Boston University
Institutional Biosafety Committee (IBC)
April 26, 2022 Meeting Minutes
Location: Zoom and/or by phone
Start time: 12:03 PM End time: 1:34 PM

Members Present:	R. Ingalls, B. Slack, E. Muhlberger, R. Davey, T. Winters, R. Morales, C. Thurman, S. Niemi, J. Keeney (joined 12:10PM), R. Timmerman (joined 12:05 PM), V. Britton, P. Liu (joined 12:27 PM), X. Brown, S. Ghosh
Guests Present:	T. Killeen, M. Fitzgerald, P. Richmond, S. Benjamin, J. Wood, A. Ahmad, J. Davis, N. Dey
Staff Present:	S. Ghosh, L. Campbell, C. McGoff

I. Review of March 22, 2022 IBC Meeting Minutes

No comments or questions were voiced.
Motion: Approve
For: 11; Abstain: 2; Absent: 1

II. New Business:

A. SQAP Report:
   i) Review of the IBC Charter:
   Members requested that the charter be resent in order to provide them with more time for review; the charter will be added to the May meeting agenda.
   ii) Development of IBC reviewer checklist
   Members were informed that a review checklist will be developed by SQAP staff; IBC members will have the opportunity to review. The checklist is anticipated by the end of the summer.

B. Review of Research Occupational Health Program (ROHP) & Environmental Health and Safety (EHS) Incident Report
   Members were provided a copy of ROHP reported incidents prior to the meeting in a new format; ROHP and EHS representatives will continue to be present at IBC meetings and available to discuss any questions or comments. No comments or concerns were voiced.

III. Protocol Review

1. Bhz – Annual Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2439		Storage, Propagation and Distribution of BSL-3 Emerging Pathogens	3	N/A	BUMC

Primary Reviewer: Rob Davey	Secondary Reviewer: Shannon Benjamin
Applicable NIH Guidelines: N/A	
Meeting Comments: The goal of this protocol is to obtain, develop stocks and storing viruses that can cause human disease and providing these to researchers. Work is also done (previously approved) with mosquitoes to study infection cycle and isolate viruses from these insects. Main risks in the protocol include direct exposure of personnel to the virus agent or to the bite of an infected mosquito. Engineering and PAPR based precautions are in place to mitigate each of these risks. PAPR is used when working in the BSL3 and biosafety cabinets are used to contain infected material when being worked with. Under sharps, glass capillaries are used to inject mosquitoes with the viruses. The capillaries are heated and drawn out into a fine needle. To prevent needle sticks, double gloves are used. It is indicated that the capillary is so fine that it will not penetrate this although caution is used when handling capillaries. As disinfectants, 5% Microchem Plus or 10% bleach is used, which is appropriate for the viruses used but 10 minute contact time is indicated for each. Overall, the experiments described are sufficient for risk mitigation. There were no other concerns voiced by the members.	

Motion: Approve	For: 13	Recuse: 0	Against: 0	Abstain: 0	Absent: 1

2. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2113		Zika virus growth and characterization; development and evaluation of diagnostic tests for positive and negative sense RNA viruses.	2+	N/A	BUMC

Primary Reviewer: Robin Ingalls	Secondary Reviewer: Jim Keeney

Applicable NIH Guidelines: Section III-D-1-a, III-D-2-a, and III-D-3-a; Appendix B-II-D, and G-II-B-3

Meeting Comments: The goals of this research are to better understand the behavior of Zika virus-infected cells so that they can identify therapeutics and prevention strategies to reduce suffering caused by Zika infection. They have expanded these studies to also include questions about other positive sense RNA viruses, including flaviviruses and coronaviruses. Biohazards in the protocol include wild type and recombinant Zika virus; Dengue virus; Snowshoe hare virus; clinical samples from Zika-infected individuals which in theory could have Dengue or Chikungunya (BSL3) virus present; SARS-CoV-2 virus and clinical samples (NP, OP swabs; serum). PI clarified that the clinical samples have been already tested negative for Chikungunya but if they are identified to have the virus, they will be stored separately and will be disposed off properly with the assistance of NEIDL EHS. Biohazards in the protocol also include human cell lines, Vero cell line, plasmodium positive clinical samples and vaccinia virus. Protocol also involves recombinant DNA work, but are only related to zika virus. However, risk mitigation plan and disinfection procedures are described in great detail and are acceptable. The following will be communicated to the PI:

- Remove from shared laboratory space.
- Remove mention of from project description (he left BUMC) and if mouse studies are no longer being done, that should be removed from the project description and detailed procedures sections (sections 2 and 3).
- Update BSC certification dates.
- Provide time for bleach treatment of liquid waste.
- Is ongoing IRB approval still needed for this protocol or is this protocol closed?

BUA Site Assessment: All members have medical clearance. Some members do not have zika virus agent-specific training which have been indicated to the individuals. Biosafety cabinets are duly certified. There were no other concerns voiced by members.

Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

3. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2118		Genetic modification of zebrafish	1	1	BUMC

Primary Reviewer: Xin Brown	Secondary Reviewer: Colleen Thurman

Applicable NIH Guidelines: Section III-F-8, Appendix C-II

Meeting Comments: The long-term goal of the project is to use zebra fish as model system to study skeleton and skull birth defects in human. Zebra fish embryos at 1-2 cell stage will be genetically modified using either Tol2 transposable element or CRISPR/Cas9 system. The fishes are then allowed to mature and reproduce. Live fishes are studied by using fluorescent microscope. Some are euthanized and fixed specimens are analyzed through sectioning and staining. The project poses minimum risk to the lab personnel or the environment. The following will be communicated to the PI:

- All members need to update their ROHP clearance.
- Check animal handling and cage changing in the PPE section if animals (zebra fishes, not the embryos) are net transferred.

- State briefly how the euthanized fishes are disposed off as a BSL2 waste.

BUA Site Assessment: Entire work is done under BSL1 conditions. All required trainings are current. However, ROHP clearance for all members need to the updated. It was noted that is no longer a BSL2 facility.

Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

4. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2363		Exploring molecular mechanisms of the immune system	2	2	BUMC

Primary Reviewer: Elke Muhlberger | Secondary Reviewers: Steve Niemi

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

Meeting Comments: The goal of this protocol is to investigate how the immune system responds to different type of stimuli for which they use wide array of assays. They use mouse infections as well as cultured mammalian cells, including human cell lines and bronco alveolar lavage (BAL) cells. They either infect live animals or treat cells with various stimuli and collect blood, tissues or BAL cells from animals or analyze cells by immunohistochemistry, RT-PCR and FACS analysis for identifying changes in signaling pathways in treated cultured cells. As the stimuli, they use *S. pneumoniae*, look worm or LPS. They also use viruses that cause lung disease such as influenza virus or RSV to induce inflammation. They also use transgenic mice, which they use for infection purpose. It was clarified that the hook worm *Nippostrongylus brasiliensis* is a risk group 1 agent. Overall, this is a nicely written large protocol which include wide variety of experiments but are all described in great detail, addressing all biohazard concerns. The following will be communicated to the PI:

- Since the protocol use human blood, all lab members must complete blood borne pathogens training.
- If the protocol is involved in shipping any biohazardous materials, at least one member should have updated Shipping training. Currently listed shipping training for Bosmann and Kontodimas have expired.
- All previous amendments, including the revisions posted in the Comments section, should be updated and integrated in the protocol.
- Consider revising the statement “mice will be lethally irradiated”, as stated. Instead, the statement should indicate that they will be irradiated at a level high enough to destroy hematopoietic cells in their bone marrow to permit reconstitution with new, transplanted hematopoietic cells. While it’s true that these mice could eventually die if not reconstituted, higher doses would be needed to kill them sooner.
- In the PI comments section, please keep only those statements that are specific for the current submission.
- It is stated that biological material will be handled in a fume food. This is not acceptable as fume hoods do not have HEPA filters. These statements should be removed.
- Remove BSL-1 agents from biohazard list, such as *Nippostrongylus brasiliensis*, *E. coli* K12. Remove non-biological substances from the biohazard list, such as 5’TP dsRNA, LPS, recombinant proteins, poly I:C, etc.
- H1N1 PR8 is listed twice in biohazard list. Is the Puertorican strain being used in the protocol? If not, remove the name.
- If Adenovirus vectors are not used, replace the Adenovirus from the list with adeno-associated virus (AAV).
- All human cell lines are considered BSL-2 at BU and must be added to biohazard list (all human and NHP cell lines listed in the rDNA eukaryotic experiment Host section).
- Recombinant DNA section should match project description. Use of adenoviral vectors in mice to express hACE2 has been proposed in the Research Project Description VII.2 and VII.3, but this work is not indicated in the recombinant DNA section.
- Recombinant DNA section, #17 – Since transgenic mice are being used in this study, the rDNA section question 17 must be checked.
- Please add Section III-D-4-a in question 19 for the use of recombinant viruses in animals.

BUA Site Assessment: Blood borne pathogen training for two personnel needs update. Biosafety cabinets are duly certified. PI indicated that they do not plan to use the irradiator (the permit will be expired in June 2022).					
Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

5. rDNA/Bhz – Three Year Renewal

BUA	(PI)	Title	BSL	ABSL	Campus
2110		Molecular mechanisms of adipocyte metabolism	2	1	BUMC

Primary Reviewer: Inna Afasizheva	Secondary Reviewer: Colleen Thurman
Applicable NIH Guidelines: Sections III-D-2 and III-D-3-a	

Meeting Comments: The project seeks understanding of ribosome dependent quality control mechanism that regulate adipocyte functional homeostasis. Study aims to develop new therapeutic strategies to decrease the high risk of obese patients developing metabolic diseases. Protocol describes standard molecular biology and cell biology procedures in sufficient details. Replication incompetent lentivirus will be used for transduction of mouse cell lines and human HEK293 cells. Work with the mice is limited to phenotyping, euthanization and collecting organs and tissue for *in vitro* experiments. Committee was informed that no biohazardous materials or recombinant nucleic acid manipulated material are being injected into the mice and as such checking of the "live animal use" box in Materials Used in Research section is not needed. However, reference of specific IACUC approval information must be mentioned, so that any unfortunate occurrence of incidents related to handling of mice be accounted for. The following will be communicated to the PI:

- Update title for in the Personnel Information section.
- Update the protocol with current version of the IACUC Approval number and date. IACUC PROTO201800404 (good until 6/20/2022) uses multiple strains of mice (Adrx KO conditional PTRF KO, conditional adipocyte ribosome nucleus labeling, conditional p53 KO). Clarify if these are being euthanized for *in vitro* experiments only.
- VIII.1. Check Animal Handling, cage changing box.
- VIII.4. Safety goggles may not be necessary for animal handling since animals will only be euthanized and tissues collected.

BUA Site Assessment: Safety training and medical clearances are current for all members. Use of equipment and PPE are appropriate.

Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

6. rDNA/Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2541		Molecular mechanisms of protein ubiquitination in breast cancer	2	N/A	BUMC

Primary Reviewer: Sajal Ghosh	Secondary Reviewer: Bob Timmerman
Applicable NIH Guidelines: Section III-E-1, Appendix B-1	

Meeting Comments: The goal of this protocol is to discover important molecular mechanisms of ubiquitination in breast cancer. They want to develop chemical proteomics strategy that can precisely quantitate protein ubiquitination changes in cells. They also are developing an enzyme catalyzed uniquitination method to site-specifically and stoichiometrically add ubiquitin onto proteins using bacterial subtiligase enzyme. Protocol involves peptide synthesis, protein expression and purification, cell culture and rDNA work. Biohazards are human cell lines. In this protocol they synthesize peptides that are known to be important for cellular signaling. Those will be used for enzyme catalyzed ubiquitination method. They also express several recombinant proteins in *E. coli* BL21 and purify them. They prepare cell lysates from several breast cancer cell lines and then treat them with LBpro, sortase, and other peptides to assess differences in ubiquitination patterns by mass spectrometry and western blotting. All solid

and liquid waste from peptide synthesis work (such as dimethyl formamide, dichloromethane and others) will be disposed off in labeled hazardous waste containers in their designated satellite accumulation area with secondary containment followed by scheduled hazardous waste pick up. Liquid wastes from cell culture work will be treated with 10% bleach (final) before discard. Potassium cyanide (KCN) is used for the peptide synthesis work. It was discussed that safe handling SOP for the use of KCN must be posted in the lab. Precautions taken and safe handling procedures are well described. The following will be communicated to the PI:

- Human cell lines MDA-MB-468, MCF7, BT474, MDA-MB-436, MCF 10A, 184A1 must be listed in the biohazard table.
- Mention of vector and donors in the eukaryotic experiments in the rDNA section implies some eukaryotic rDNA work (such as cloning of DNMT1, MDM2 or p53), but nothing in that regard is mentioned in the laboratory procedure. Please clarify.
- Use of viral vector (baculovirus) is mentioned in question 15 of the rDNA section, but no detail is described in the laboratory procedure section.
- Please clarify if the lab has safe handling SOP for KCN in place.
- Please be aware that dimethyl formamide used in peptide synthesis is a teratogen. Pregnant or expected women in the lab must be made aware of this information.

BUA Site Assessment: The lab has exposure control plan. Safety training and medical clearances for the lab members are current. PI is new to the BU and the lab is still being set up. EHS will provide more information about the existence of SOP for the use of potassium cyanide.

Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

7. Bhz – New Application

BUA	(PI)	Title	BSL	ABSL	Campus
2547		Matrix Remodeling and Biosynthesis in Human Fibroblasts	2	N/A	CRC

Primary Reviewer: Ed Loechler	Secondary Reviewer: Ron Morales

Applicable NIH Guidelines: N/A

Meeting Comments: The goal of this proposal is to understand the mechanisms of extracellular matrix remodeling in soft tissues and how these processes are altered in the case of disease, injury or in natural aging. Functional differences in matrix synthesis and degradation in cells derived from human keloids are a focus. The PI will work collaboratively with other investigator in the BUMC, who is studying epigenetic regulation in melanoma and keloid formation. Epigenetic reprogramming in keloid cells will be pursued via application of small molecule inhibitors of epigenetic modifying enzymes. The biohazardous materials in this protocol include human dermal fibroblast cell lines derived from healthy skin and keloids from unnamed sources. Dermal fibroblasts are also isolated from patients with keloids in collaborator's lab via approved IRB protocol and then are transported to the PI's lab and stored in liquid nitrogen until use. Fibroblasts are treated with EMT-modifying drugs and functional assays are performed to assess cell proliferation and matrix biosynthesis using pulse labeling with radioisotopes. DNA and collagen synthesis are measured in cells using 3H-Thymidine, 3H-Proline, or 35S-Sulfate via appropriate radioisotope use permit. Cells are lysed and collected to measure radioisotope decay via liquid scintillation counting. New workers will be trained by senior members of the lab. Proper PPE will be used. Experiments with aerosol hazards will be done in a chemical fume hood, using aseptic technique for cell culture. Sharps will be handled and disposed off properly. The following will be communicated to the PI:

- Since the protocol involves work with human cells and blood products, all members must have current blood borne pathogen training.
- Please provide brief description of safe handling of tritiated thymidine and other radiolabeled samples.
- The laboratory procedure section indicates use of sharps, but the PPE section question 6 says no sharps will be used. Please make it consistent.

- For surface disinfection, please indicate that the 10% bleach solution to be used will always be freshly prepared.
- Please indicate the use of a Biological Safety Cabinet (BSC) for cell culture work, not the chemical fume hood.
- Provide most recent biosafety cabinet certification date.

BUA Site Assessment: The ROHP clearance and safety training are not complete for some lab members and all have been notified of the requirement. Biosafety cabinet certification has been scheduled.

Motion: Conditional Approval (Administrative Review)	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0

8. rDNA/Bhz - Amendment

BUA	(PI)	Title	BSL	ABSL	Campus
1409		Replication strategies and host response mechanisms of RNA viruses with a focus on filoviruses	2	N/A	BUMC

Primary Reviewer: Barbara Slack	Secondary Reviewer: Tom Winters Robin Ingalls

Applicable NIH Guidelines: Sections III-D2, III-D3, III-E1, III-F, App. B, App. G

Meeting Comments: The overall goal of this project is to identify and characterize virus- and cell-specific factors that contribute to the virulence and pathogenicity of filoviruses and other highly pathogenic negative sense RNA viruses, such as henipaviruses and arenaviruses. They analyze host responses to viral infection in various cell culture infection models. However, this protocol does not culture any of those viruses directly. They have established minigenome systems which they use to study replication of these viruses under BSL2 conditions. In this amendment, they are adding new infection model to study virus replication such as a transcription-replication competent virus-like particle to study henipaviruses and filoviruses, as well as adding four RG2 negative strand RNA viruses that serves as surrogate for many RG3 and RG4 viruses, to make recombinant versions of these viruses. PI is well-experienced in making minigenome and other recombinant viral genomes for variety of molecular studies. Committee noted though that lymphocytic choriomeningitidis virus (LCMV) added in this amendment is associated with birth defects. It was discussed that PI and her lab will receive health advisory from the ROHP office in this regard. Safe handling of different agents used in this protocol is well addressed. Any validation work involving growth of BSL4 viruses will be done in BSL4 containment via multiple approved BSL4 IBC protocol. No concerns were noted. All new added viruses were also reviewed for the their potential to cause Laboratory Acquired Infection. The Chair and the medical director indicated that LCMV will be reviewed by the LAI subcommittee in their upcoming meeting.

PI recused herself from voting.

Motion: Approve	For: 13	Recuse: 1	Against: 0	Abstain: 0	Absent: 0

9. rDNA/Bhz - Amendment

BUA	(PI)	Title	BSL	ABSL	Campus
2408		Rapid Detection of Pathogens by Microfluidics-Based Nucleic Acid Amplification Tests	2	N/A	CRC

Primary Reviewer: Robin Ingalls	Secondary Reviewer: Sajal Ghosh

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

Meeting Comments: This protocol describes development of low cost point-of-care diagnostic reagents for bacterial and viral pathogens with greater sensitivity and accuracy. Inkjet-printer compatible bioink (Fluorescent biosensors) are printed on chips, which are soaked into DNA or RNA preparations of the test sample. Results are visualized using a microscope. The process involves two major steps; 1) nucleic acid sample preparation from the clinical samples and 2) Amplification of nucleic acids in the samples on specially designed chips using loop-mediated isothermal amplification (LAMP) and subsequent microscopic evaluation of the test. This protocol already designed methods to detect pathogens like Ebola and Zika viruses or *Chlamydia trachomatis* bacteria and is described well in the protocol.

To expand the diagnostic potential of their design, they are adding two more viruses to their protocol in the current amendment, namely Influenza virus and human rhinovirus. The biohazardous materials in this protocol include Zika Virus, Chlamydia, two strains of Influenza virus (a lab adapted virus) and two strains of human. For Ebola they only use a vector that express a 150bp fragment of Ebola virus L protein. Seasonal flu vaccine covers both the influenza viruses they use and the two rhinoviruses they use are common cold viruses. Most importantly, they do not culture any of these viruses. Rather, they purchase the viruses from ATCC and extract nucleic acids directly from those vials in biosafety cabinet using BSL2-specific PPE. They are aware that RNA from +ve strand RNA viruses (like Zika or rhinovirus) can produce viruses upon transfection and therefore no transfection work will be done with them. Since no particular gene that express viral protein from either ebola or Zika is being cloned, the rDNA gene expression question is marked 'No'. This was a well described protocol. No issues were noted.					
Motion: Approve	For: 14	Recuse: 0	Against: 0	Abstain: 0	Absent: 0