignment Search Tool (BLAST) search. The committee determined that a 3 base pair mismatch is acceptable given the nature of the targeted sequences.
- **Off-Target Effects:** The committee discussed the potential off-target effects associated with the gene-editing procedure. It was recommended that the registration be amended to specify a requirement of at least six mismatches outside the target region.
- **Environmental Release:** The committee discussed the possibility of environmental release. It was noted that the manipulated animals are manipulated in secondary containers and maintained in secure cages within sealed, restricted-access rooms designed to prevent escape. In addition, the building in which they are housed is equipped with glue traps to further reduce the risk of escape. Finally, the geckos are not native to Wisconsin and are incapable of surviving the winter climate. For these reasons, the likelihood of environmental release is low.
- **Escape Incidents:** The committee inquired about any prior instances of gecko escape. The PI confirmed that no such incidents have taken place.
- **Capture Training:** The committee reviewed the training provided by the PI on the capture of escaped geckos and determined it to be acceptable.

iii. Required changes:

- **“People” Section**
 - Ensure that all listed individuals complete Biosafety 101 Training as well as any expired training modules.
 - Ensure that the phone number is included for all listed individuals.
- **“Project Details” Section**
 - Remove mentions of 2% Chlorhexidine Gluconate to ensure alignment with the corresponding IACUC protocol.
 - Provide an assessment of the likelihood of an injection into the nucleus of a cell of the lab personnel, as well as any possible effect on the cell resulting from the manipulation.
 - Reference the attachment outlining the proposed procedures in the body of the registration.
 - Describe the aliquoting procedure.
 - Specify that the gRNAs must be designed to ensure a minimum of six base pair mismatches with the human genome outside the intended target region.

- In the body of the registration, provide a brief overview of the experimental procedures to accompany the attachment outlining the procedural details.
- Delete surgical procedure details from the “Facilities Description” subsection.
- Provide additional information about the facility to convey that it is appropriately equipped for the proposed work (e.g., address presence of sink, eyewash station, etc.)
- Reference the location of the freezer used to store animal carcasses.
- Revise PPE subsection to indicate that face mask and hair nets will be worn during surgical procedures.
- Specify where and how PPE is stored.
- Address the removal of PPE, noting that gloves and other PPE must be removed in a manner that minimizes personnel contamination and transfer of infection materials.
- Specify that the sharps containers will be disposed of in the biohazardous waste stream.
- Specify the disposal method for PPE that comes in contact with biohazardous substances.
- Switch to an EPA-approved disinfectant such as Rescue and specify the contact time.
- Include a spill response procedure.
- Explain why the risk of environmental release is minimal.
- Provide additional details regarding the transportation of the utilized biomaterials, such as the delivery location, location in which the packages are opened, and the location in which the materials are stored.

• **“Health Surveillance” Section**

- Choose “yes” in answer to “do you monitor the health of personnel listed on this protocol?” and answer the subsequent questions, indicating that all individuals working under this registration complete the Occupational Health and Safety risk assessment as well as the yearly follow-up.

iv. Motion to provisionally approve the protocol subject to the provisions discussed following the review of the designated member reviewer:

- Approve: 9 , Deny: 0, Abstain: 0
- Conflicts of interest: None

XI. MODIFICATION(S)

A. N/A

XII. DESIGNATED REVIEW(S)

A. New protocol(s)

i. N/A

- B.** Three year renewal(s)
 - i.** Nick Raddatz, BISC; #5129: "The Role of Extrasynaptic Glutamate Transmission in Neuron-Astrocyte Communication"
 - ii.** Kristi Streeter, PT; #5124: "Phrenic afferents and diaphragm pacing-induced recovery of breathing following spinal cord injury"
- C.** Modification(s)
 - i.** Murray Blackmore, BISC; #4598: "Combinatorial Manipulation of Transcription Factors to Promote CNS Regeneration"
 - ii.** Lisa Petrella, BIOL; #4589: "Temperature-sensitive germline structures and temperature thresholds of fertility in *Caenorhabditis nematodes*"
- XIII. TERMINATION(S)**
 - A.** Krassimira Hristova, BIOL; BR-211: "Investigating the effects of disinfection on the persistence of surface-associated pathogens"
- XIV. ADDITIONAL BUSINESS**
 - A.** **CTB:** The committee discussed whether an IBC registration should be required for the use of CTB. It was noted that CTB lacks the toxic A subunit and is therefore non-toxic. It was also noted that many institutions choose to include the use of CTB in IBC registrations, even though this is not explicitly required under the NIH Guidelines. The matter was deferred for further discussion at the September IBC meeting.
- XV. TRAINING**
 - A.** N/A
- XVI. PUBLIC COMMENTS**
 - A.** N/A
- XVII. INSPECTIONS/ONGOING OVERSIGHT**
 - A.** N/A
- XVIII. ADJOURN 11:42 AM**

Office of Research
Compliance