Boston University
Institutional Biosafety Committee (IBC)
February 15, 2022 Meeting Minutes
Location: Zoom and/or by phone
Start time: 12:00 PM End time: 1:53 PM

Members Present:	C. Abraham, R. Ingalls, B. Slack, I. Afasizheva (joined 12:12 PM), R. Davey, E. Loechler, P. Liu (joined 12:25 PM), T. Winters, C. Thurman, S. Niemi, J. Keeney (joined 12:15 PM), R. Timmerman, V. Britton (joined 12:05 PM), J. Barton, S. Ghosh
Guests Present:	A. Ahmad, J. Davis, L.T. Watson, P. Richmond, S. Benjamin
Stuff Present:	S. Ghosh, L. Campbell, C. McGoff

I. Review of January 25, 2022 IBC Meeting Minutes (C. Abraham)

No comments or questions were voiced.
Motion: Approve
For: 11; Abstain: 1; Absent: 3

II. New Business:

A. SQAP Report:
Committee members were informed they will be contacted for new member suggestions. S. Ghosh suggested seeking new members experienced in microbiology, cancer biology and molecular biology.

B. Research Occupational Health Program (ROHP) & Environmental Health and Safety (EHS) Report
ROHP reported three (3) incidents to the committee:

- A visiting researcher cut left thumb with razor blade while cutting unfixed human kidney tissue;
- A graduate student sustained a puncture to left 2nd digit with a needle that had been used to inject lentivirus vector on transgenic mice;
- A PhD student sustained percutaneous injury to left 5th finger from unknown glass shard.

EHS reported on a near-miss incident to the committee:

- Needles found in biohazard trash

EHS provided follow-up on the root cause of these incidents and corrective actions. It was noted that the needle stick injury with lentiviral vector inside is a NIH reportable incident and the report will be sent to NIH and BPHC.

III. Protocol Review

1. rDNA/Bhz – Annual Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2286		Biomolecule Production Core - Propagating BSL4 pathogens	4	N/A	BUMC
Primary Reviewer: Rob Davey			Secondary Reviewer: Guillermo Madico Shannon Benjamin		
Applicable NIH Guidelines: Section III-D-1-a, III-D-1-b, III-D-1-c					
Meeting Comments: The goal of this protocol is to propagate and evaluate wild type and recombinant viruses that will be shared with NEIDL investigators. Viruses are typically BSL4 agents but does include BSL3 agents SARS-CoV-1 (a select agent) and SARS-CoV-2 (non-select agent). Dual Use Research of Concern (DURC) aspects are described and while recombinant work is not expected to generate viruses of altered tropism or pathogenesis, they will look out for such and have an action plan in place: Stop the work, contact EHS and if decided, destroy the material. Training documentation is excellent which also clearly describes the path for new trainees. In this amendment they are adding Lloviu virus, which is a filovirus found in bats. This work will help to understand the virus reservoir. It does not alter					

the biosafety level of the work being done and will be performed in the BSL4. Work with SARS-CoV-1 (select agent regulations) will be performed in the BSL4 only. All work with agents follows BSL4 procedures which involves use of 5% Microchem Plus as disinfectant. This is approved for all the viruses used in this protocol. All waste is autoclaved. All work is performed in BSC within BSL4 laboratory by personnel wearing BSL4 suits. BSC certification date is from September 2021, which is up to date. As NEIDL is approved to work with the SARS-CoV-1 but not the genomic genetic material, the PI clarified that they will not remove inactivated material derived from SARS-CoV-1 out of containment because it contains genomic genetic material. Overall, there are no biosafety concerns for this protocol.					
Motion: Approve	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 1

2. rDNA/Bhz – Three-Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
1702		iPS Cells: Novel Applications for Blood Bourne Disease	2	2	BUMC
Primary Reviewer: Barbara Slack			Secondary Reviewer: Colleen Thurman		
Applicable NIH Guidelines: Sections III-D-1-a, III-D-2-a, III-D-4-a, III-E-1: Appendix B-II-D, G-II-B;					

Meeting Comments: The lab specializes on creating induced pluripotent stem cells from differentiated adult cells. In this protocol they use the technology to create hematopoietic progenitor cells and follow their differentiation leading to blood development in health and disease. They do rDNA cloning work in E. coli 12 and use 3rd generation lentiviral vectors to express proteins required for reprograming of embryonic genes. They also use AAV vectors that express these reprogramming proteins and use them to transduce human cell lines or inject them in to mice via approved IACUC protocol to test transgene expression. Once established, human PBMCs will be transduced with these engineered vectors and then either will be tested *in vitro* for hematopoietic differentiation or *in vivo* (in mice) to test if they can lead to end-stage hematopoietic differentiation. All experiments with lentiviral vectors and AAV (with helper virus) will be carried out in BSL2 containment. It was noted that this protocol is described in great detail. Committee recommended clarification on which transduced cell lines to be injected in mice and to add them in the hazardous biological agent list. The following will be communicated to the PI:

- In the Overview and Grant Funding information section the box for amendments should be left blank for 3 year resubmittals.
- The rDNA training dates for and need renewal.
- The ROHP clearance for is missing and update needed for and
- VII.3. says “IACUC PROTO201800151 is pending until this IBC amendment is approved”, but that protocol is approved until 8/13/2023. Please update.
- Section VIII.1- check 'animal handling and cage changing’. Check this box as the last change on this section before saving.
- VIII.4. Will face shield and disposable scrubs be used if animals are inoculated in a biosafety cabinet? This is beyond the normal PPE requirement for the ABSL2 mouse space.
- Section VIII.5- update BSC certification date.
- Section A. The table indicates that only AAV will be injected into mice, but the Lab Procedures section also states that transduced differentiated PBMCs will be used in mouse assays. Please add Human PBMC transduced with AAV as another entry in the Section A. table and also respond to the associated questions.
- Section H. rDNA table- Lab Procedures states that 'mouse or human cells will be transduced' with various vectors, but only HEK cells are listed in table. Please list all cell types that will be transduced with viral vectors under 'host strain' in the Eukaryotic Experiment section.

BUA Site Assessment: Done: Biosafety cabinet and fume hoods are duly certified. Training deficiencies have been reported the members. One member needs to update ROHP clearance and have been notified of this requirement.

Motion: Conditional Approval (Administrative Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

3. Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2357		Providence/Boston CFAR/BU School of Dental Medicine Collaboration: Oral Health in Persons Living with HIV	2	N/A	BUMC

Primary Reviewer: Tom Winters	Secondary Reviewers: Jim Keeney

Applicable NIH Guidelines: N/A

Meeting Comments: The goal of this study is to validate self-reported measures of periodontal disease in different cohorts of patients living with HIV especially in underserved populations experiencing oral health disparities. They will characterize the oral health status – tooth count, dental caries, periodontal disease and oral lesions of these people examined by a dentist. They will also establish the feasibility of participants self-collection of saliva samples, packing and mailing of samples in cohorts of these participants. The study which include two phases; a) collection of saliva samples (which has been concluded now) and 2) recording of all the information from the clinical exam and from during collection of saliva samples. Appropriate PPE for working blood materials will be worn. The collected sample tubes will be cleaned with Cavi wipe and placed in a biohazard bag. The samples will be carried over to a freezer in the W-building. No laboratory procedures will be performed and samples will be stored for future use. The blood borne pathogen standard is evoked as saliva samples are collected. It was noted that the study no longer involve any shipping. EHS clarified that Cavi wipe contains cavicide, which is a EPA approved disinfectant for HIV. EHS also indicated that BMC clinical space is no longer used in this study. There appears to be no other biosafety risks in this protocol. The following will be communicated to the PI:

- Please state briefly what is Cavi wipe and their effectiveness as anti-HIV disinfectant.
- Update biosafety cabinet certification date.
- Since BMC clinical space is no longer used in this protocol, choose 'No' to your response to BMC clinical space use question in Section IX.

BUA Site Assessment: Safety trainings, including shipping training and ROHP clearance are current for all members.

Motion: Conditional Approval (Administrative Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

4. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
487		Analysis of negative strand RNA virus transcription and replication mechanisms	2	N/A	BUMC

Primary Reviewer: Rob Davey	Secondary Reviewer: Bob Timmerman

Applicable NIH Guidelines: Sections III-D-1-a; III-D-2-a; III-D-3-a; Appendices B-II-D; B-V; G-I; G-II-B

Meeting Comments: This study will investigate mechanisms by which gene expression and genome replication of RNA viruses are controlled by the viral polymerase. Four types of work will be done: 1) Expression of virus polymerase proteins in cells, 2) Use of a minigenome (miniaturized form of the virus genome lacking virus proteins) to study how the genome is replicating by the virus polymerase 3) Making mutant viruses in the virus genome (for RSV and HMPV only) 4) Challenge of cell lines with the recombinant viruses. The laboratory procedures include conventional recombinant DNA work in *E. coli*, use of vaccinia virus or cells expressing T7 polymerase to make RNA copies of genomes in mammalian cells, For step 4 virus work, the viruses are BSL2 level agents and work is performed in the same BSC.
All virological work that include human cell lines or insect cells are performed in a recently certified BSL2 BSC using appropriate PPE used in BSL2 containment. *E. coli* and other tissue culture liquid waste is treated with fresh 10% bleach, wescodyne or vesphene. Treatment time is 30 minutes and is appropriate. Transport of biohazardous materials are done in shatterproof, leakproof containers. Personnel are well trained and is documented well. Training for members is provided by the PI or other experienced personnel in the lab. Overall, the work is performed appropriately. The PI indicates that recombinant work will be unlikely to increase viral pathogenesis, instead typically attenuate the virus. This is likely true but to be consistent with other similar protocols, the committee recommended

that a statement should be added that identification of any elevation of pathogenesis will result in halt of the work, and consultation with EHS. The following will be communicated to the PI:

- Is Room freezer still being shared with Dr. Duprex? If not, please remove his name.
- Please add a statement in the laboratory procedure section VII.3.5 that 'identification of any elevation of pathogenesis will result in withholding the work until a mitigation plan has been determined following consultation with the EHS.

BUA Site Assessment: The lab has exposure control plan in place. The ROHP clearance is current for all members. Safety trainings are also current for all members. Their biosafety cabinet and fume hood are duly certified.

Motion: Conditional Approval (Administrative Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

5. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2109		Generating full-length clones of negative-sense RNA viruses	2	N/A	BUMC

Primary Reviewer: Robin Ingalls	Secondary Reviewer: Sajal Ghosh

Applicable NIH Guidelines: Section III-D-2-a

Meeting Comments: The work in this protocol involves the generation and storage of cDNA clones containing DNA copies of the full-length genomes of Risk Group 4 (RG4) non-segmented negative-sense RNA viruses of the family of filovirus and paramyxovirus (henipavirus). The goal is to use these constructs to modify the viral genomes by inserting mutations in viral genes of interest to analyze the impact of these mutations on different aspects of the viral replication cycle. Importantly, these cDNA clones are not infectious and will not create virus particles if expressed in an eukaryotic cell as they do not contain the sequence for the required support proteins, viral RNA polymerase and the nucleoprotein. Per the NIH requirements for RG4 rDNA work, the PI's lab generates and stores the full-length clones in a separate full-length cDNA laboratory (FLCL) which meets BSL3 requirements and is physically separated from the PI's regular BSL2 laboratory space. For the purpose of sequence verification only smaller restriction enzyme digested fragments will be sent out, not the full-length plasmids. Plasmids containing full-length viral genomes will only be removed from the FLCL to be transported to the BSL-4 facility of the NEIDL for viral rescue, or to be shipped to off-site BSL-4 facilities. All rDNA work utilizes standard DNA cloning procedures in either *E. coli* K12 bacteria or yeast TAR cloning system in commercially available cloning vectors. In this particular protocol, there are no biohazards as the bacteria and yeast used are BSL1, and the rDNA work is standard and adequately explained. The rDNA work does create synthetic DNA from RG4 agents using RT-PCR, but this cDNA is not infectious. No concern was noted.

BUA Site Assessment: All required safety training are current for all members. Everyone has appropriate ROHP clearance. All biosafety cabinets and fume hoods are duly certified.

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

6. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
634		Programmed cell death in Drosophila development	1	N/A	CRC

Primary Reviewer: Inna Afasizheva	Secondary Reviewer: Pinghua Liu

Applicable NIH Guidelines: Section III-D-4-a; Appendix B-1, Appendix C-II, Appendix G-II-A, Appendix G-IIIM,

Meeting Comments: The goal of this protocol is to study the mechanisms of programmed cell death and cell clearance in the Drosophila ovary and brain. Drosophila is a well-established model organism. Investigation of the signaling pathways initiated by cell death involves genetic and *in vitro* approaches including fluorescent microscopy, genetic screening, and RNA sequencing. Drosophila genes generated by PCR amplification or from cDNA preparations will be expressed in the *E. coli* K-12 using standard cloning techniques. These methods do not involve any serious

biohazard risks. Transgenic Drosophila flies will be created by using transposable *P-element* containing vectors that cannot replicate in mammalian cells. Transgenic flies will be generated by third party company using these constructs. PI will receive the larvae containing the transgene. Same protocol is described for CRISPR/Cas9 experiments where PI's lab will clone gRNAs into plasmids and send the constructs to the company for transgenic flies production. It was clarified that because Cas9 and gRNA are delivered into the cells via different expression cassettes, there is no risk of creation of a gene drive event. Cell culture experiments include non-pathogenic insect cells. They also occasionally create drosophila mutants using chemical carcinogen. Committee recommended that lab should have plan to provide appropriate guidance to the lab members about the risk potential of this carcinogen. The following will be communicated to the PI:

- ROHP clearance update needed for .
- Please include more protocol detail for lab work with flies from the moment of larvae are received. Specifically: colony growth conditions, phenotyping, incubator usage, precautions to prevent escape of flies.
- For the use of ethyl methane sulfonate to produce Drosophila mutants, please provide more details on the dosage, safety protocols and use restrictions on its handling and waste disposal plan (because this is a carcinogen that may impact pregnant women).

BUA Site Assessment: ROHP clearance needs update for one personnel and is being processed currently. Training is up-to-date for all members. Biosafety Cabinet and fume hoods are duly certified. EHS will communicate with the PI about safe handling of ethyl methane sulphonate.

Motion: Conditional Approval (Administrative Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

7. rDNA/Bhz – Three-Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2094		New Molecular Tools for Imaging Biochemical Events in Live Cells	2	N/A	CRC

Primary Reviewer: Ed Loechler	Secondary Reviewer: Barbara Slack

Applicable NIH Guidelines: Sections III-D-1-a and III-D-2-a

Meeting Comments: The protocol is to develop tools to understand how cells receive and process information from their surrounding environment. Molecular probes and genetically encoded tools will be generated to visualize (using fluorescence microscopy) biomolecules and biochemical events inside living cells, including signaling molecules (such as ions and reactive oxygen species) and important cellular parameters (such as membrane potential and organelle age). Recombinant DNAs encoding reporter proteins will be generated using standard cloning procedures, including PCR amplification and cloning including Gibson assembly. Reporter genes including standard fluorescent proteins (GFP, YFP, mCherry, etc.) will be fused to mammalian proteins of interest, such as histone H2B, actin, etc. The resulting constructs will be transfected into mammalian cells and selected for stable integration which will then be evaluated using a combination fluorescence imaging and flow cytometry analyses. Work with biological agents and hazardous chemicals will be carried out using proper PPE including safety goggles, appropriate gloves, and laboratory coats. When appropriate, work will be carried out in a biosafety cabinet or chemical fume hood. All cultures of bacterial cell strains and mammalian cells will be properly decontaminated by at least 20 minutes incubation in freshly prepared 10% beach prior to disposal. The committee noted that the lack of detail in the proposal impeded proper risk assessment. It was not clear how a GFP-H2A fusion could help measure the changes in signaling by reactive oxygen species (ROS), membrane potential or organelle age. Committee requested more detail of the procedures to be used. The following will be communicated to the PI:

Committee recommended more detail of all the laboratory procedures so that a proper risk assessment can be done.

- Please briefly describe how GFP fusion proteins are used to measure each of ROS, membrane potential or organelle age? Describe all safety issues associated in carrying out those experiments.

- It is stated in the last line of the laboratory procedure section that 'all steps involving hazardous reagents will be carried out in fume hood'. What hazardous agents will be used in the protocol?
- Except , all members need to update their ROHP clearance.
- , and need to update LST, BSL1/2, BBP and Chem Safety training.
- Sloas needs to update the rDNA/IBC policy training.
- Update biosafety cabinet certification date.
- Liquid waste must be treated with bleach at a final concentration of 10% for a minimum of 30 minutes before disposing of them in the sink.
- What is the "other" disinfectant? Clarify which disinfectant is used for what purpose.

BUA Site Assessment: The lab has appropriate exposure control plan. ROHP clearance is being updated for all members. Biosafety cabinet is duly certified but needs to be updated on the application.

Motion: Conditional Approval (Primary and Secondary Reviewer Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

8. rDNA/Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2539		Neural, behavioral, and developmental mechanisms of affective regulation	1	1	CRC

Primary Reviewer: Carmela Abraham | Secondary Reviewer: Steve Niemi

Applicable NIH Guidelines: Sections III-D-1-a, III-D-2-a, III-E-1, App. B-II-D, App. G-II-B

Meeting Comments: The project focuses on studying learning and memory related to fear and reward. The PI utilizes novel genetic tools and screens several mouse behaviors to study how animals learn and use previous learning to guide decision making and behavior. Adeno-associated viruses (AAV, serotype 1, 5, 8, or 9) will exclusively be used to incorporate light sensitive proteins, which permits imaging and activation or inhibition of brain cells, respectively, as well as a fluorophore (for example, GFP or mCherry) into the neurons of mouse brains during a survival surgery. Behavioral testing will then follow. Animals will be euthanized and the brain of the mice will be separated and slices prepared for immunofluorescence. The application is very well written. The committee noted that formaldehyde treated brain slices does not need to be treated with bleach prior to their disposal as tissues treated with formaldehyde overnight or longer essentially inactivates all living organisms. Further AVV is only a risk group 1 (RG1) agent. The following will be communicated to the PI:

- In the survival surgery 2nd paragraph, it is stated that "the pipette is retracted from the brain and soaked in 10% bleach". Please modify to state that it will be soaked in 10% bleach for 30 minutes.
- Similarly change the statement "cardboard boxes, which when full", to "when ¾ full".
- Section VIII.1: Check "animal handling" and "centrifugation"
- Section VIII.6: How will reusable sharps be disinfected? Sharp container may be disposed of directly in the red biohazard boxes without autoclaving.
- Section VIII.7A: Check typo "poured down the sink".
- Section VIII.8: Add percentage of Wescodyne. Should be (2%).
- Recombinant DNA Section: Applicable NIH guidelines should be: Sections III-D-1-a, III-D-2-a, III-D-4-a, III-E-1, App. B-II-D, App. G-II-B

BUA Site Assessment: Surgical room and the lab room was also evaluated as a part of this assessment. Both rooms need to be added to the protocol. Certified fume hood is available for the formaldehyde treatment work. The lab is part of a shared space where other work requiring BSL2 containment are also done. In this type of situation, out of convenience, generally waste disposals are handled as BSL2-containment waste.

Motion: Conditional Approval (Administrative Review)	For: 15	Recuse: 0	Against: 0	Abstain: 0	Absent: 0