85 E. Newton Street
Fuller Building, [redacted]
Boston, MA 02118
Phone 617.638.4263
Fax 617.638.4226
Email IBC@bu.edu

Boston University
Institutional Biosafety Committee
Biological Use Authorization

Policy

All research work involving recombinant DNA; microbiological agents infectious to humans, animals or plants; select agents and biological toxins; materials from humans and non-human primates, transgenic animals, human gene clinical transfer; xenotransplant clinical studies and field studies involving animals must be reviewed and approved by Boston University's Institutional Biosafety Committee (IBC) and other applicable regulatory agencies before work can commence.

Responsibility

The Principal Investigator (PI) must complete, sign and submit the Biological Use Authorization to the IBC via the Research Information Management System (RIMS). The protocol will be reviewed and discussed at the next scheduled monthly committee meeting if it is received by the submission deadline. A PI who is submitting a new protocol for the first time must also provide a copy of the most current NIH Biosketch. The Biosketch should follow the National Institute of Health (NIH) two-page format. The IBC may contact the PI for questions and comments prior to the scheduled monthly meeting. The PI must provide the information requested to avoid any delay in the review of the protocol. The PI should contact the IBC Office at 617-638-4263 or the Biosafety Office, Research Safety Division of Environmental Health and Safety (EHS) at 617-638-8830 for assistance in completing the protocol. The PI must be a faculty member. Applicants who are not faculty members may be listed as an Associate Investigator under the supervision of the PI. Sponsored Personnel are individuals that are sponsored by the PI for an individual project or grant. Post Docs and Fellows that apply for grants under their own names can not apply for an IBC protocol. They must have a faculty sponsor.

Renewals and Updates

Once the protocol is approved, it will be active for three years. The PI must resubmit a completed protocol for review by the IBC after three years before it expires. The IBC Office will send the PI a renewal notice to request an annual update before each annual anniversary date of the approval. The form must be promptly completed and submitted back to the IBC Office.

Amendments

Amendments must be submitted (electronic & hard copy) for changes within an approved project. All changes should be detailed in the amendment form which must be reviewed and approved by the IBC.

Compliance

The laboratory facilities must be inspected within the year prior to approval of the protocol. All laboratory personnel must also complete their annually-required Laboratory Safety Training prior to approval of the

protocol. PI's should call the Biosafety Office, Research Safety Division of EHS for questions or assistance in this matter.

Principal Investigator (PI):

I. OVERVIEW & GRANT FUNDING INFORMATION

1. **Project Title**:
   Host Response to Filovirus Infection

2. **Specify if the research project is a**:
   - (•) New Project
   - ( ) 3 Year Re-submittal
   - ( ) Annual Renewal
   - ( ) Amendment

For Amendments and Annual Renewals:

Changes?	Instructions
Personnel	Please update the IBC personnel page
Lab Space	Please update the Research Laboratory Facility page
☐ Biological Agents	Please update the Materials Used in Research, Hazardous Biological Agents, Other Potentially Infectious materials, and/or Recombinant DNA page
☐ Procedures	Please update the Research Project Description page

Please summarize changes:

3. **What is your source of funding?**
   - ☑ Federal: NIH/NIAID U01 AI082954, UC7 AI070088
   - ☐ Non-Federal:
   - ☑ Other: start-up funds

4. **Is your grant administered through**:
   - ☑ BUMC (ORA)
   - ☐ CRC (OSP)
   - ☐ BMC (ORA)
   - ☐ Other (Specify):

INSTITUTIONAL BIOSAFETY COMMITTEE USE

DO NOT COMPLETE THIS SECTION. This section is for the Institutional Biosafety Committee use only.

Receipt Date: 08/06/2014

Approval Number:

Approval Date:

Review Date: 11/12/2013

Laboratory Biological Safety Level (BSL): BSL 4

Animal Biological Safety Level (ABSL):

Primary Reviewer: [redacted] E m a[redacted]

Secondary Reviewer: [redacted] E m a[redacted]

IBC Recommendation: APPROVED PENDING

IBC Stipulations:

_____ _____

[redacted] IBC Chair Date

II. INVESTIGATOR CONTACT INFORMATION

1. **Principal Investigator** (MUST be BU Faculty member):

Name: [redacted] **University ID Number**: [redacted]
School: School of Medicine **Department**: [redacted]
Section:
Address: [redacted]

Phone: **Fax**: [redacted]
Email (@bu.edu): [redacted]

2. **Laboratory Safety Coordinator**:

Name: [redacted] **Email:**
Phone: [redacted]

3. **24 Hour Contact for Emergencies**:

Primary:
Name: [redacted] **Email:** [redacted]
Phone: [redacted] **Mobile**: [redacted]

Secondary:
Name: **Email:**
Phone: **Mobile**:

4. **Department Administrator***:

Name: [redacted]

Phone: 617 638 [redacted] **Fax**:
Email: [redacted]

Is the Department Administrator a Boston University employee: ◉ YES ○ NO

*Administrative Contact Information Is For Contact Purposes Only In The Event That The PI Cannot Be Contacted

5. **Associate Principal Investigator**:

Address:

Name: **University ID Number**:

School:
Department:
Section:
Center:
Address:

Phone:
Fax:
Email:

6. **Sponsored Personnel**:

Name:
University ID Number:
School:
Department:
Section:
Center:
Address:

Fax: **Phone**:
Email:

III. PERSONNEL INFORMATION

1. List the names of all personnel involved in the project starting with the Principal Investigator and indicate the individual's anticipated work

Name (Last)	rDNA	Infectious Agents	Select Agents	Experienced	State how many years experience, when and where
[redacted]				Yes	BSL4 experience: University of Marburg (1990-2009)
[redacted]				Yes	BSL4 experience: 8 years; 2005-2009: University of Marburg, Marburg, Germany; 2010-present: Rocky Mountain Laboratories, NIAID, Hamilton, MT
[redacted]				Yes	BSL4 experience: UTMB; Galveston, TX: approx. 140 hours NIH/NIAID RML, Hamilton, MT: 50 hours
[redacted]				Yes	BSL4 experience: Texas Biomed, San Antonio, TX: 80 hours

2. Training Dates and ROHP Clearance

Name (Last)	BU LST	Univ	BSL1 & 2	BBP	Chem	rDNA/IBC	Ship
[redacted]	08/22/2013		08/22/2013	08/22/2013	08/22/2013	07/22/2013	
	07/19/2013		07/19/2013	07/19/2013	07/19/2013		10/16/2012
	04/10/2013		04/10/2013	04/10/2013	04/10/2013		12/13/2012
	04/03/2013		04/03/2013	04/03/2013	04/03/2013		04/16/2013

Name (Last)	BSL-3	SA	Agent: FT	Agent: TB	Agent: YP	ROHP Status	ROHP Date	ROHP #'s
[redacted]		04/04/2013				Cleared	09/26/2013	1409
		04/08/2013	09/05/2012			Cleared	09/25/2013	1409
		04/05/2013				Cleared	01/11/2013	n/a
		04/04/2013	04/08/2013			Cleared	10/22/2013	1409

a[redacted]nd

experience with the following:

Name (Full)	BU Alias	Title	Descriptive Role
[redacted]	[redacted]	PhD	PI
[redacted]	[redacted]	PhD	postdoctoral fellow
[redacted]	[redacted]	PhD	postdoctoral fellow
[redacted]	[redacted]	PhD	Postdoctoral Fellow

IV. RESEARCH LABORATORY FACILITY INFORMATION

1. Laboratory locations and corresponding Biological Safety Levels (BSL):

Building	Room	BSL	Animal BSL	Shared PI Name	Shared Other	Function of Room
NEIDL	[redacted]	BSL 4	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 4	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 4	N/A			Storage of Biohazardous Materials Fumigation Airlock
NEIDL	[redacted]	BSL 4	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 4	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 4	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 1	N/A			Tissue culture, Microscopy, Centrifugation
NEIDL	[redacted]	BSL 4	N/A			Storage of Biohazardous Materials rack staging room containing second autoclave

2. Off-site Location:

Facility/Institution	Address	Lab Safety Training	Emergency Care	Biosafety Officer	Phone

VI. DUAL USE RESEARCH OF CONCERN

Please review the eight categories below and indicate if your research falls into any of the dual use research concern category:

1. Enhance the harmful consequences of a biological agent or toxin. If Yes, please explain. ○ YES ◉ NO

2. Disrupt immunity or effectiveness of an immunization without clinical and or agricultural justification. If Yes, please explain. ○ YES ◉ NO

3. Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against agent or toxin. If Yes, please explain. ○ YES ◉ NO

4. Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies. If Yes, please explain. ○ YES ◉ NO

5. Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin. If Yes, please explain. ○ YES ◉ NO

6. Alter the host range or tropism of a biological agent or toxin. If Yes, please explain. ○ YES ◉ NO

7. Enhance the susceptibility of a host population. If Yes, please explain. ○ YES ◉ NO

8. Generate a novel pathogenic agent or toxin, or reconstitute an eradicated or extinct biological agent. If Yes, please explain. ○ YES ◉ NO

VII. RESEARCH PROJECT DESCRIPTION

1. Describe the project in Layman's Terms (NIH requirement of 3 to 4 sentences about the goal of the experiment. This should be written in non-technical language (6th grade reading level).

The filoviruses Marburg- and Ebolavirus cause a severe feverish bleeding disease in humans. Currently, there is no therapy available to treat or prevent filovirus disease. The aim of this project is to study how cells react to filovirus infection and which cellular pathways involved in the antiviral defense are blocked or activated by filoviruses. The long term goal is to identify potential viral or cellular targets for antiviral drug development.

2.Provide a brief description of the project scientific objectives in 200 words or less.

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. The filoviruses Ebola (EBOV) and Marburg virus (MARV) cause a severe hemorrhagic disease in humans. Due to the high case fatality rate and the lack of treatment and approved vaccines, filoviruses are classified as BSL4 agents and select agents. A hallmark of filovirus infection is the massive impairment of the host antiviral response. In this project, we will characterize the earliest events that occur in the interaction of the virus with the host cells. This includes cytokine/chemokine responses, Toll-like receptor responses, and interaction with other cellular pathways involved in antiviral responses. We will also use different filovirus species that vary in their virulence to determine if there is a correlation between the capability of these viruses to antagonize the host immune response and pathogenicity. We will not perform recombinant DNA work with BSL4 agents.

3. Describe laboratory procedures and manipulations involved in the study. Provide sufficient detail for the reviewer to fully understand the potential health and environmental hazards associated with the project and any steps or procedures in place to limit the potential hazards.

BSL2 procedures related to this protocol such as standard tissue culture procedures are described in IBC protocol 1409.

This IBC protocol focuses on handling the BSL4 pathogens MARV and EBOV. The protocol includes propagating filoviruses in cell culture, virus titration, infection of human and animal cell lines and human and animal primary cells. BSL4 work requires agent- and procedure-specific training and BSL4-specific PPE. There is an SOP in place for each laboratory procedure that is vetted and approved by Environmental Health and Safety. Only personnel signed off for BSL4 work are allowed to work in the BSL4 facility. All staff must follow general SOPs for BSL4 operation as well as agent- and procedure-specific SOPs. All staff must wear a positive pressure suit and at least 2 pairs of gloves while performing the procedures in BSL4. After work is completed, personnel must exit the BSL4 laboratory through a decontaminating chemical shower, remove scrubs worn under the positive pressure suit which are autoclaved, and take a personal shower before dressing and leaving the BSL4.

Work with infectious material is generally performed in class II biosafety cabinets (BSC). All items in the BSC will be wiped down or sprayed down with an appropriate disinfectant (5% Microchem-Plus, 70% ethanol) prior to removal from the BSC. 70% EtOH will mainly be used for sensitive equipment (e.g. rotors) and to remove traces of Microchem-Plus from surfaces. Liquid waste is collected in containers located inside the BSC and containing an appropriate disinfectant (e.g. Microchem-Plus; final concentration at least 5%). Liquid waste will be disposed of by pouring directly into a BSL4 sink after sufficient contact time. All BSL4 sinks are connected to cook tanks in which the liquid waste is heat-inactivated again before discharge. Solid waste that was in direct contact with infectious material (pipette tips, pipettes, tubes, tissue culture vessels) will be submerged in containers containing an appropriate disinfectant (e.g. Microchem-Plus; final concentration at least 5%). After sufficient contact time, the solid materials will be separated from the disinfectant, placed into waste

collection containers outside of the BSC that are double-lined with red bags and autoclaved. Solid waste that was not in direct contact with infectious material (gloves, tissues) will be collected inside the BSC in containers lined with an autoclave bag. The bag will be sprayed down prior to removal from the BSC, placed into a waste collection container outside of the BSC double-lined with red bags and autoclaved.

Viruses: We will use filoviruses for this study. Filoviruses belong to the group of negative-sense RNA viruses and cause a severe hemorrhagic disease in humans with high fatality rates. The only members of the filovirus family are Marburg- and Ebolavirus. Ebolaviruses are subdivided into five species, Zaire ebolavirus, Sudan ebolavirus, Tai forest ebolavirus, Reston ebolavirus, and Bundibugyo ebolavirus. The different EBOV species vary in terms of virulence and pathogenicity. The most pathogenic species in humans is Zaire ebolavirus with a case fatality rate of about 80%, followed by Sudan ebolavirus with a case fatality rate of about 50%, and Bundibugyo ebolavirus with a fatality rate of about 30%. To date, there is only one reported non-fatal human case of Tai forest ebolavirus infection and no human disease cases of Reston ebolavirus infection, although individuals, who were in contact with infected animals, developed an antibody response against Reston ebolavirus. Marburgvirus is closely related to EBOV. There is a single MARV species comprising various viral strains, including marburgvirus Musoke, marburgvirus Angola and several MARV isolates from bats. Filoviruses (except Reston ebolavirus) are endemic in central Africa. For some of the index cases it has been reported that they became exposed to the virus through contact with blood or organs of infected animals. Spread of the virus between individuals is the result of direct contact with blood or other body fluids (saliva, sweat, stool, urine, tears, and breast milk) from infected patients. Filoviruses are not transmitted by aerosols. Currently there is no vaccine or treatment against filovirus infection. Since filoviruses have a lipid envelop, they can be easily inactivated by disrupting the envelope with chemical reagents, including alcohols and detergents, or heat.

Virus strains used in this study
Zaire ebolavirus (strain Mayinga 76, strain Kikwit 1995); Reston ebolavirus (strain Pennsylvania, strain 2008); Sudan ebolavirus (strain Gulu); Tai Forest ebolavirus; Bundibugyo ebolavirus; Marburgvirus (strain Musoke, strain Angola, strain Popp, strain bat)

Laboratory procedures inside the BSC
Infection: Infection of cells with filoviruses will be performed in the BSL4 suite inside a biosafety cabinet (BSC). We will use human, nonhuman primate and animal cells for infection. We will also use human primary blood cells, human umbilical vein endothelial cells and bat primary blood cells for infection. For most experiments, the infected cells will be inactivated after an appropriate incubation time in the CO2 incubator using standard inactivation procedures (see below). Infected cells will be used for immunoprecipitation assays (live material), Western Blot analysis (inactivated material), microscopy (live and inactivated material), RNA analysis (inactivated material), and FACS analysis (see below). Supernatants will be used for cytokine and chemokine analysis using Bioplex technology (see below).

Virus purification and titration: Cells seeded in standard cell culture vessels will be infected with filoviruses. After an appropriate incubation period at 37 C, supernatants will be clarified by low speed centrifugation (sealed buckets). Viral particles will be purified by sucrose cushion ultracentrifugation (sealed buckets) and gradient ultracentrifugation, if required. Titration of virus stocks will be done by plaque assay, focus forming assay, or tissue culture infectious dose 50 (TCID50) assay. The titers of filovirus stocks generally range between 106 108 infectious units/ml. In most cases, BSL-4 virus stocks are stored in volumes ranging from 0.5 ml to 1.0 ml per vial.

For purification of large amounts of viral RNA, clarified supernatants will be subjected to polyethylene glycol (PEG) precipitation. PEG precipitation will be performed in 50ml screw-cap tubes overnight on a rotator placed in the refrigerator. Precipitated viral particles will be pelleted by low speed centrifugation and resuspended in TRIZOL.

Laboratory procedures outside the BSC

Incubation of cells in CO2 incubator or refrigerator: Infected cells seeded in standard tissue culture vessels will be placed into CO2 incubators for virus propagation. For some protocols, samples in closed containers will be incubated at 4 C in a refrigerator.

Centrifugation: We will use microcentrifuges, minifuges, and ultracentrifuges for centrifugation. All centrifugation steps are performed using buckets and rotors equipped with sealed covers. Leak proof centrifuge tubes are loaded into the buckets in a BSC and, after centrifugation, buckets are opened inside the BSC. There are SOPs in place describing how to operate the centrifuges and how to proceed in case any problems occurred during the run.

Bioplex assay: Supernatants will be clarified by low speed centrifugation. Preparing the supernatants for bioplex analysis will be performed inside the BSC. Since the use of the Bioplex instrument might lead to aerosol formation, samples will be treated with 4% paraformaldehyde for 30 min prior to analysis on the BioPlex 200. Microchem-Plus (final concentration at least 5%) will be added to the waste tank.
It has been shown for Hantaan virus, a BSL-3 pathogen that shares some physicochemical features with filoviruses, including an encapsidated negative-sense RNA genome and a lipid envelope, that 30 min incubation in 1% PFA are sufficient for inactivation (Kraus et al., Intervirology 2005; 48:255261). However, since our standard PFA inactivation procedure for cell culture includes a minimal incubation time of 12 hours, samples treated with 4% PFA for a shorter period of time than 12 hours are not considered to be inactivated.

FACS analysis:Preparation of cells for FACS analysis will be performed inside the BSC (antibody staining). Since operating the LSRII FACS instrument might lead to aerosol formation, samples will be treated with 4% paraformaldehyde for 30 minutes prior to FACS analysis (please see comment to Bioplex assay about 30 min treatment). Microchem-Plus (final concentration at least 5%) will be added to the waste tank.

Luminometer: The luminometer will only be used for training purposes to test its function under BSL-4 conditions. Cells will be lysed in the BSL-2 laboratory in the respective reporter lysis buffer and the cell lysates will be brought into the BSL4 lab where they will be handled with BSL4 precautions. Measurements will be performed using 96-well format with small volumes of lysate (10 microliter). Microchem-Plus (final concentration at least 5%) will be added to the waste tube.

Microscopy: Microscopy of live material will only be performed with closed tissue culture vessels inside the BSL4.

Immunoprecipitation: To analyze protein-protein binding (viral-cellular, viral-viral), we will perform coimmunoprecipitation analysis. All procedures except incubation at 4 C will be performed inside the BSC. 4 C incubation of samples will be performed on a rotator placed in the refrigerator. Tubes containing samples (1.5 or 2 ml tubes) will be placed in secondary containers (50 ml screw cap tubes equipped with paper towels) for 4 C incubation. Supernatants and washing buffers will be removed from beads using plastic tips. No needles will be used.

Inactivation Procedures
All steps after inactivation are performed outside the BSC to avoid contamination with live agents. Inactivated samples are removed from the BSL4 laboratory through dunk tanks or chemical showers. 5% Microchem-Plus is used as disinfectant in dunk tanks and chemical showers. Removal of inactivated samples from the BSL4 laboratory has to be documented (name of researcher, date, inactivation procedure, number of samples).

Heat inactivation: This inactivation method is usually used for Western blot samples. Cell lysates will be transferred into fresh tubes containing SDS loading buffer (e.g., Laemmli buffer). The final concentration of SDS must exceed 1%. The tubes will be placed in a pre-heated heat block with the temperature set to 120C/248F. The temperature of the heat block must be at least at 110C/230F before the tubes are placed into the heat block. Samples will be incubated for at least 10 minutes in the heat block and removed from the BSL4 lab through the dunk tank or chemical shower.

Paraformaldehyde (PFA) and formalin inactivation: This inactivation method is commonly used for immunofluorescence and FACS samples. Adherent cells seeded on coverslips, chamber slides etc. will be incubated in 4% PFA or 10% formalin for at least 12 hours at 4 C. Cell pellets will be resuspended in 4% PFA or 10% formalin and incubated for at least 12 hours at 4 C. Then, cells will be transferred into fresh containers containing 4% paraformaldehyde or 10% formalin. All inner surfaces of the containers must be wetted with PFA/formalin. Samples will be removed through the dunk tank or chemical shower.

RNA extraction: Cells. Media will be removed from cells and TRIZOL Reagent (Invitrogen) or a similar RNA extraction buffer (E.g. RNAzol; Molecular Research Center) will be added onto cells (1ml TRIZOL per well of a 6-well plate). Cells will be lysed by pipetting and transferred into tubes. After vortexing, cell lysates will be incubated for 10 min at RT for inactivation. For removal form the BSL4 lab, lysates will be briefly spun down and transferred into clean tubes outside the BSC. Tubes will be shaken to make sure that all inner surfaces had contact with TRIZOL mixture and removed through the dunk tank or chemical shower. Supernatants. Cell supernatants will be clarified by low speed centrifugation and transferred into tubes. If required, samples will be diluted 1:2 with RNase free water (1 vol/ 1vol). 0.75 ml TRIZOL LS Reagent (Invitrogen) will be added per 0.25 ml of sample volume. Samples will be vortexed, incubated for 10 min at RT and processed as described for RNA extraction from cells.

Storage

Short-term storage. Infectious samples will be stored at 4 C, -20 C, or -80 C in screw-cap tubes or cryovials inside the BSL4 facility. Long-term storage of virus stocks will be in a -80 C freezer. Vials will be transported form the freezer to the BSC in racks. Due to security concerns, room numbers for long term storage cannot be released. Short-term and longterm stored samples will be inventoried according to the requirements for storage of select agents.

Transport

For room-to-room transportation, screw cap tubes or cryovials containing biological samples are placed into leak proof, shatter proof secondary containers. These samples are then transported by the investigator to adjacent rooms within the BSL4 through the corridors.

Transport of infectious material to BU

There is a detailed Hazardous Materials Transportation Plan in place regulating the transport of BSL-4 pathogens (attached). Filoviruses are classified as select agents. Transport of these agents will be done in accordance with all laws and regulations including the approval from the U.S. Department of Health and Human Services, Center for Disease Control and Prevention, prior to shipment, and notification within 24 hours of receipt. The transport will also include the utilization of appropriate forms and the reporting of registration numbers of all parties involved in shipping, transporting and receiving packages.

Environmental Health and Safety (EHS), Emergency Response Planning and Public Safety (PS) at BU are in charge to organize the transport of filoviruses. EHS will train all users of the laws, regulations, polices and requirements involved in the shipping and receiving of subject materials and will manage the tightly controlled, pre-approved, scheduling of shipment and delivery times. EHS will train all BU/BMC users in the approved procedures for the packaging of materials, the approved contracted services to be used in the transport of such materials and the penalties of failing to follow all aspects of this policy.

EHS and PS will ensure that BU/BMC staff involved in the high risk materials shipping/ receiving areas will undergo a background clearance check, as appropriate, consistent with the Select Agent law requirements prior to being approved to work in these locations.

EHS and PS will determine the best location for the receipt, control, audit, transport, and shipping of all BSL4 items. Such location(s) will be operated or provided with oversight by representatives of EHS and other related user departments. These areas will be routinely audited. Transport to and from this location will be by major routes of travel that immediately border BU/BMC and are limited to Albany Street, Massachusetts Avenue and the highway/connector system in the rear of BioSquare.

EHS and PS will determine the packaging requirements to be used in the shipping and receiving of subject materials. These requirements will comply with all applicable regulatory standards. EHS, PS and the Office of Purchasing will select contractors for the transportation of BSL4 pathogens based on a number of criteria that qualify the transporter to provide transport service for highly pathogenic agents. Delivery of BSL4 pathogens will be tracked and may be escorted. DPS will provide security at the point of receipt of BSL4 pathogens and escort the package from the point of entry to the final destination in the NEIDL.

Prior to the transport, the Emergency Response Planning Division will ensure that the appropriate Commonwealth having jurisdiction and the Boston Public Health Commission are notified. It is the responsibility of the Boston Public Health Commission to notify the appropriate city of Boston departments.

There are emergency plans in place dealing problems or incidents on route.

VIII. PERSONAL PROTECTIVE EQUIPMENT AND SAFETY EQUIPMENT

1. Indicate all laboratory manipulations involved in the research protocol that have the potential to produce aerosols or droplets:

Laboratory Procedure	Performed

Homogenizing, tissue grinding	☐
Vortexing	☑
Vigorous mixing, blending	☐
Freeze drying, lyophilizing	☐
Sonicator, ultrasonic cleaners	☐
Animal handling, cage changing	☐
Pipetting infectious liquid	☑
Centrifugation, ultra centrifugation	☑
Opening containers under pressure	☐
Culture stirrers, shakers	☐
Plating, colony counting	☐
Animal inoculations	☐
Animal aerobiology exposure	☐
Other (Specify):	

2. Indicate the engineering controls in place to prevent potential exposure from procedures described.

- ☑ Work that produce/or potentially produce aerosols are done in the Biological Safety Cabinet or other containment equipment.
- ☑ Use of centrifuges with sealed rotors or sealed cups.
- ☑ HEPA and hydrophobic filter protection on the vacuum line.
- ☐ Gasket blenders/ homogenizers.
- ☐ Others (describe):

BSL4 containment

3. Indicate the personal protective equipment *to be used in the laboratory* to prevent potential exposure from procedures described.

- ☐ Laboratory coats
- ☑ Disposable gloves
- ☐ Goggles
- ☐ Safety glasses
- ☐ Face shield
- ☐ Surgical mask
- ☐ Respirator (i.e. N95)
- ☐ Shoe cover
- ☐ Head cover
- ☐ Powered Air Purifying Respirator (PAPR)
- ☐ Disposable scrubs
- ☐ Double gloves
- ☐ Back fastening gowns
- ☐ Other (describe):

Standard BSL4 PPE

4. Indicate the personal protective equipment to be used in the animal containment to prevent potential exposure (if no animals are used, do not check)

- ☐ Laboratory coats
- ☐ Disposable gloves
- ☐ Goggles

- ☐ Safety glasses
- ☐ Face shield
- ☐ Surgical mask
- ☐ Respirator (i.e. N95)
- ☐ Shoe cover
- ☐ Head cover
- ☐ Powered Air Purifying Respirator (PAPR)
- ☐ Disposable scrubs
- ☐ Double gloves
- ☐ Back fastening gowns
- ☐ Other (describe):

5. Will Biological Safety Cabinets (BSCs) be used for this work? If YES, provide the following information:

◉ YES ○ NO

Make: Baker

Model: Steril GARD III Advance

Serial Number: 95508, 95673, 95663, 95506, 95543,94341

Recent Certification Date: 07/11/2013

6. Will sharps be used in the studies?

◉ YES ○ NO

If YES, describe the safety precautions to be followed:

Chamber slides consisting of a removable plastic chamber attached to a glass microscope slide and glass coverslips placed into tissue culture plates will be used for immunofluorescence analysis. Coverslips will be handled with tweezers only. Plastic chambers will not be removed from glass slides in BSL4 lab. Chamber slides and coverslips will be handled with extreme caution. After inactivation of infectious virus with 4% PFA, chamber slides and coverslips will be removed from the BSL4 lab. No sharp waste will be generated.

7. Describe how you will treat and dispose the biological or biohazardous wastes:

A. Liquid Wastes:

Since waste management is key to BSL4 safety, waste management procedures are briefly described in the laboratory procedure section. There are EH&S-approved SOPs in place describing all waste management procedures.

B. Solid Wastes: see above

8. What disinfectant will be used?

5% Microchem, 70% EtOH

9. Is there a spill kit in the laboratory?

◉ YES ○ NO

10. How and where are biohazardous materials stored?

Short-term storage. Infectious samples will be stored at 4C, -20C, or -80C in screw-cap tubes or cryovials inside the BSL4 facility. Long-term storage of virus stocks will be in a -80C freezer. Vials will be transported form the freezer to the BSC in racks. Due to security concerns, room numbers for long term storage cannot be released. Short-term and long-term stored samples will be inventoried according to the requirements for storage of select agents.

11. Describe how biohazardous materials are transported:
(indicate nature of primary and secondary containers)

For room-to-room transportation, screw cap tubes or cryovials containing biological samples are placed into leak proof, shatter proof containers. These samples are then transported by the investigator to adjacent rooms within the BSL4 through the corridors.

12. Are all equipment used for biohazardous materials work affixed with biohazard warning labels?

◉ YES ○ NO

IX. MATERIALS USED IN RESEARCH

The Principal Investigator must state the type of materials to be used in the study and complete only the appropriate Sections:

If your research involves the following materials or activities (check all that apply)...	Example/Description
☑ Hazardous Biological Agent including Human Cells and Cell Line	Viruses, Bacteria, Fungi, Parasites, Rickettsia, Prion, Human Cell Lines, Non Human Primate Primary or Cell Lines
☑ Other Potentially Infectious Materials	Other Human Material, Other Non-Human Primate Material, Sheep Material: Blood, Plasma, Serum, Unfixed Tissue, Brain, Organs, Unfixed Cells
☐ Human Embryonic Stem Cell	Human Embryonic Stem Cell
☐ Select Biological Toxins	Abrin, Botulinum neurotoxins, Conotoxin, Clostridium perfringens epsilon toxin, Diacetoxyscirpenol (DAS), Ricin, Staphylococcal enterotoxins, Saxitoxin, Shiga-like ribosome inactivating proteins, Shigatoxin, Tetrodotoxin, T-2 toxin
☐ Field Study with Animals or Insect Vector	Environmental or field studies with animals
☐ High Hazard Chemical	BU High Hazard Chemical List
☐ Radiation and X-Ray	Radioactively-labeled compounds; Inject animals with radioactive-labeled compounds; X-ray or other imaging of specimens; Use of the irradiator
☐ Inactivated biological samples	Use of inactivated, non-infectious biological samples derived from Biological Safety Level 3 (BSL-3) or Biological Safety Level 4 (BSL-4) agents
☐ Recombinant DNA	Recombinant DNA molecules aredefined as molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell or those resulting from such replication.
☐ Synthetically derived nucleic acid molecules	Creation of synthetically derived nucleic acid molecules; synthetic DNA segments that are likely to yield a potentially harmful polynucleotide or polypeptide are considered as equivalent to their natural DNA counterpart.
☐ Live Animal Use	Work involving the use of biohazardous materials or recombinant DNA in live animals

Highest Biosafety Level (BSL) required for this project:

○ BSL-1 ○ BSL-2 ○ BSL-2 with special practices of BSL-3 ○ BSL-3 ○ BSL-4

Highest Animal Biosafety Level (ABSL) required for this project:

○ ABSL - 1 ○ ABSL-1+ ○ ABSL-2 ○ ABSL-3 ○ ABSL-4 ○ N/A

Section A. Hazardous Biological Agents

Is this a

Specific Name	Strain	Agent Class	Obtained From	BSL
Blood from Rousettus aegyptiacus and Pteropus vampyrus bats		Other	Lubee Bat Conservancy, Gainesville, FL	BSL-2
Fruit bat cell lines R05T, R06E, RoNi		Other	company; Institute of Virology, Bonn, Germany	BSL-2
HeLa, HEK293, HEK293T, U2OS, Huh7, THP-1		Human Cell/Lines	BU researcher, Harvard researcher, ATCC	BSL-2
Vero, Vero C1008		Non Human Primate Cell/Lines	ATCC	BSL-2
Marburg virus	Musoke, Angola, bat	Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4
Zaire ebolavirus	Mayinga, Kikwit	Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4
Sudan ebolavirus	Gulu	Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4
Reston ebolavirus	Pennsylvania, 08	Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4
Tai Forest ebolavirus		Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4
Bundibugyo ebolavirus		Virus	NIH/NIAID Rocky Mountain Laboratory	BSL-4

2.)
hospital-based project? Yes No

If Yes, you must inform Physician for Hospital Epidemiology by contacting 617-414-5037 on the agent/s used and please provide the following information:

Physician contacted: **Date:**

1.)

Specific Name	Cause Human Disease?	Attenuated?	Introducing Antibiotic Resistance?	Antibiotic Resistance Profile	IRB for Agent?
Blood from Rousettus aegyptiacus and Pteropus vampyrus bats	No	No	N/A		No
Fruit bat cell lines R05T, R06E, RoNi	No	No	N/A		No
HeLa, HEK293, HEK293T, U2OS, Huh7, THP-1	No	No	N/A		No
Vero, Vero C1008	No	No	N/A		No
Marburg virus	Yes	No	N/A		No
Zaire ebolavirus	Yes	No	N/A		No
Sudan ebolavirus	Yes	No	N/A		No

3.)

Reston ebolavirus	Yes	No	N/A		No
Tai Forest ebolavirus	Yes	No	N/A		No
Bundibugyo ebolavirus	Yes	No	N/A		No

Specific Name	Select Agent?	Attenuated BSL3/BSL4?	Verification Lab	Verification Contact
Blood from Rousettus aegyptiacus and Pteropus vampyrus bats	No	No		
Fruit bat cell lines R05T, R06E, RoNi	No	No		
HeLa, HEK293, HEK293T, U2OS, Huh7, THP-1	No	No		
Vero, Vero C1008	No	No		
Marburg virus	Yes	No		
Zaire ebolavirus	Yes	No		
Sudan ebolavirus	Yes	No		
Reston ebolavirus	Yes	No		
Tai Forest ebolavirus	Yes	No		
Bundibugyo ebolavirus	Yes	No		

4.)

Specific Name	Live Animals With Agent?	Description	IACUC Approval#	Species	ABSL	USDA permit to import/transport	Permit #
Blood from Rousettus aegyptiacus and Pteropus vampyrus bats	No						
Fruit bat cell lines R05T, R06E, RoNi	No						
HeLa, HEK293, HEK293T, U2OS, Huh7, THP-1	No					No	
Vero, Vero C1008	No						

Bundibugyo ebolavirus	No						

Marburg virus	No					No	
Zaire ebolavirus	No					No	
Sudan ebolavirus	No						
Reston ebolavirus	No						
Tai Forest ebolavirus	No						

Section B. Other Potentially Infectious Materials

Class	Type	Other Type	Source of Material
Other Human Material	Blood		Blood is obtained as anonymous samples from New York Biologics.

Yes

1.) Do you have IRB (Institutional Review Board) approval related to this project?

If YES or Pending please supply the following:

IRB Approval Number: [redacted]

IRB Expiration Date:

X. AGREEMENT POLICY

As the Principal Investigator of this project, I certify that the information contained in this application is accurate and complete. I agree to comply with any requirements posed by the Institutional Biosafety Committee (IBC) and pertinent regulatory

In addition, I agree to abide by the following requirements:

☐ I will not initiate experimentation until this research project has been approve by the IBC.

☐ I will follow appropriate Biosafety Level laboratory techniques required for this project.

☐ I will comply with all shipping requirements for materials, as appropriate.

☐ I will provide to the laboratory staff copies of the approved protocols which describes the potential biohazards and the precautions that must be taken.

☐ I will train the staff in good microbiological practices and techniques required to ensure safety for this project, and in the procedures for dealing with accidents and waste management.

☐ I will ensure that all laboratory workers are registered with the IBC.

☐ I will supervise the staff and correct work errors and conditions that could result in breaches of the *Biosafety Manual, Exposure Control Plan, Chemical Hygiene Plan* and other plans as appropriate.

☐ I will submit an amendment for any changes/ additional work to be performed that go beyond the range of the current protocol (before work begins).

☐ I will obtain required additional approvals if my work involves animals from institutional Animal Care & Use Committee (IACUC) for the use of primary human tissues or cells from the Institutional review Board (IRB)

☐ I will contact Research Occupational Health Program (ROHP) 24/7 at (617) 414-7647 immediately after a potential exposure or accident in my lab.

[redacted] Electronically submitted through secure, password-protected login to BU Research Information Management System (RIMS) 08/06/2014

Name of Principal Investigator

PUBLIC HEALTH COMMISSION

ENVIRONMENTAL HEALTH OFFICE

1010 Massachusetts Avenue
Boston, MA 02118
Phone (617) 534-5965

FAX (617)
534-2372

REGISTRATION FORM FOR rDNA PROJECTS

Project Title:

Anticipated Starting Date:

Brief Description of Project:

Institution Name:

Lab Facility Address(es):

Building(s):

Room(s):

Principal Investigator(s):

Are Large Scale Volumes Used (>= 10 liters)?

Is an rDNA gene product efficiently expressed?

Containment levels (date and subsection of applicable NIH Guidelines):
Highest BSL:

NIH Guideline:

Host-Vector_Donor System:

Lab Personnel to contact in emergency situations requiring immediate remedial action:

Name: Phone:

Certification:

1. I am familiar with and agree to abide by the provisions of the Boston Public Health Commission Regulations for Recombinant DNA Use & Technology. The information above is accurate and complete.

Principal Investigator: Electronically submitted through secure, password-protected login to BU Research Information Management System (RIMS), by [redacted] Date: 08/06/2014

2a. I certify that the Institutional Biosafety Committee (IBC) has reviewed on 11/12/2013 the proposed project for DNA and has by a majority vote found it in compliance with the provisions of the Boston Public Health Commission Regulations for Recombinant DNA Use & Technology. The IBC will monitor the project throughout its duration to ensure its compliance with the Boston Public Health Commission Regulations.

Chairperson, Biosafety Committee: _____ Date: _____

OR

2b. I certify that I have been authorized by the IBC to administratively review and approve Class III-E, and F experiments or by an Institutional Review Body (IRB) to administratively review and approve Class III-F experiments. The proposed project has been found to be in compliance with the Boston Public Health Commission Regulations for Recombinant DNA Use & Technology. The institution will monitor the project throughout its duration to ensure its compliance with the Boston Public Health Commission Regulations.

Officer: _____ Date: _____