Boston University
Institutional Biosafety Committee (IBC)
November 14, 2023 Meeting Minutes
Location: Zoom and/or by phone
Start time: 12:03 PM End time: 1:34 PM

Members Present:	B. Slack, I. Afasizheva, E. Muhlberger, R. Davey, W. Lu, V. Gouon-Evans, T. Winters, P. Liu (joined 12:31 PM), E. Loechler (joined 12:35 PM), R. Morales, C. Thurman, S. Niemi (left 12:10 PM), J. Keeney, R. Timmerman, V. Britton, N. Dey, S. Ghosh
Guests Present:	A. Henderson, P. Richmond, A. Ellis, J. Wood, M. Fitzgerald
Staff Present:	C. McGoff, L. Campbell

I. Review of October 17, 2023 IBC Meeting Minutes
No concerns were voiced.
Motion: Approved
For: 14**; Against:** 0**; Abstain** 1**; Absent:** 2

II. Chair's Report: Nothing to report.

III. New Business:
A. IBC Office Updates: Members were informed that the IBC office is working with the Office of the General Counsel on the information to be sent to an individual requesting a report of IBC meeting minutes, member rosters, and member bio sketches.
B. Incident Report: Members were informed that there will be follow-up at the next meeting pertaining to the two incidents projected today.
C. Review of Research Occupational Health Program (ROHP) Report: Nothing to report
D. Environmental Health and Safety (EHS) Report: Nothing to report

IV. Protocol Review

1. rDNA/Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2469	Elke Muhlberger	Pathogenesis studies of BSL-4 viruses using rodent and humanized mouse models	4	4	BUMC

Primary Reviewer: Rob Davey	Secondary Reviewer: Colleen Thurman Additional Reviewer: Guillermo Madico Additional Reviewer: Jim Keeney
Applicable NIH Guidelines: Section III-D-4-a	
Meeting Comments: In this new protocol rodent models engrafted with human tissues will be used to study disease processes for BSL4 pathogens. The animal work will involve tissue engraftment and recovery before animals are placed in the ABSL4 and challenged with high containment pathogens. The engraftment work will be done by another NEIDL PI, who is also listed as member of this protocol but is covered under his protocol (2397). The virus challenge and analysis of virus loads will be performed by current PI's as listed in their other approved BSL4 IBC protocol (2286). Pathology work will be performed by yet another NEIDL PI, through their approved protocol (2299), who is also a listed member in this protocol. Animal protocols and veterinary staff are covered under the ABSL4 IBC protocol 2620. Filoviruses, Henipaviruses, CCHFV and Lassa viruses will be used. Each are BSL4 agents that have similar pathogenic outcomes and there is no difference in the way each will be handled. Some recombinant viruses will also be used. However, they will generated through another BSL4 IBC approval from the PI. Decontamination will be by 5% Microchem plus which is appropriate. All work is performed in BSC within BSL4 laboratory by personnel wearing BSL4 suits. All centrifugation steps are performed using buckets and rotors equipped with sealed covers. Animals are sedated for all virus challenge procedures to reduce risk to personnel from bites or unexpected movements of animals. Caging will be HEPA filtered. Preparation of tissue for analysis is done within tubes so that scissor blades are	

not exposed. Areas and instruments are cleaned and decontaminated with Microchem and may involve autoclaving if deemed necessary. Tissues are inactivated in formalin or TRIzol. Serum and plasma is inactivated using TRIzol LS using approved methods and only after validation of the process. Overall, the work appears to be performed appropriately. Further, NEIDL Scientific Safety Officer informed the committee that an amendment request has been made to the Federal Select Agent Program's ABSL4 work permit in the NEIDL to include current PI. The following will be communicated to the PI:

- Please remove the ambiguity about the use of Lassa and CCHFV in this protocol. Committee recommends words like 'will be used' instead of 'might be', or provide a reason when they might be used.
- It is indicated that virus for challenge will be drawn up into syringes without needles attached and these will be placed into plastic bags. Indicate what will be used to prevent the virus containing liquid from escaping the syringe, such as a syringe cap. Use of a flexible oral gavage "needle" should also be considered to suck up liquid as this prevents excessive contamination of the syringe mouth. The syringe should be wiped down with Microchem before being placed into the bag.
- For the recombinant viruses it is indicated that a reporter gene will be used but no description is provided in the recombinant DNA section. The genes used should be indicated as this will be important to know during an exposure. Minimally, it should be written as fluorescent and luciferase proteins and indicate origins.
- Under question 17 it is indicated that source of transgenic animal is Jackson labs or other PI. Since a NEIDL colleague is one of the sources, their name should be indicated.
- It was noted that there are some minor discrepancies in proposed animal handling procedures compared to what is currently practiced in NEIDL Animal Research Center. The Committee recommends that PI should discuss the issues with ARC manager and establish working procedures that is safe and acceptable and modify the protocol language accordingly. These include: a) use of non-sharps during intranasal inoculation, b) whether blood sampling is done by 'survival blood collection' versus by 'terminal collection', 3) whether rodent temperature is measured by thermometers or by implanting microchips in the abdomen.
- If PI decides to carry on experiments on guinea pigs in the future, please provide brief description of the work in research project description 'Laboratory Procedure section.'

BUA Site Assessment: All trainings are up to date. BSC certifications are current. ABSL-4 laboratory was last inspected on Oct 4, 2023.

PI recused herself from voting.

Motion: Conditional Approval (Administrative Review)	For: 13	Recuse: 1	Against: 0	Abstain: 0	Absent: 3

2. rDNA/Bhz – Amendment

BUA	(PI)	Title	BSL	ABSL	Campus
1888	Elke Muhlberger	Host Response to Filovirus and Henipavirus Infections	4	N/A	BUMC

Primary Reviewer: Andy Henderson	Secondary Reviewer: Robin Ingalls Additional Reviewer: Guillermo Madico

Applicable NIH Guidelines: Section III-D-1-c, III-D-1A

Meeting Comments: This amendment is to add the use of a recombinant Dehong virus in PI's existing approved BSL4 protocol to study filoviruses. Dehong virus is a newly identified bat filovirus found in fruit bats in China and genomic RNA sequence information made it phylogenetically related to Marburg virus (MARV). No human cases of Dehong virus have been identified in humans. However, given it is related to MARV it will be handled under BLS4 conditions for infection studies described for other BSL4 viral pathogens. This includes purification of virus and infection of human cells and cell lines. Comparisons will be made to MARV and other filoviruses. Studies of individual gene product of this virus and full-length genome are being studied under other IBC protocols from the PI. Inactivation procedures for all the BSL4 work are described in detail, and approved SOPs are referenced. The virus cDNA clone will be generated under Dr Muhlberger's BSL2 protocol which covers generation of cDNA clones for other viruses

used in the laboratory. NIH OSP has been informed about this proposed work. The committee found it is appropriate to use this virus as proposed in the amendment under BSL4 conditions. *PI recused herself from voting.*

Motion: Approve	For: 15	Recuse: 1	Against: 0	Abstain: 0	Absent: 1

3. Bhz – Annual Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2361	Anthony Griffiths	Testing medical countermeasures against high consequence pathogens in rodents	4	4	BUMC

Primary Reviewer: Elke Muhlberger	Secondary Reviewer: Sajal Ghosh
Applicable NIH Guidelines: N/A	
Meeting Comments: The original protocol is to test variety of antivirals in animal models, mostly in mice. The viruses to be use are risk group 4 (RG4) viruses as well as some RG3 viruses. These viruses are grown in the lab for the purpose of using them as challenge inoculum. All work will be done in BSL4 containment. The current submission is an annual renewal where no changes have been made, except to remove one personnel and to update biosafety cabinet certification dates. The following minor issues will be communicated to the PI: • Please remove [redacted] from the shared room list. • Update the liquid waste management section because the minimal incubation time for 5% MicroChem liquid waste has been shortened in the corresponding SOP. • Will the work with the RG2 coronaviruses be performed in BSL4?	

Motion: Conditional Approval (Administrative Review)	For: 16	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

4. rDNA/Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2619	Lynne Chantranupong	Signaling and Metabolic Adaptations of the Brain	2	N/A	CRC

Primary Reviewer: Sajal Ghosh	Secondary Reviewer: Steve Niemi
Applicable NIH Guidelines: Section III-D-1-a, Section III-D-2-a, Section III-E-1; Appendix B-II-D; Section III-D-4-a	
Meeting Comments: The goal of this protocol is to study how structural and functional diversities of various neuronal cells arise and how cellular biochemical pathways in different neuronal cell types are linked to these specializations. Their long-term goal is to understand how neuronal cell functions are perturbed causing cellular death leading to neurodegenerative diseases. Mouse primary neurons isolated from normal and transgenic mice brain and MEF will be used. Cellular organelles will be isolated from homogenized cells and analyzed through metabolomics. For protein biochemistry analysis, proteins of interest will be expressed in E. coli BL21 cells and isolated by Glutathione-Sepharose beads. Replication incompetent AAV or lentivirus vectors expressing proteins of interest will be prepared in the lab by transfection of component plasmids in HEK293 cells. They will also perform survival surgery in mice brain where recombinant lentivirus or AAV viruses expressing protein of interest will be injected and animal behavior will be followed for 4 days post-surgery. IACUC protocol is being prepared at this time. All personnel working in the laboratory will wear lab coats, gloves, and safety glasses to prevent exposure of personnel to biohazards listed in this protocol. Liquid wastes will be disinfected bleach at a final concentration of 10% for 30 minutes. The following will be communicated to the PI: • Please add shared room [redacted] as biological materials will be stored in that room. • Section VII. Research Project Description, 3. laboratory procedures: intracerebral AAV injection in mice “will be performed in the main lab space ([redacted]), and mice will recover and be housed in a non-barrier facility” – where is the “non-barrier facility” and how long will mice be housed there?	

- VII.3. HHC tetrodotoxin is listed in Section D, but the purpose of its use has not been described in the laboratory procedure (Section VII.3). Please describe briefly.
- VIII.1. Check "Animal inoculation" and uncheck "Opening containers under pressure".
- VIII.3. Why N95 respirators are used in the lab? Please uncheck if not needed or used.
- VIII.5. Update Biosafety Cabinet Certification date.
- VIII.8. Disinfection practice appropriate for prion is unnecessary in this protocol. Please remove those statement.

BUA Site Assessment: The lab is currently being set up and this is PI's temporary lab space. PI's future lab space will be in the 6th floor of the same building. There is no need to add prion disinfection procedure for the reusable surgical tools. Usage of tetrodotoxin is not mentioned in the protocol. PI should add its usage in the protocol.
The 10% bleach contact time should be 30 min. instead of 20 minutes as mentioned in Section VIII point 8 and 11.
Storage of biological materials will be done in Room # [redacted] (a shared space), it should be added to the protocol.

Motion: Conditional Approval (Administrative Review)	For: 16	Recuse: 0	Against: 0	Abstain: 0	Absent: 1

5. Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2625	Dennis Jones	Enhancing Lymphatic Function with Transplanted Muscle Cells After Bacterial Infection	2	2	BUMC

Primary Reviewer: Weining Lu	Secondary Reviewer: Colleen Thurman Additional Reviewer: Bob Timmerman

Applicable NIH Guidelines: N/A

Meeting Comments: This new protocol aims to study how bacterial skin infections on the skin affect lymphatic vessels, which are vital to our immune system. Specifically, they will examine how methicillin-resistant *Staphylococcus aureus* (MRSA) affects lymphatic vessel contractility, lymphatic muscle cells (LMCs) recovery, and LMC cell regeneration after MRSA infection. The lab experiments include single-cell RNA sequencing, immunostaining of proliferation markers, and use of calcium-reporter mice to measure the calcium flux of LMCs in mice subcutaneously infected with MRSA. General lab safety procedures are described in the protocol to limit potential contamination and infection of MRSA, such as using PPE, biosafety cabinets, and frequent hand washing. However, some laboratory procedures and manipulations in this project need to be described in greater detail, such as preparing samples from MRSA-infected cells for single-cell RNA sequencing with and use of calcium-reporter mice to measure the calcium flux of LMCs in MRSA-infected mice. Several other apparently minor concerns were also noted. However, a declaration by PI that they have not used S. aureus in the lab before made the committee seriously concerned about the safe execution of the proposed research activity with MRSA, that are known to cause havoc if handled improperly. Committee wanted clear statement on prior experience of the PI and lab members on handling MRSA or plan to get hands-on training before IBC approval may be considered. The following will be communicated to the PI:

The committee expressed serious concern with PI statement that no MRSA work has been carried out in PI's lab, as it was interpreted as PI or the lab members do not have hands-on experience with MRSA. Since MRSA is a serious health hazard if handled improperly, the committee wanted clear statement on prior experience of the PI and lab members on safe handling of MRSA. If indeed the lab members do not have prior hands-on experience on safe handling of MRSA, the lab must arrange for getting hands-on training on safe handling of MRSA from experienced research professional before IBC would consider re-reviewing the protocol. Please address the following concerns urgently so that the revised version can be re-reviewed in the next IBC full-committee meeting.
Add room [redacted] to the room list.

- Please define acronym MRSA at its first use. MSSA is defined.
- It needs to be clarified if the PI and the graduate student are well-trained to handle drug-resistant bacteria such as MRSA. The PI also commented in the protocol that he had not worked with Staphylococcus aureus,

especially MRSA. It is unclear if new training on working with MRSA has been planned and if there are collaborators who are experts in handling drug-resistant bacteria such as MRSA.

- The project will isolate the previously MRSA-infected mouse cells using the flow cytometry core. Although the protocol mentioned that infection would be resolved before core use, it is unclear what lab procedures would be used to determine the status of MRSA infection.
- The protocol also mentioned, "In the event of an infection, any lesions must be fully covered to prevent further spread of the infection." It is unclear if this is MRSA infection in humans (i.e., researchers) or animal models (i.e., the MRSA-infected mice).
- Several disinfectants are described in the protocol, such as 70% ethanol, chlorhexidine, sodium hypochlorite (chlorine bleach), and benzalkonium chloride. However, glutaraldehyde and formaldehyde are tissue fixatives and toxic to humans. Please state what precautions will be undertaken. Please remove those that are not being used.
- The protocol mentioned, "Animals infected with MRSA will undergo clearance of the infection before tissue collection for subsequent analysis." It is not clear when infection clearance will happen after animals are infected with MRSA, how will it be monitored or whether tissues collected after recovery from infection will remain useful for experimental objectives.
- Three different MRSA are mentioned In Section IX. Please clarify what's the difference among three drug-resistant MRSA bacteria?
- The protocol lists Ketamin-Xylazine dose for mice as 10 mg/kg ketamine and 100 mg/kg xylazine. This is incorrect and appeared reversed. Standard mouse dosing for ketamine/xylazine cocktails is 70-100 mg/kg of ketamine and 10-20 mg/kg xylazine. Please correct this statement.
- Since animals are being infected subcutaneously, what is the risk to personnel handling those animals post-infection and prior to tissue harvest? How often are they screened for monitoring and how (or are they)? If any manipulations are made prior to tissue harvest, please describe any precautions related to handling animals that may be contaminated (for example, residual MRSA on the surface of the animal).
- For Ca2+ channel agonist experiment, does that occur pre or post-mortem in Ca2+ reporter mice? Please briefly describe the procedure.
- Protocol describes use of surgical instruments, although no surgery is described in the procedure section. Are these for necropsy?
- How are animal housing racks disinfected? Do they need to be autoclaved? Or is it just standard cage sanitation via the cage-washer?
- Section VIII.3. Shoe cover, head cover, and disposable scrubs are not required in the BSL2 laboratory.
- VIII. 4. Will mask, head cover, eye protection be worn or will work occur in a BSC? Is use of disposable scrubs accurate or will personnel wear street clothes with PPE over?
- In Section VIII.6. Needles may be discarded in designated sharps containers without any disinfection. Please clearly mention what disinfectant will be used to clean and disinfect surgical instruments.
- In Section VIII.7A. Liquid wastes should be treated with bleach at a final concentration of 10% for 30 minutes before disposal down the drain, not in a double-bagged biohazard bin.
- VIII. 7B. Glass or potential sharp items (some pipettes) should be disposed of in sharps container.
- VIII. 8. A 70% ethanol may not be adequate disinfectant for this pathogen. If you are using 1:10 bleach, just write bleach to a final concentration of 10% (which is 0.5-0.6% sodium hypochlorite. Is there a reason this is 1%?).

BUA Site Assessment: The location # [redacted] should be added to the protocol as it is the main lab. All used syringes and needles should be disposed of in the red sharps container. Type of disinfectant used to dispose of liquid waste has not been mentioned. For liquid waste, 30 min contact time with final concentration of 10% bleach is needed for decontamination before its disposal. The lab is currently not using the following disinfectants in the lab: 2% glutaraldehyde, 0.25% benzalkonium chloride, and formaldehyde. No transportation of biological materials is anticipated in this protocol.

Motion: Withhold Approval	For: 16	Recuse: 0	Against: 0	Abstain: 0	Absent: 1

VI. List of Protocols reviewed by DMR (not discussed in the meeting)

List of protocols that were reviewed by DMR was displayed in the meeting.