VCU Environmental Health and Safety
Safety and Risk Management

BIOSAFETY MANUAL

TABLE OF CONTENTS

I. ACKNOWLEDGMENT FORM.

Principal Investigators (PIs) and all personnel active in research within laboratories under their jurisdiction must sign and date the following statement to acknowledge policies and conditions set forth in this document.

1. Principal Investigator: I am familiar with and agree to comply with the provisions of the VCU Biosafety Manual and have added information where required to address hazard conditions which are specific to the laboratory spaces under my jurisdiction. I have thoroughly discussed the content of this manual with those working under my supervision and have given them the opportunity to ask questions and voice concerns regarding their job description/work environment:

Principal Investigator printed name	Principal Investigator signature	Date

2. Laboratory Personnel: I am familiar with and understand the potential hazards, emergency procedures, and proper use of the work methods, personal protective equipment (PPE), and engineering controls detailed within this document. My PI has provided me with further site-specific training regarding potential hazards which may present within my workplace and job description.

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II. INTRODUCTION.

A. Scope. Safety and Risk Management - Environmental Health and Safety (EHS) under the auspices of the Institutional Biosafety Committee (IBC) has developed this model biosafety manual to assist PIs and laboratory directors in limiting staff and student exposure to biohazardous agents and to better ensure university compliance with all applicable regulations promulgated by governmental regulatory and credentialing agencies. This biosafety manual serves as the university model: through adding requested information and applicable attachments (Appendices) a document which meets the IBC mandate for development of a laboratory-specific biosafety manual can be satisfied. This manual does not address issues of radiation or chemical safety. These are covered in the university Radiation Safety Manual and the Chemical Hygiene Plan and can be accessed at https://srm.vcu.edu/.

B. Regulatory Forces. The guidelines developed by the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), Occupational Safety and Health Administration (OSHA), and the Virginia Department of Environmental Quality (VDEQ) are key components of this biosafety manual. Compliance with the minimum conditions set forth in this biosafety manual is mandatory for all university-owned or leased facilities. All university research laboratory personnel are strongly advised to fully review and understand the following information offered by regulatory agency websites:

1. NIH: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

2. CDC-NIH: Biosafety in Microbiological and Biomedical Laboratories

3. OSHA: CFR 29 1910.1030, Bloodborne Pathogens Standard

4. VDEQ: Regulated Medical Waste Management Regulations

C. Biological Safety Program at VCU. Principal investigators are ultimately responsible for ensuring implementation of a comprehensive biological safety program for all laboratories under their supervision. Development of a complete biosafety program requires cooperation and interaction among the flowing university entities:

1. Institutional Biosafety Committee (IBC). All institutions awarded NIH funding for recombinant DNA research are required to form IBCs which function in accordance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. While the NIH Guidelines are specific to rDNA materials, the VCU IBC has elected to exercise at its discretion jurisdiction over certain other protocols containing chemical or biological hazards. Failure to comply with the requirements established during the IBC review process may result inthe suspension of research involving chemical, biological, rDNA, and/or institutional privilege to use animals.

2. Institutional Animal Care and Use Committee (IACUC). Reviews all *in vivo* research protocols to ensure ethical treatment of animals and compliance with applicable regulations. Approval from IACUC is required prior to initiating new *in vivo* research or altering existing *in vivo* research protocols. The approvals concerning *in vivo* use of hazardous materials are awarded by the IBC following protocol approval by the IACUC.

3. Safety and Risk Management - Environmental Health and Safety (EHS/SRM). Oversees the university Biological Safety Program, administers university Select Agent Program, manages the university Lab Safety Program, administers university Respiratory Protection Program, and

appoints an Institutional Biosafety Officer (BO) who is responsible for establishing/interpreting university biosafety policies. EHS/SRM staffs the "Biosafety Office" (contact mtelliot@vcu.edu) which works under the direction of the BO in the research protocol approval/registration process and performs protocol and laboratory inspections to confirm availability of proper conditions and facilities.

4. The PI must register all protocols involving biohazardous/rDNA agents and Select Agents (including those maintained at *de minimus* quantities) with EHS/SRM annually through submission of biological registration under the VCU Bioraft Safety Management System (vcu.bioraft.com). No new or modified research procedures involving these hazards may commence prior to receiving approval from the EHS/IBC. Principal investigators must submit a completed IACUC protocols for all research that involve the use of animals and biohazardous/rDNA materials and select agents. Copies of all active IBC approvals involving biohazardous/rDNA materials are attached to Appendix A of this manual.

5. The PI must provide, at no cost to the employee, all required training, PPE, engineering control devices, immunizations, emergency response equipment, and any other necessities to adequately address recognized or anticipated hazards. The PI shall ensure that manuals containing thorough/up-to-date SOPs, emergency response plans (ERPs), Exposure Control Plan (ECPs), Worker's Right to Know, and Job Safety Analyses maintained in locations familiar to all staff within all research spaces where manipulations with biohazardous/rDNA agents are performed. Principle investigators shall ensure applicable SOPs, ERPs, ECPs, and JSAs are updated annually or whenever new procedures involving biohazardous agents are performed. Laboratory staff shall receive thorough training regarding the content of the SOP/ERP/ECP/JSA manual annually and whenever new procedures are added to the laboratory regimen. Copies of updated SOPs for all procedures involving biohazardous/rDNA materials are attached as Appendix C to this document, a copy of the ECP (if applicable) must be attached to Appendix E to this manual.

III. DEFINITION OF BIOHAZARDS

For the purpose of this manual, potentially hazardous biological agents and by-products are called biohazardous agents. The VCU IBC's working definition of a biohazardous agent includes the following:

1. Pathogenic agents (bacteria, rickettsia, fungi, viruses, protozoa, parasites, prions, and Select Agents)

2. Recombinant or synthetically derived nucleic acid, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos) or both. The NIH defines synthetically derived nucleic acid molecules as follows:

    a. Molecules that (a) are constructed by joining nucleic acid molecules and (b) can replicate in a living cell (i.e., recombinant nucleic acids);

    b. Nucleic acid molecules that are chemically or otherwise modified but can pair with naturally occurring nucleic acid molecules (i.e., synthetic nucleic acids);

    c. Molecules that result from the replication of those described in (a) or (b) above

3. Recombinant DNA molecules, organisms, vectors (e.g., plasmids, viral vectors), and viruses containing recombinant DNA molecules

4. Human and non-human primate blood, tissue, body fluid, and cell culture (primary and established cell lines)

5. Plants, animals, or derived waste which contains or may contain pathogenic hazards

This manual also includes guidelines for containment of biohazards to control the spread of contamination. The control practices contained in this manual are meant to supplement conventional safety efforts, including accident and fire prevention**.**

IV. PRINCIPLES OF BIOSAFETY

A. Containment. The term "containment" refers to utilizing routine safe methods when handling infectious material in the laboratory. Containment is the first line of defense for reducing exposure potential to laboratory personnel and the possible contamination of the laboratory or beyond. The Centers for Disease Control and Prevention identifies the following two types of containment:

1. Primary Containment.

2. Personal Protective Equipment.

a. Gloves. Gloves must be worn whenever manipulations involving potentially biohazardous agents or hazardous chemicals are performed. Select glove type based on specific biohazardous agents and chemical compound(s) to be handled as not all glove materials are suitable for every type of exposure. As required by the laboratory-specific Exposure Control Plan, two pairs of gloves may be specified to adequately protect the laboratory employee. Gloves must be removed prior to leaving the laboratory.

b. Safety Glasses/Goggles. Safety glasses/goggles are required for all procedures involving potentially biohazardous agents. Select eye protection which provides side shielding and is ANSI-approved (bears Z-87 certification). Manipulations with the potential for splashing and/or spattering of biohazardous agents shall require the use of a face shield in addition to safety glasses or chemical splash goggles.

c. Laboratory Coats. Protective laboratory coats, smocks, or other protective apparel designated for the work area use must be worn while working with any hazardous materials. Protective clothing must be removed before leaving the work area unless you are conducting research-related activities outside the work area (e.g., waste disposal, animal transport outside the laboratory or vivarium, stockroom pick up, maintenance activities, etc.). Individual departments may establish more stringent requirements for personal protective equipment. Non-disposable personal protective equipment items may not be taken home for laundering or laundered in public facilities (e.g., laundromats).

B. Disposable gowns/scrubs. Disposable gowns/scrubs must be utilized whenever potential for splashing of hazardous materials exists.

C. Respirators. Respirators shall be utilized whenever potential for aerosolization or other airborne biohazard exposure exists and cannot adequately be controlled through engineering controls. Utilization of respiratory protection devices is subject to the review and approval of EHS/SRM under the university Respiratory Protection Program. All staff participating in the Respiratory Protection Program must be identified in Appendix D of this Biosafety Manual.

D. Laboratory Attire. Shorts and other clothing exposing feet or legs, sandals, and other open-toed shoes areprohibited laboratory attire.

1. Engineering Controls.

    a. Biological Safety Cabinets (BSCs). Manipulations potentially generating biological aerosols must be performed within certified BSCs, these procedures may include, but are not limited to: centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption (sonication), opening containers of infectious materials with internal pressures that may differ from ambient pressures, *in vivo* administration of agents via intranasal or gavage procedures; and harvesting infected tissues from animalsor embryonate eggs. Complete information regarding BSC certification requirements, maintenance procedures, and classifications may be viewed in the BMBL.

    b. Centrifuge safety cups must be utilized when centrifuging materials having the potential for producing biohazardous aerosols. The safety cups will be loaded within a BSC prior to centrifuging and will be opened within a BSC following centrifuging.

2. Secondary Containment.

    a. Facility Construction. New or renovated research facilities where manipulations involving potentially biohazardous agents are performed and/or test animals potentially infected with biohazardous agents must be constructed to satisfy the requirements outlined in the current edition of the BMBL.

    b. Waste Disposal. All regulated medical waste generated within university research facilities must be disposed of through the Physical Plant Department, Customer Service Center (828- 9444), and must be packaged and labeled in accordance with federal as well as state requirements. The total weight of individual incineration boxes may not exceed 40 lbs. Upon filling, boxes must not bestored in unrefrigerated spaces for over 5 days, and in refrigerated space, for no longer than 10 days.

E. Standard Microbiological Practices and Techniques. The CDC has developed "biosafety levels" (BSL)/"animal biosafety levels" (ABSL) which specify standard operating procedures and facility requirements for work involving biohazardous agents/infected research animals. These biosafety levels range from BSL/ABSL-1 (low individual risk, low community risk), involving agents posing minimal risk to normal, immunocompetent individuals and to the environment through BSL/ABSL-4 (high individual risk, high community risk), which involves biohazardous agents that are extremely dangerous to humans and/or the environment (note that research involving BSL-3 is permitted at VCU with the approval of the IBC while ABSL-3 and BSL/ABSL-4 work is not permitted at VCU). Persons working with infectious agents or infected materials must be aware of potential hazards, recommended biosafety level for the agent(s) being manipulated, and must be trained and demonstrate proficiency in the practices and techniques required to handle such material safely. The PI is responsible for providing or arranging appropriate training of laboratory personnel assigned to each protocol. Personnel must be advised of special hazards and shall be required to read and follow required practices and procedures.

F. Standard Microbiological Practices

1. The laboratory supervisor enforces the institutional policies that control safety in and access to the laboratory.

2. The laboratory supervisor ensures that laboratory personnel receive appropriate training regarding their duties, potential hazards, manipulations of infectious agents, necessary precautions to minimize exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosolization) and that appropriate records are maintained. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are advised of the potential hazards, are instructed on the appropriate safeguards, and read and follow instructions on practices and procedures. An institutional policy regarding visitor training, occupational health requirements, and safety communication is considered.

3. Personal health status may affect an individual's susceptibility to infection and ability to receive available immunizations or prophylactic interventions. Therefore, all personnel, and particularly those of reproductive age and/or those having conditions that may predispose them to increased risk for infection (e.g., organ transplant, medical immunosuppressive agents), are provided information regarding immune competence and susceptibility to infectious agents. Individuals having such conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. See Section VII.

4. A Safety manual specific to the facility is prepared or adopted in consultation with the facility director and appropriate safety professionals. The safety manual is available, accessible, and periodically reviewed and updated as necessary.

    i. The safety manual contains sufficient information to describe the biosafety and containment procedures for the organisms and biological materials in use, appropriate agent-specific decontamination methods, and the work performed.
    ii. The safety manual contains or references protocols for emergency situations, including exposures, medical emergencies, facility Section IV—Laboratory Biosafety Level Criteria 53 malfunctions, and other potential emergencies. Training in emergency response procedures is provided to emergency response personnel and other responsible staff according to institutional policies.

5. A sign incorporating the universal biohazard symbol is posted at the entrance to the laboratory when infectious materials are present. Posted information includes: the laboratory's Biosafety Level, the supervisor's or other responsible personnel's name and telephone number, PPE requirements, general occupational health requirements (e.g., immunizations, respiratory protection), and required procedures for entering and exiting the laboratory. Agent information is posted in accordance with the institutional policy.

6. Long hair is restrained so that it cannot contact hands, specimen, containers, or equipment

7. Gloves are worn to protect hands from exposure to hazardous materials. a. Glove selection is based on an appropriate risk assessment. b. Inner gloves are not worn outside the laboratory. c. Change inner gloves when contaminated, glove integrity is compromised, or when otherwise necessary. d. Do not wash or reuse disposable gloves, and dispose of used gloves with other contaminated laboratory waste.

8. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or manipulated.

9. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food is stored outside the laboratory area.

10. Mouth pipetting is prohibited. Mechanical pipetting devices are used.

11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware are developed, implemented, and followed; policies are consistent with applicable state, federal, and local requirements. Whenever practical, laboratory supervisors adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions are always taken with sharp items. These include:

12. Plasticware is substituted for glassware whenever possible. 54 Biosafety in Microbiological and Biomedical Laboratories

13. Use of needles and syringes or other sharp instruments is limited in the laboratory and is restricted to situations where there is no alternative (e.g., parenteral injection, blood collection, or aspiration of fluids from laboratory animals or diaphragm bottles). Active or passive needle-based safety devices are to be used whenever possible.

14. Uncapping of needles is performed in such a manner to reduce the potential for recoil causing an accidental needlestick.

15. Needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

16. If absolutely necessary to remove a needle from a syringe (e.g., to prevent lysing blood cells) or recap a needle (e.g., loading syringes in one room and injecting animals in another), a hands-free device or comparable safety procedure must be used (e.g., a needle remover on a sharps container, the use of forceps to hold the cap when recapping a needle).

17. Used, disposable needles and syringes are carefully placed in puncture-resistant containers used for sharps disposal immediately after use. The sharps disposal container is located as close to the point of use as possible.

18. Non-disposable sharps are placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving. d. Broken glassware is not handled directly. Instead, it is removed using a brush and dustpan, tongs, or forceps.

19. Perform all procedures to minimize the creation of splashes and/or aerosols.

20. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant. Spills involving infectious materials are contained, decontaminated, and cleaned up by staff who are properly trained and equipped to work with infectious material. A spill procedure is developed and posted within the laboratory.

21. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method, consistent with applicable institutional, local, and state requirements. A method for decontaminating all laboratory wastes is available in the laboratory Section IV—Laboratory Biosafety Level Criteria 55 (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). See B. Special Practices, #7 in the following sub-section for additional details.

22. An effective integrated pest management program is implemented. See Appendix G. 16. Animals and plants not associated with the work being performed are not permitted in the laboratory.

1. **Biosafety Level 1 (BSL-1)** includes agents not known to cause disease in normal, healthy adults. Use of these agents requires the use of standard microbiological practices, which include the use of PPE (Protective laboratory coats, gowns, or uniforms; protective eyewear & gloves). Protective eyewear is required to be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. No specific safety equipment is required and these agents can be manipulated on an open laboratory bench. However, a biosafety manual containing required laboratory procedures and decontamination requirements/methods should still be maintained. A sink for hand washing must be readily available as well as signage posted at the entrance when the agent is present.

2. **Biosafety Level 2 (BSL-2)** agents are associated with human disease, hazard, or threat of auto-inoculation, ingestion, or mucous membrane exposure, and low aerosol-exposure risk. In addition to BSL-1 practices, the laboratory must limit access, post biohazard warning signs, follow universal "sharps" precautions, and maintain a biosafety manual containing required laboratory procedures, decontamination requirements/methods, waste disposal requirements, immunization requirements, medical surveillance requirements, etc. Safety equipment required for BSL-2 procedures include a certified Class I or II BSC or other physical containment devices used for any manipulations of agents that may create splashes or aerosols of infectious materials. Required PPE includes laboratory coats, gloves (latex and latex alternatives, based on risk assessment, and use two pairs when appropriate), and face or eye protection as needed or as specified in the laboratory in the laboratory exposure control plan. The laboratory facility must meet BSL-1 standards and have a suitable means available for management of any generated biohazardous waste materials (autoclave/orange bagging or red bag disposal).

3. **Biosafety Level 2 Enhanced (BSL-2E or BSL-2+)** is required for agents that are associated with human disease, have a threat from autoinoculation, ingestion, or mucous membrane exposure as well as an aerosol-exposure threat, but do not fit the criteria for inclusion in BSL-3. However, BSL-2+ requires additional precautions not found at the BSL-2 level. BSL-2+ applies primarily to viral vectors including replication competent strains and systems with varying degrees of engineered safety controls (e.g., second and third generation lentiviral vectors). BSL-2+ practices include BSL-2 practices plus minimization of sharps use, elimination of sharps wherever possible or replacement with safety-engineered devices, verification that work is strictly conducted within a certified biosafety cabinet, confirmation that laboratory access is restricted to properly trained and protected individuals, and confirmation that the laboratory has developed a laboratory-specific biosafety manual. Task-specific training is required for all staff with potential for exposure. This training should cover basic epidemiology of the agent, possible associated oncogenic risks, and a review of written SOPs for specific tasks involving the agent with special focus placed on manipulations posing greatest potential for harmful exposure including tasks involving needles/other sharps, tasks with potential for generating significant amounts of aerosol. Dates of this training must be indicated on the IBC Memorandum of Understanding or Bioraft biological registration (vcu.bioraft.com) as well as signed records of the training (e.g., initialed attendance roster for PI and staff) must be available. Specific written SOPs shall be developed for all procedures involving the vector system including preparation/administration of doses (with emphasis on

needle/sharp safety), disinfection of surfaces/equipment, orange/red-bag disposal of waste, spill response, exposure reporting and exposure report follow-up, etc. All surfaces will be immediately disinfected with freshly prepared 10% bleach solution (one part household bleach to nine parts deionized water), 70% ethanol, or other effective disinfectant following completion of tasks involving the vector system and all laboratory coats/other laboratory protective attire and equipment used during manipulations will be removed prior to exiting the laboratory unless other required research-related activities are to be conducted outside the laboratory. Details of this effort should be documented in a central laboratory filing system.

4. **Biosafety Level 3 (BSL-3)** agents are indigenous or exotic agents with potential for aerosol transmission and where the disease may have serious or lethal consequences. BSL-3 practices consist of BSL-2 practices plus decontamination of all waste prior to transfer out of laboratory, enhanced access/security controls, and special engineering and design features. The IBC has determined that baseline sera will not be collected preferring instead to collect acute and convalescent sera following suspected exposure. Safety equipment includes Class II or III BSCs used for all manipulations of the infectious agent: protective laboratory attire, gloves (type based on risk assessment), face and eye protection, and respiratory protection as dictated by risk assessment. The BSL-3 facility must meet all BSL-2 requirements plus physical separation from access corridors, self-closing, “air lock” access, special security requirements, negative pressure conditions within laboratory, 100% exhausted air from laboratory, and nonporous/seamless laboratory surfaces.

G. Risk Assessment. "Risk" implies the probability that harm, injury, or disease may occur.In the context of microbiological and biomedical laboratories, the assessment of risk focuses primarily on prevention of laboratory acquired infections (LAIs). When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical exercise that assigns an appropriate biosafety level (facilities, equipment, and practices) in order to reduce the risk of exposure aswell as the environmental threat of laboratory staff to an acceptable minimum. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level. The PI is responsible for assessing risks in order to set appropriate biosafety levels. This process should be conducted in close collaboration with the IBC to ensure compliance with established guidelines and regulations. Determining factors in risk assessment include:

- *Pathogenicity* of the infectious agent pertaining to disease frequency and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe the disease, the greater the risk associated with that pathogen.

- *Route of transmission* (e.g., parenteral, airborne, or by ingestion) with novel agents may not be definitively established. Extensive epidemiological research has indicated that agents readily transmitted via aerosol route have caused most laboratory-acquired infections making critical the consideration of aerosol transmission potential of novel agents. The greater the potential for aerosolization, the higher the risk of transmission, and correspondingly, the higher the required biosafety level.

- *Agent stability* is a consideration that involves aerosol infectivity (e.g., from spore-forming bacteria), and an agent's ability to survive over time in the environment. Factors such as desiccation, temperature, exposure to ultraviolet light, and exposure to chemical disinfectants must be considered.

- *Infectious dose* can vary from one to hundreds of thousands of infectious units. There is a complex interaction between pathogen and host; the resultant issues present a significant challenge even to the healthiest immunized laboratory worker and may pose a serious risk to those with lesser resistance. The laboratory worker's immune status is in direct correlation to his/her susceptibility to disease when working with an infectious agent.

- The *concentration* (number of infectious particles per unit volume) is important in determining risk of infection. Items to consider are as follows: the milieu containing the organism (e.g., solid tissue, viscous blood or sputum; or liquid medium), volume of concentrated material, and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation).

- The *origin* of infectious materials is critical when preparing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign), host (e.g., infected or uninfected human or animal), or nature of source (potential zoonotic or associated with a disease outbreak).

- The *availability of animal data* in the absence of human data may provide useful information in a risk assessment.

- The established *availability of an effective prophylaxis or therapeutic intervention* is an essential factor to be considered. The most common form of prophylaxis is immunization.

- *Medical surveillance* ensure that the safeguards implemented produce the desired health outcomes. Medical surveillance is part of risk management and may include serum banking, monitoring employee health status, and participating in post-exposure management.

H. Risk Groups. Microorganisms are classified according to degree of risk in terms of infectivity, pathogenicity and the availability of preventive measures and effective treatments for the disease. The NIH Guidelines have established a classification and assigned human etiological agents into four risk groups based on hazard. The risk groups correlate with, but do not always equate to, biosafety levels. A risk assessment will determine the degree of correlation between an agent's risk group classification and biosafety level. The NIH Guidelines, Section II provides additional information on the differences and relatedness of risk groups and biosafety levels. There is no research with Risk Group 4 agents at VCU.

The NIH Guidelines defines the risk groups as:

Risk Group 1 (RG1) - Agents that are not associated with disease in healthy adult humans. This group includes a list of animal viral etiologic agents in common use. These agents represent no or little risk to an individual and no or little risk to the community.

Risk Group 2 (RG2) - Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. These agents represent a moderate risk to an individual but a low risk to the community.

Risk Group 3 (RG3) - Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. These agents represent a high risk to an individual but a low risk to the community.

Risk Group 4 (RG4) - Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. These agents represent a high risk to the individual and a high risk to the community.

For more information, please refer to:

The NIH Guidelines for Involving Recombinant or Synthetic Nucleic Acid Molecules

Biosafety in Biomedical and Microbiological Laboratories

The WHO Biosafety Manual

OIE Terrestrial Manual 2012

V. BIOHAZARD DECLARATION.

A. BIOHAZARD CLASSIFICATION. Biohazards are infectious agents or biologically-derived infectious materials that present a potential risk to the health of humans or animals, either directly through infection or indirectly through environmental contamination. Infectious agents have the ability to replicate and give rise to potentially large populations when small numbers are released in nature from a controlled situation. Principal investigators should indicate below any of the hazard categories which are stored or in use within laboratories under there charge. **If boxes are checked, identity and biosafety level of agents meeting classification should be listed in the space provided below each category, additional spaces may be added if required:**

□ **Pathogens**: human, animal, and plant pathogens, including: bacteria, prions, rickettsia, fungi, viruses, and parasites.

□ **Human Blood and Other Potentially Infectious Materials (OPIM)**: All human blood, blood products, unfixed clinical tissues, and certain body fluids (specified in OSHA 29 CFR 1910.1030).

□ **Cells/Cell Lines:** Cultured cells from humans, non-human primates, other mammalian species, lymphoid or tumor cells, and the potentially infectious agents these cells may contain. See BMBL Appendix H for additional handling recommendations.

□ **Allergens**: Adjuvants, animals dander, latex, etc.

□ **Toxins:** Includes bacterial, fungal, plant, other toxins.

□ **rDNA:** Recombinant DNA, synthetic nucleic acids, and related products.

□ **Clinical Specimens:** To include all patient derived tissues, cells, body fluids, and other materials.

□ **Infected animals**: Including live animals, animal tissues, animal bedding/waste materials, and other materials derived from known or potentially infected animals.

□ **Select agents**: Any select agent material or toxin on the current CDC or USDA listing.

B. UNIVERSITY BIOHAZARD POLICIES: All PIs must comply with NIH/CDC standards regarding biological hazards through:

1. Limiting laboratory access to authorized personnel.

2. Limiting unauthorized access to known or potentially biohazardous materials.

3. Limiting handling of biohazardous materials to the minimum possible amount.

4. Ensuring proper disinfection, decontamination, and/or disposal of material after usage.

5. Ensuring proper usage of appropriate safety equipment, precautions, and procedures when handling biohazardous materials.

6. Maintaining appropriate levels of identification, warning, and security during storage of the material.

7. Posting of universal biohazard sign on the outside door of each laboratory of BSL-2 and above.

8. Maintaining proper ventilation of the laboratory.

9. Keeping laboratory doors closed during operations involving biohazards.

10. Following additional standards and special practices as described in the BMBL or imposed by the IBC.

C. BIORAFT REGISTATION/MEMORANDUM OF UNDERSTANDING AND AGREEMENT (MUA)

1. In keeping with NIH policy, implemented at the university through the IBC, PIs must complete a BioRaft biological registration (vcu.bioraft.com) prior to commencing new research involvingbiohazardous agents, rDNA, or gene therapy. New registrations utilizing the WORD "MUA" form werediscontinued as of March 1, 2018. MUAs approved prior to the March 1, 2018 deadline however, will remain valid for full three-year cycle.

2. In order to receive written approval from the IBC, the completed Bioraft biological registration for work involving biohazardous agents, rDNA, or gene therapy must be submitted in advance of anticipated start-date as commencement of the proposed research project." Copies of all active BioRaft biological registrations and MUAs should be attached to Appendix A of this manual.

D. SAFETY DATA SHEETS. Safety data sheets (SDS, formally known as Material Safety Data Sheet, or MSDS) contain health hazard information such as infectious dose, viability (including decontamination), medical information, laboratory hazard, recommended precautions, handling information, and spill procedures. The intent of the SDS is to provide a safety resource for laboratory personnel working with these infectious substances and ready accessibility is required by OSHA. Proper utilization of SDSs in association with prudent work practices fosters a safer, healthier working

environment. The following URL provides SDSs for a wide array of biohazardous agents, for additional resources and assistance contact the Biosafety Office at 400-4984: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html An inventory of all hazardous/biohazardous materials and/or agents and copies of all SDSs for all biohazardous agents and biological toxins stored or in use should be attached to Appendix B of this manual.

E. SELECT AGENTS: In accordance with the DHHS "Possession, Use, and Transfer of Select Agents and Toxins; Final Rule; March 18, 2005 and NIH/CDC: "Additional Requirements for the Transfer or Receiving of Select Agents" (42 CFR Part 72), all facilities which transfer, use, or store specific agents (referred to as 'select agents') which are considered capable of causing substantial harm to humans are subject to strict regulatory requirements. The regulations are intended to act as a deterrent to biological terrorism through controlling access to acutely toxic and infectious "select agents." Failure to comply with these requirements could pose significant legal penalties (mismanagement of select agents is a federal felony) to negligent individuals as well as to the university. Further information can be obtained from the CDC Select Agent Program or BioRaft biological registration. The complete list of Select Agents can be obtained from http://www.cdc.gov/od/sap/docs/salist.pdf.

1. Biomedical Research Exemption.

a. According to 42 CFR Part 72: "Toxins for medical use, inactivated for use as vaccines, or toxin preparations for biomedical research use at an LD_{50} for vertebrates of more than 100 nanograms per kilogram body weight are exempt so long as specified exemption quantity limits for toxin(s) involved are not exceeded. National standard toxins required for biologic potency testing as described in 9 CFR Part II 3 are exempt."

b. Legislation ("Possession, Use, and Transfer of Select Agents and Toxins; Final Rule; March 18, 2005") states that designation of "exempt" status for institutions following September 12, 2002 be declared by DHHS/USDA on a case-by-case basis. The current exempt status for toxins at limited quantities is subject to reinterpretation and/or revision. Thus, VCU researchers must register all select agents with EHS/SRM CDC Select Agent Program whether of exempt or nonexempt status.

c. The National Institutes of Health advise that the Registry of Toxic Effects of Chemical Substances (R-TECS) tables, maintained by the National Institute for Occupational Safety and Health, be used as a guide when determining the LD_{50} of a select agent.

d. Possession prior to 42 CFR 72. Since the new DHHS regulations include use, *possession,* and transfer of select agents, claims of exemption due to possession prior to 42 CFR 72 are not valid.

2. University Policy. The university must identify and track all the select agents on both campuses. The university relies upon several methods for identifying/tracking select agents such as PI registration, Bioraft biological registration other protocol review, and through annual laboratory inspection. Select agents are toxic at extremely low concentrations; research involving these materials in excess of *de minimis* quantities is restricted to staff who have submitted required forms and been approved by Department of Justice, received proper training, have access to adequate, secure work facilities, and are provided suitable personal protective equipment (PPE). The exact degree of these requirements is specified in the research protocol which must be approved by the IBC prior to initiating any procedures involving non-*de minimus* quantities of select agents or etiologic agents and/or other biohazardous materials. Additionally, work with select agents exceeding *de minimis* quantities can only

be carried out in laboratories specifically inspected and approved by the CDC. The National Institutes of Health/Centers for Disease Control direct facilities to deplete or destroy all select agents stocks onsite prior to disposal which includes depletion, destruction, and recycling.

F. DUAL USE RESEARCH OF CONCERN:

1. Defining DURC: Dual Use Research of Concern (DURC) is life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security. The United States Government's oversight of DURC is aimed at preserving the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.

2. DURC Policy: VCU has instituted a DURC Oversight Policy which is intended to direct researchers in the identification of potential DURC research and in implementation of prudent biosecurity and biosafety safety measures for mitigating the associated risks to staff, the public, and the environment.

3. Principal Investigator – DURC Responsibility: PIs are responsible for registering all applications involving biological and rDNA hazards under their Bioraft registration – thorough completion of the registration will provide the IBC with sufficient detail for determining if an institutional DURC review is warranted. Protocols identified as having potential DURC will be reviewed by the Institutional Review Entity (IRE) to ensure that sufficient biosecurity and biosafety precautions are in place.

VI. RECOMBINANT DNA AND SYNTHETIC NUCLEIC ACIDS

A. The National Institutes of Health: NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules are designed to specify practices for constructing and handling the following agents: recombinant deoxyribonucleic acid (rDNA) molecules as well as organisms and viruses containing rDNA molecules. NIH further defines rDNA as: *"(i) 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 (ii) molecules that result from the replication of those described in (i) above. Synthetic DNA segments which are likely to yield a potentiallyharmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.*
Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH guidelines unless the transposon itself contains recombinant material."

B. University Requirements: Section III of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules separates rDNA research into sixbasic classifications: Section III A through F.

1. "Nonexempt" activities: All rDNA which falls under research Sections III.A – E is considered to be "nonexempt" (regulated under the guidelines) and **must be registered with the IBC via** Bioraft biological registration and receive full IBC review/approval prior to commencing work.

Activities requiring NIH and IBC approval:

(1) Section III-A: Applications involving deliberate transfer of drug resistance may require preapproval from the NIH Recombinant DNA Advisory Committee (NExTRAC) and the NIH Director submission of an MUA/IBC approval prior to commencement.

(2) Section III-B: Research involving formulation of genes for biosynthesis of highly lethal toxin molecules may require prior approval from the RAC in addition to submission of an MUA/IBC approval prior to commencement.

(3) Section III-C: Experiments involving human subjects will require notification and approval of the RAC and submission of a Memorandum of Understanding and Agreement HUMAN Use/IBC approval prior to commencement.

b. Experiments requiring only IBC approval prior to initiation: rDNA activities falling under Sections III-D and III-E may be initiated upon receiving IBC approval via submission of Bioraft registration. Experiments involving *in vivo* applications will require additional IBC approval/registration via completion of the standard IACUC protocol.

2. "Exempt" Activities: Activities included under Section III-F are considered to be exempt from NIH purview but do require IBC registration via submission of an Bioraft registration/MUA (however, approval by the full committee is typically not required). Copies of all active Bioraft biological registrations and MUAs are attached in Appendix A of this manual.

3. Transgenic Species: All activities involving transgenic animals which do not fall under Section III-F (transfer and purchase of transgenic mice which do not require housing in excess of BSL-1 precautions) will require IBC approval/registration via submission of a Bioraft biological registration. This will include all transgenic lines generated through the use of viral vectors and any other animals requiring handling at ABSL-2 or greater containment conditions.

VII. GENE THERAPY.

A. Research proposals involving the deliberate transfer of rDNA into human subjects (human gene transfer) must be preapproved by the NIH NExTRAC. Principal investigators shall submit their relevant information on the proposed human gene transfer experiments to NIH/OBA.

B. The IBC will be notified prior to the submission of review documents to NIH and/or any other outside review agencies.

C. Approval from the Institutional Review Board (IRB) is mandatory prior to engaging in research involving human subjects.

D. Approval from the IBC via completion of Bioraft registration or submission of Memorandum of Understanding Agreement HUMAN Use must be obtained prior to administering rDNA material to human subjects. Copies of all active MUAs are attached to Appendix A of this manual.

E. In accordance with RAC guidelines and university policy, **genetic research involving germ line alteration(s) shall not be permissible within university facilities.**

VIII.EMERGENCY PROCEDURES.

A. LABORATORY EMERGENCY POSTINGS

1. Names of responsible individuals to be contacted in case of emergencies must be posted outside of entrance doors leading into each laboratory. A phone number for the contact individual is helpful, but optional, as long as the contact person has verified that their contact info is up to date in the VCU Employee Banner Self-Serve contact section.

2. A list of the significant hazards found within the laboratory must to be posted for notification of staff and emergency response personnel. The list of hazards that must be identified by signage posted at entrances to laboratories includes (but is not limited to):

a. Use/storage of biohazardous agents, acute carcinogens and toxic chemicals, radiological agents, and flammable materials.

b. Presence of strong magnetic equipment.

c. Emission of X-rays.

d. Required PPE.

e. A list of all alarms in the laboratory and whom to contact if an alarm is sounding.

3. To assist researchers in meeting posting requirements, EHS/SRM has developed fill-in-the-blank laboratory safety and biohazard signage which are available upon request (contact Biosafety Office).

4. The following emergency contact numbers should also be posted in an area convenient to all laboratory staff:

<u>Biological, Chemical, and Radiation Emergencies:</u>

VCU and Hospital: 828-9834

<u>Fire Emergencies:</u>

Hospital: *50

Medical (east) campus: *50 or 828-1234

Monroe Park (west) campus: 828-1234

<u>Medical Emergencies:</u>

Immediate Emergencies: 828-1234

Employee Health: 828-0584

Poison Control: 828-9123

MCVH Emergency Room: 828-9000

Security Contact Numbers:

VCU Campus Police: 828-1234

MCV Hospital Security: 828-6595

VCU Communications Center: 828-1234 or 828-4357

Laboratory Contacts:

Principal investigator contact numbers:

Other important contact numbers:

B. EMERGENCY EQUIPMENT.

1. The PI is responsible for the verification that proper emergency equipment is provided within laboratory spaces. Specific emergency-response equipment requirements will depend upon the nature of research conducted within each laboratory space. Principal investigators are responsible for assessing and acquiring all necessary emergency response equipment prior to initiating new research projects. The PI must ensure that all laboratory personnel are familiar with locations and proper use of emergency equipment within their laboratory.

2. **Chemical Spill Emergency Response Plan:** Templates for creating Standard Operating Procedures (SOPs) for response to spills involving biological/rDNA materials in general laboratory areas and within BSCs have been provide in Appendix C: PIs should complete the templates to make them lab specific.

A. Each area or department of VCU or VCUHS must be prepared to handle a spill of chemicals widely or routinely used within that department. Please note that pre-packaged emergency spill kits are available to handle small spills of solvents, biologicals, and corrosives. These spill kits can and should be purchased through a local laboratory safety supply company. Contact EHS/SRM for advice if uncertain of needs.

"**Home-made" spill kit:**
Absorbent pads ("blue" pads)
*Vermiculite (available at garden centers of Wal-Mart, Lowes, etc)
Heavy-duty gloves (to handle broken glass) & exam gloves (preferably nitrile)
Safety glasses/goggles
Hand broom & dustpan
Trash bags
Put these items together in a box/container, label "spill kit", and place in an easily accessible spot in the lab.

*Used to absorb large amounts of liquid

B. For certain hazards, respiratory protection may be required for routine and emergency operations. If respirators (including N-95 filtering face pieces) are provided, the laboratory must have a written Respiratory Protection Program and all users must have been medically evaluated, fit-tested, and trained within the past twelve months to qualify for respirator use. Use of respirators without fulfilling all requirements of the Respiratory Protection Program is not allowed. All staff participating in the Respiratory Protection Program must be identified in Appendix D of this manual.

C. EVACUATION ROUTES: Principal investigators must ensure that staff receives adequate training regarding emergency evacuation procedures that includes the following elements:

1. Familiarization with primary and secondary (alternate) evacuation routes.

2. Awareness of alarm method(s) used to signal a building evacuation.

3. Designation of post evacuation meeting areas for laboratory staff.

4. For assistance in determining proper evacuation procedures contact the VCU Fire Safety Office.

D. BIOHAZARDOUS MATERIAL SPILL RESPONSE PROCEDURES. A summary of recommended precautions is provided below, templates for creating spill-response SOPs have been provided under Appendix C; the SOP templates must be completed to make them laboratory specific. Principal investigators are responsible for developing emergency response procedures and ensuring that laboratory personnel are thoroughly trained in the event of incidents involving biological and chemical spills. The essential elements of a biohazardous spill response plan suitable for addressing the two most common types of incidents encountered within university laboratories are listed below:

1. SPILL IN A BIOLOGICAL SAFETY CABINET. A spill that is confined to the interior of a properly operating biological safety cabinet (BSC) may present little or no hazard to personnel in the area. In the event of a biohazardous spill within a BSC, the following procedures shall be followed:

(a) Leave the cabinet on. While wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with suitable disinfectant as specified by the SDS or as recommended in the CDC Disinfecting Guidelines . If necessary, flood the work surface, as well as drain pans and catch basins below the work surface with disinfectant and allow a contact time of at least 20 minutes.

(b) Soak up disinfectant and spill with spill pad or paper towels. Drain catch basin into an appropriate container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.

(c) Autoclave all clean-up materials before disposal in the biohazard waste container. Remove gloves and wash hands thoroughly with soap and water after the clean-up procedure.

2. SPILLS IN OPEN LABORATORY AREAS. Biohazardous spills occurring in open laboratory areas pose a greater potential for exposure than spills occurring within biological safety

cabinets and as such a greater degree of care and preparedness is required for safely responding to open area incidents. Essential elements of open area biohazard spill response are detailed below:

(a) When potentially biohazardous materials are spilled in open areas of the laboratory, evacuate the laboratory immediately and close the door to limit exposure to aerosols.

(b) Upon exiting the laboratory, warn other personnel in the area of the incident and post a sign alerting others of the spill inside.

(c) If clothing and/or skin is known or suspected to have been contaminated during incident, proceed immediately to full immersion emergency shower or changing area providing shower suitable for personal decontamination.

(d) Remove contaminated clothing with gloved hands, folding contaminated area inward. Discard clothing in a red biohazard bag or place clothing directly in an autoclave.

(e) Thoroughly wash all potentially contaminated areas, arms, face, and hands with soap and warm water.

(f) Avoid reentry into the laboratory for at least 20 minutes to allow for the settling of aerosols potentially generated by the spill.

(g) Don appropriate PPE for cleaning operation. This must include at a minimum: gloves, eye protection, and laboratory coat. Spills involving high risk biohazardous agents with high potential for aerosol transmission may require additional PPE including respiratory protection. All staff participatingin the Respiratory Protection Program must be identified in Appendix D of this Biosafety Manual.

(h) Cover spill gently with paper towel(s), apply disinfectant as specified in product MSDS or recommended by the CDC Disinfecting Guidelines onto adjacent surfaces working toward spill. Complete action by applying copious amount of disinfectant to actual spill area.

(i) Allow disinfectant to stand for at least 20 minutes, proceed with thorough wipe-down of spill and adjacent surface areas. Note: however, whenever sharps materials are involved, wipe down and collection of waste materials shall be conducted via mechanical means. Refer to the EHS/SRM Bloodborne Pathogens and Infectious Waste Management Procedure for complete instructions on cleaning spills involving sharps materials.

(j) Repeat steps (h) and (i), above.

(k) If the floor and sink are affected by the spill, flood these areas with disinfectant.

(l) Dispose of all liquid and solid waste generated during spill cleanup as biohazardous waste through VCU Customer Service (828-9444).

4. Advance Planning. Advance preparation for management of a spill is an essential element of laboratory biosafety. A "spill kit" which includes necessary PPE, disinfectant, and other materials required for responding to biohazardous spills must be available in all areas where manipulations involving potentially biohazardous agents are conducted. The standard elements of a typical spill kit are detailed in Chapter VIII, Section B, of this manual.

5. Emergency Telephone Numbers. Whenever spills involve personal injury or biological contamination, call 8-1234 or 8- 4357 from any campus telephone and request medical assistance and request that the Control Center initiate the Chemical Emergency page. Be sure to state the type of contaminant involved in the incident. The caller should remain available to brief emergency responders on the type of contamination and proper procedures for handling the material. SOP templates have been provided in Appendix C, PIs should review the SOPs included, select applicable documents, and provide all requested information to make the SOPs lab specific.

IX. STERILIZATION/DECONTAMINATION

A. STERILIZATION. Sterilization is the total destruction of all viable microorganisms from a surface or given volume of gas or liquid. For protection of personal health and the integrity of research, laboratory personnel must understand this concept when working with potentially biohazardous agents and ensure proper autoclaving procedures are followed. When sterilizing glassware and other reusable instruments, autoclave operators must ensure that cycle times and temperatures are adequate and that autoclave units are functioning properly. Whenever biohazardous (regulated medical) waste materials are sterilized onsite for disposal via orange bags (domestic waste stream), the VDEQ Regulated Medical Waste Management Regulations (9VAC20-120) are applicable. Proper autoclaving procedures are detailed in Section XI (Biohazard/Biohazardous Waste Management).

B. DECONTAMINATION: Decontamination is the process whereby viable microorganisms are removed from solutions, surfaces, or materials by filtration, heating, radiation, or chemical removal. A freshly prepared dilution of household bleach is a frequently employed, and is a quite effective decontaminant for a number of biological agents. Researchers should; however, refer to the agent/product SDS and the CDC Disinfecting Guidelines whenever determining appropriate disinfectants. If bleach is selected, EHS/SRM recommends use of a bleach-water 10% solution (i.e., one part household bleach to nine parts water) freshly prepared. Decontaminants are an essential component of an emergency spill response kit. Spill kits are required in all labs conducting research involving potentially biohazardous agents. A listing of the required elements within a spill kit includes:

1. A sufficient reserve to produce at least four liters of 10% bleach solution or other suitable decontaminant. Even in un-opened original containers, undiluted bleach will lose its potency over time; therefore, concentrated bleach held on hand for spill decontamination should be rotated every six months.

2. Absorbent materials, such as absorbent pads, vermiculite, or disposable towels for containing and treating spills.

3. Spray/mist bottles for disinfectant application.

4. "Red bags" for receiving biohazardous waste generated during spill response or for over packing leaking containers.

5. Leak-proof, puncture-resistant, closable, and properly labeled containers for receiving contaminated broken glass and other sharps materials.

6. Protective clothing, and equipment including:

   a. Liquid impermeable disposable coveralls (e.g., Tyvek®).

b. Eye protection gear, including splash resistant safety glasses and face shields.

c. Gloves suitable for protection from biological/chemical hazards. Be aware that glove materials are variably resistant to chemical penetration and degradation; therefore, the glove material must be appropriate for the chemical against which it is intended to provide a barrier.

d. Rubber boots and/or impervious shoe covers.

e. Protective breathing devices such as N-95 respirators. *

*For certain hazards, respiratory protection may be required for routine and emergency operations. If respirators are to be provided, the laboratory must have a written respiratory protection program which addresses all aspects of an OSHA-compliant respiratory protection program. Information about respirators and respiratory protection programs can be viewed at respiratory protection.

7. Forceps, broom, heavy-duty brush, and dustpan (for spills involving sharps materials).

8. Extra clothing to replace items contaminated during spill/cleanup (e.g., scrubs, etc.).

Additional details regarding sterilization and decontamination are available on the EHS/SRM Bloodborne Pathogens and Infectious Waste Management Procedure.

An SOP template covering spill decontamination has been provided in Appendix C, PIs should review the SOP and provide requested information to make lab-specific.

X. BIOHAZARD/BIOHAZARDOUS WASTE MANAGEMENT

A. Regulated Medical Waste: The VDEQ Regulated Medical Waste Management Regulations and university policy designate the following seven classes of regulated medical (i.e., biohazardous, infectious) waste**:**

1. Cultures and stocks of microorganisms and biologicals. Discarded cultures, stocks, specimens, vaccines, and associated items likely to have been contaminated with potentially pathogenic organisms potentially.

2. Human blood/blood products, other potentially infectious material (OPIM), and animal blood/blood products. This includes wastes consisting of human and animal blood/blood products (includes serum, plasma, etc.) and items contaminated by *significant* amounts human and animal blood/blood products. “Significant” quantities of blood are present whenever materials render visible release of liquid or dried blood upon being subjected to wringing and/or typical handling procedures. Under this definition, materials stained with small quantities of embedded blood/blood products do not require disposal as RMW.

3. Tissues and other anatomical waste. This includes all human anatomical wastes and all human tissues, organs, body parts, or body fluids.

4. Sharps materials. Includes all discarded needles and scalpels (regardless of blood borne pathogen contamination potential), any other sharps materials likely to be contaminated with pathogenic organisms, and all sharps used in patient care and veterinary practice.

5. Intentionally infected animal carcasses, body parts, urine, feces, bedding, and related waste. This applies when source animals are known or suspected to be infected with organisms potentially pathogenic to healthy humans.

6. Residues, soils, liquids, and other debris resulting from cleanup of spilled regulated medical waste.

7. Solid waste contaminated by, or mixed with, regulated medical waste.

8. Other Regulated Biological Materials: In accordance with NIH standards, all rDNA/gene therapy waste that may have come into contact with rDNA molecules or gene therapy waste will be considered regulated medical waste. Surgical instruments and other reusable materials should be sterilized in accordance with the CDC Disinfecting Guidelines and applicable institutional policies prior to reuse.

B. Proper Management and Disposal Procedures: All of the above listed materials shall be considered regulated medical waste and shall be managed/disposed of as detailed below.

C. Sharps Materials: Virginia Commonwealth University has adopted a policy that all needles, scalpels, blades, scissors, pipette tips, Pasteur pipettes, slides, and other items that could pose laceration or puncture hazards will be managed and disposed of according to “infectious sharps” disposal requirements.

1. Management and disposal requirements for infectious sharps must include the following elements:

a. Infectious sharps materials must be placed in an approved container. Approved infectious sharps containers must have the following properties:

(1) Containers must be rigid and puncture resistant.

(2) Containers must be leak resistant on sides and bottom.

(3) Containers must be capable of being readily (without coming into contact with sharps materials) closed and securely sealed properly prior to disposal.

(4) Containers must be clearly marked with the following labeling: "BIOHAZARD: INFECTIOUS SHARPS."

b. Fingers/hands must never be placed inside sharps containers. In the unlikely event that an item would have to be retrieved or dislodged from a sharps container, forceps or another mechanical device must be utilized.

c. Sharps containers must not be overfilled. Sharps material must fit completely into the container; portions of sharps materials shall not be allowed to protrude from the top of the vessel.

d. Upon filling, infectious sharps containers must be securely sealed and placed within a red bag-lined incineration box units for disposal via VCU Customer Service (828-9444).

e. Needles and other sharps materials must not be recapped unless deemed essential to research project by PI. Where alternative means are not available, needle recapping may be performed under the following conditions:

(1) Recapping of needles may be conducted only by the use of mechanical devices or one-handed scoop technique under the approval of the IBC. Principal investigators wishing to include needle recapping in research protocols are required to complete/submit a Needle Recapping Waiver Form. Upon IBC approval, a copy of the waiver form should be maintained in the laboratory exposure controlplan and/or biosafety manual. Waivers are valid for three years from date of issue and must be re- submitted if the need persists beyond three years.

(2) Needles, scalpels, and other mounted sharps materials may not be removed from mountings or remounted.

f. Breaking, bending, or shearing of needles is strictly prohibited.

g. All employees involved in incidents with known or potentially infectious sharps materials shall report immediately to Employee Health for medical evaluation. Incidents involving rDNA material must be reported to the Biosafety Office (mtelliot@vcu.edu) within 24 hours of occurrence.

h. All sharps-related injuries shall be recorded in a sharps injury log which must be obtained from Employee Health.

i. The university model ECP should be included to cover all staff with reasonablepotential for exposure to blood borne pathogens.

2. Pipette tip disposal:

a. Pipette tips with the potential for contamination with infectious materials will be handled by either of the two following means:

(1). Direct disposal into an incineration box which has been double-lined with red bags. Both bags must be individually sealed to prevent possible leakage of residual fluid. Tips with potential for retaining significant amounts of fluids should be handled as indicated below.

(2). Disinfection in bleach solution (freshly prepared stock at 10% or greater concentration) with minimum contact time of 30 minutes, and disposal as “noninfectious broken glass” (refer to line IX.C.3)

b. Pipette tips with no potential for contamination with potentially infectious materials must be discarded as “noninfectious broken glass” (refer to line IX.C.3).

3. “Non-infectious broken glass” includes all broken glassware which has not come into contact with potentially infectious agents. These materials may include such items as: glassware broken during or after cleaning, glassware broken while containing noninfectious materials (water, buffers et. al.), broken coffee cups, broken soda bottles, etc. Glass Pasteur pipettes, pipette tips, glass and plastic serological pipettes, test tubes, flasks and Petri dishes which have not come into contact with potentially infectious agents may also be classified as non-infectious broken glass, if handled accordingly as detailed below:

a. Place all noninfectious broken glass items into puncture resistant containers (e.g. sturdy cardboard/fiberboard box) which has been lined with plastic bag (do not use a red or orange bag).

b. Do not fill above the top of the box; when approaching full, seal box and wrap box with several strips of packing or duct tape.

c. Clearly label box "NON-INFECTIOUS BROKEN GLASS" (use an indelible black marker or other clearly visible permanent pen type).

d. Dispose of noninfectious broken glass box through housekeeping (or place in regular "domestic" trash dumpster).

D. Utilization of Reusable Containers for Staging of Biohazardous Waste Materials.
Regulated medical waste materials including red-bagged material and orange-bagged materials prior to autoclave sterilization may be conveyed and/or staged in reusable containers or carts under the following conditions:

1. Waste in containers must be fully packaged in compliance with VDEQ and DOT requirements (within suitable red bags). Discrete packages of waste and the reusable container must each be labeled in accordance with DEQ requirements.

2. Immediately following each time that a reusable container is emptied and prior to being reused, it must be thoroughly cleaned, rinsed, and effectively sanitized with a hospital grade disinfectant effective against mycobacteria. The area in which this takes place shall be considered a regulated medical waste staging area; therefore, it must be regularly sanitized and placed under restricted access during times when disinfectant is in use.

3. Unloading of large-volume reusable carts and containers must be accomplished through mechanical means that do not involve manual handling of bags or packages. Mechanical means may consist of tipping floors, chutes, snares, and other simple mechanisms.

4. Unloading of small-volume containers such as pails or trashcans must be performed with proper personal protective equipment (gloves, laboratory coat, safety glasses, etc.) utilizing techniques which minimize handling of red/orange bags and while avoiding contact with bag contents.

XI. SHIPPING OF BIOHAZARDOUS MATERIALS.

A. Regulatory Requirements. Materials in commerce deemed to have a reasonable potential for being contaminated with infectious agents are classified by the U.S. Department of Transportation (DOT) as "dangerous goods." The shipment of dangerous goods is regulated primarily by DOT although several other federal agencies (USDA, CDC, OSHA, etc.), international organizations (United Nations, World Health Organization, International Air Transport Association (IATA), etc.), and foreign governments also have requirements which apply to the shipment of dangerous goods under certain circumstances.

B. University Policy. As indicated in the section above, employees who participate in the packaging, shipping, receiving, transportation, or otherwise handle packages containing dangerous goods must be trained, tested, and certified. Detailed information regarding packaging, shipping, and labeling requirements for dangerous goods (infectious materials, diagnostic specimens, dry ice, etc.) training/certification requirements and related topics may be reviewed at the EHS/SRM Dangerous Goods Webpage.

C. Contact the EHS Dangerous Goods Program. To arrange for dangerous goods training and certification and/or assistance with questions/problems involving the packaging, shipping, receiving, labeling, or other dangerous goods issues, contact the Biosafety Office (mtelliot@vcu.edu).

D. Accredited Staff: Laboratory personnel who have been certified for shipping/receiving of dangerous goods via the EHS platform presentation or other approved course should be listed below (note: certification must be renewed every two years):

1. _____ Certification expires: _____

2. _____ Certification expires: _____

3. _____ Certification expires: _____

4. _____ Certification expires: _____

5. _____ Certification expires: _____

6. _____ Certification expires: _____

XII. **TRAINING REQUIREMENTS.** The OSHA Blood Borne Pathogens Standard and Laboratory Safety Standard specify that employers (PIs, laboratory managers, and other supervisory staff) are responsible for ensuring that employees are trained regarding the hazards associated with their job descriptions. University laboratories conducting research involving known or potentially biohazardous agents must meet the following training requirements:

A. General Laboratory Safety Training. EHS has provided a platform for comprehensive laboratory safety training under the Bioraft Safety Management System (vcu.bioraft.com). Through completion of Bioraft laboratory registration PIs will be provided with prompts indicating the required level of training for each registered laboratory worker.

B. Hazard Communication Training: The OSHA Hazard Communication Standard requires that general laboratory safety training be complemented with job-specific/hands-on safety training. Principal Investigators must ensure that employees are made aware of all hazards associated with their job and other hazards present within the work area through completion of a Worker's Right to Know form for each employee. Thorough hazard communication training includes incorporation of the record keeping elements described in Section XI.

C. Special Training Requirements: Laboratory staff working under special conditions may require additional training and/or certifications. Examples of duties requiring special training would include:

1. Preparing/receiving dangerous goods shipments (refer to Section IX).

2. Work in BSL-3 or greater biological containments (refer to Section III).

3. Work involving select agents (refer to Section IV.E.).

4. Working with agents and or applications with potential Dual Use Research of Concern (DURC) with require additional training for all staff involved. If you are working with potential DURC contact the Biosafety Office for additional training information.

5. Issuance of respiratory protection in order to reduce exposure to biological and/or chemical hazards will require participation in a respiratory protection program. **All staff participating in the Respiratory Protection Program must be identified in Appendix D of this Biosafety Manual.**

XIII. RECORDKEEPING REQUIREMENTS

A. Standard Operating Procedures (SOPs) Manual. **Laboratory SOPs for procedure involving biohazardous and rDNA materials should be attached in Appendix A of this manual.** Standard operating procedures (SOPs) must be developed for all laboratory procedures that involve known or potentially biohazardous agents. Laboratory staff must be trained annually in regards to each SOP or whenever new procedures are added to the work regimen. Required elements of an SOP include:

1. Descriptive title defining purpose of operation.

2. Preparation and revision dates.

3. Identification of department/laboratory for which SOP is applicable.

4. Brief statement of purpose.

5. Indication of potential undesirable outcomes.

6. Identification of regulatory standards that apply to procedures.

7. Listing by category of all materials, tools, and equipment required to complete SOP – it is critical that all safety equipment be identified (required PPE, BSCs, etc.).

8. Listing of environmental conditions, time constraints, or other factors which may have a negative impact on the execution of the SOP.

9. An overview of the sequence of the SOP describing major functions and anticipated/potential health and safety and environmental impact.

10. Definitions of terms.

11. Prominent display of warnings and cautions prior to description of each task with potential danger involved.

12. Listing of all tasks included within SOP in sequential order.

B. Biological Safety Data Sheets. In addition to the inventories of SDSs compiled for hazardous chemicals, all laboratories performing research with known or potentially biohazardous agents must include these materials on the laboratory hazardous materials inventory and maintain an appropriate MSDSs for each biohazardous agent. Questions or concerns regarding acquisition of MSDSs for biohazardous materials should be directed to the Biosafety Office.

C. Job Safety Analysis (JSA). OSHA requires that supervisors prepare assessments (JSAs) of safety issues relating to the workplace and work activities identifying the range of hazards and determining whether existing precautions are adequate. If existing safety precautions are not adequate, appropriate corrections must be identified and implemented. Supervisors are further required to explain and discuss the completed JSA with employees and to maintain the JSA within the laboratory. The Job Hazard Assessment Form (appendix A. pg 36) must be updated annually or as conditions warrant. Contact the Safety and Risk Management Occupational Safety Office for additional details.

D. Exposure Control Plan (ECP). In accordance with the OSHA Blood Borne Pathogen Standard, laboratories performing research where there is a potential occupational exposure to blood borne pathogens are required to develop and maintain an ECP. The Office of Environmental Health and Safety has developed a model ECP which provides a uniform policy for protection of university personnel who, as part of their job function, face reasonably anticipated exposure to blood borne pathogens. **If an ECP is required, a copy of the completed document should be attached to Appendix E of this manual.**

E. Sharps Injury Log: In accordance with the OSHA Blood Borne Pathogens Standard, laboratories performing research involving known or suspected blood borne pathogens must document all incidents involving potential employee exposure via sharps injury. University sharps injury logs must be obtained from Employee Health whenever sharps-related incidents occur. Information requested on the sharps injury log form must be completed by the injured worker's supervisor and reviewed/signed by the injured worker. Completed and signed forms must be submitted to Employee Health with a copy maintained within the laboratory of affected employee.

F. Medical Surveillance Program. Principal investigators must retain records of all occupational monitoring, medical examinations, vaccination/vaccination declination, and required medical treatment records for all employees involved tasks with risk for exposure to biohazardous agents. Medical surveillance (which included personal exposure monitoring) records must be maintained on file for the duration of employment plus 30 years.

G. Engineering Control Devices and Safety Equipment Testing/Certification. Laboratories must arrange for testing services required for maintaining certification of all engineering control devices and safety equipment necessary for achieving compliance with regulatory requirements. An abbreviated list of equipment requiring regular testing and/or certification includes:

1. Biological safety cabinets utilized for procedures involving containment of BSL-2 or greater biohazardous agents require annual testing/certification required (annual certification is also strongly recommended for BSCs used strictly for BSL-1 agent work).

2. Biological glove box units utilized for procedures involving containment of BSL-2 or greater biohazardous agents require annual testing/certification required.

3. Directional flow/negative pressure ventilation of laboratories operating under BSL-3 conditions require annual verification of suitable pressure relationships.

4. Chemical fume hoods receive annual testing provided by EHS/SRM.

5. Autoclave units utilized for onsite treatment of biohazardous waste must be operated per VDEQ requirements (Regulated Medical Waste Management Regulations).

H. Respiratory Protection Program. Research involving certain toxic chemicals and/or biohazardous agents which cannot be mitigated by engineering controls may require that respiratory protection be provided for employees involved in routine tasks or potential emergency response operations. If respirators are provided, the laboratory must implement a written respiratory protection program that is fully compliant with 29 CFR 1910.134.

I. Dangerous Goods Shipping Records. All shippers' declarations and waybills related to the shipment of any dangerous goods materials must be retained in the laboratory's central files for a minimum of two years post shipment. Copies of the shipper's certification must be maintained in conjunction with retention of dangerous goods declarations and waybills.

APPENDIX A: Institutional Biosafety Committee (IBC) Approvals

Attach copies of all active protocols involving biohazardous and/or rDNA materials and corresponding IBC approvals. This should include all IACUC protocols requiring reporting of *in vivo* use of biological and/or rDNA hazards, Bioraft biological registrations, Memoranda of Understanding and Agreement (MUAs), Needle Recapping Waivers, and any other documents conveying IBC instruction/interpretation.

APPENDIX B: Safety Data Sheets (SDS)

Attach an agent/product-specific MSDS copy for each biohazardous agent and/or biological toxin in use with in the laboratory. All biological toxins (even if covered under this biosafety manual) must also be addressed in the laboratory Chemical Hygiene Plan (CHP). If an SDS is not available for specific agents/toxins provide whatever hazard information is available.

APPENDIX C: Laboratory SOPs

Attach laboratory standard operating procedures for all tasks involving biological hazards and recombinant DNA materials. As a service to researchers, EHS/SRM has attached SOPs to cover several common laboratory applications involving biological/rDNA hazards. If any of the following SOPs apply to the laboratory regimen they should be completed (fill in blank spaces) in order to make them lab specific.

SOP for Centrifuging of Biohazardous Materials

Revised 06/26/24

1. Centrifuging will only be conducted by staff who have received task-specific training addressing potential hazards associated with the agent(s) and procedures involved.

2. Indicate building/room number where centrifuging will take place:_____

3. Is the space a shared facility?____It the space to be utilized for procedure is a shared space, the PI assured that precautions will be taken to alert other researchers of the potential hazard, and to limit access to designated staff during procedures.

4. Access to space will be restricted during centrifuging through posting of hazard signage, locking of door, and notification of employees in general area.

5. PPE to be donned during procedure will include examination gloves, safety glasses/goggles, lab coat, and proper laboratory attire.

6. Measures to be taken in the event of a spill: leave room immediately, restrict access, allow 20 minutes for aerosol to settle, don full PPE (as indicated under line 5.), clean area with suitable disinfectant.

7. Potential biological hazard/rDNA exposures occurring during centrifuging/other lab applications will be reported immediately to Employee Health Services and with 24 hours of occurrence to the Biosafety Office.

8. The PI verifies that only safety cups will be utilized for centrifugation of biohazardous materials: centrifuge safety cups will loaded within a certified Biological Safety Cabinet (BSC) and following centrifugation, will only be opened within a certified BSC.

9. Disinfectants to be used: 70% EtOH (replaced every 60 days) for routine cleaning, and 10% bleach solution (replaced every 7 days) or other EHS/SRM- approved disinfectant for spill cleanup.

Approximate RPMs which centrifuge will be operated at when conducting procedures involving biohazardous materials:_____

10. **For viral vector production and applications involving other potentially biohazardous agents**: Centrifugation is not 100% effective in removing cells or cell debris. For viral vector production and applications involving other potentially biohazardous agents, the supernatants will be filtered with a 0.45-micron syringe filter to ensure that there are no viable transfected cells or other biohazardous agents in the virus stock.

SOP for Cleanup of Large Biohazardous Spills

*List lab spaces to be covered under SOP:*_____

1. Leave room immediately, restrict access to space, and allow 20 minutes for aerosols which may have been generated during spill to settle.

2. Don proper PPE for cleaning of spill, minimum to include: examination gloves, lab coat, safety goggles, and proper laboratory attire (if this level of PPE is not sufficient to ensure adequate protection, contact EHS/SRM at 828-9834 for assistance).

3. Gently apply layer of paper towels which have been saturated with freshly prepared 10% bleach solution over entire spill area (note: if spill involves sharps contact EHS/SRM (828-9834) for assistance prior completing steps below).

4. Mist entire paper towel covered area and marginal surface areas thoroughly with freshly prepared 10% bleach solution, and allow 20 minutes contact time.

5. Remove towels, wipe down spill area thoroughly, and apply one final misting of bleach solution – allow final application to air dry.

6. Dispose of all waste materials generated during cleanup via red-bagging.

7. Wash hands thoroughly with soap and water.

8. If spill involves potential staff exposure to biohazardous/recombinant DNA materials, affected staff will report to Employee Health as soon as possible and the event will be reported to the Biosafety Office (mtelliot@vcu.edu) within 24 hours of occurrence.

SOP for Cleanup of Biohazardous Spill in Biological Safety Cabinet (BSC)

*List lab spaces to be covered under SOP:*_____

1. Leave BSC on.

2. Follow steps 2 - 8 above.

3. If the cabinet has a catch basin beneath the work surface and the spill resulted in liquids flowing into this area, more extensive decontamination is required.

4. Ensure the drain valve under the cabinet is closed.

5. Pour disinfectant onto the work surface and through the front and rear grilles into the drain pan. Allow 20 minutes contact time.

6. Absorb spilled fluid-disinfectant from work surface with paper towels and discard in an orange autoclave bag or red bag.

7. Prepare to empty drain pan. Place fresh disinfectant solution into a collection vessel. Attach flexible tubing to the drain valve. The tube should be of sufficient length to allow the open end to be submerged in the collection vessel to minimize aerosol generation.

8. Open the drain valve and empty the drain pan into the collection vessel containing disinfectant. Flush the drain pan with water and remove the flexible tubing. Manage contaminated materials as if they are infectious.

9. Remove protective clothing used during cleanup and place in an orange bag for autoclaving or red bag for incineration.

10. Wash hands thoroughly.

11. Notify Principal Investigator or Supervisor and the BSO to determine if vapor/gas decontamination of the cabinet and filters is necessary.

12. Allow BSC to operate for at least 10 minutes after cleanup, before resuming activity in the cabinet.

SOP for Live Imaging of Potentially Biohazardous Live Samples

*List Biohazardous/rDNA materials to be live imaged:*_____

I. Purpose: Live sample imaging is a powerful technique and is commonly performed on many confocal microscopy systems. The guidelines provided below are intended to ensure that researchers are provided with proper protection from potential to biological hazards during live sample imaging. Live sample imaging in VCU research facilities may potentially involve applications under two Biosafety Levels (BSLs):

A. BSL1: Yeast, *E coli*, well established animal (nonhuman primate) cells, and other organisms not known to cause disease in humans of normal health (refer to the BMBL for further guidance).

B. BSL2: All human cells (including cultured cell lines like HeLa) which may contain blood borne pathogens (HIV, HCV, HBV, EBV, etc.), cell lines which have been transformed by replication deficient viral vectors (which have not been confirmed to be free RCV by PCR or other approved methods), and other specimens containing microorganisms classified under BSL-2 (refer to the BMBL for further guidance).

C. Forbidden Applications: Researchers must be aware that specimens classified at BSL-3 or greater (e.g. Ebola, TB, other potentially airborne pathogens) cannot be live-imaged at VCU. Applications involving agents which are highly resistant to the limited available disinfectants suitable for use with microscopy equipment (Cryptosporidium e.g.) may also not be permitted within the core facility. The IBC and Microscopy Core Facility will ultimately determine the feasibility of applications.

II. Required Standard Operating Procedure (SOP) for BSL2 Samples (adherence to this SOP is also strongly recommended imaging applications involving BSL-1 materials):

A. All research involving agents classified at BSL-2 or greater and/or recombinant DNA materials must be approved and registered through submission of a Bioraft biological registration prior to utilization of live imaging equipment. If the research involves use of core facility equipment a copy of the Bioraft registration will be provided to core facility director by the researcher prior to initiating imaging.

B. Completion of adequate biosafety training must be verified on Bioraft prior to permitting any research staff to perform applications involving live-imaging of materials classified at BSL-2 of greater: minimal requirements include direct task-specific training from principal investigator (PI) and completion of EHS/SRM “Biosafety Training CDC/NIH Module.”

C. All sample/slide preparation must be performed within a certified biological safety cabinet (BSC) under the conditions established under the Bioraft registration.

D. Research staff will don proper PPE during preparation and transport of samples/slides to include: lab coat, examination gloves, proper laboratory attire, and eye protection where necessary.

E. Imaging dishes must be tight-fitting and or designed to be fully closable (for example, ibidi USA “microslides,” in Vitro Scientific “Multiwell” glass bottom plates), with parafilm utilized to further secure lids and minimize risk of spills. The outer surfaces of the dishes must be wiped with an appropriate disinfectant (refer to biological registration) prior to packaging for transfer to core facility.

F. Transfer of Potentially Biohazardous Materials: If transportation from laboratory to microscopy facility (or vice versa) is required, samples must be transported within a closed secondary container displaying universal biohazard signage on the exterior surface, with sufficient absorbent materials in the bottom of the container to absorb contents in the case of leakage.

G. Signage must be posted on entry door of room housing microscope by research staff while conducting procedures involving potentially biohazardous agent(s).

H. Examination gloves will be worn when removing dishes from secondary container and placing onto the stage, the gloves will be removed and hands washed thoroughly prior to contacting the microscope, computer, or other equipment.

I. After completing imaging, research staff will remove samples from the microscope and wipe down the microscope stage with tissue saturated with disinfectant. Unless otherwise specified (by the conditions of the biological registration and with written verification from the Microscopy Core), 70% ethanol (EtOH) solution will be utilized for disinfection of microscope surfaces and components. Researchers will be required to provide their own disinfectant: stocks of 70% EtOH solution expire 60 days after preparation.

J. Spills: In the event of any spill, notify core staff immediately. Researchers are responsible for providing a "spill kit" which includes all materials required for conducting response in the event of a spill. The spill kit should at a minimum include disinfectant (70% EtOH), paper towels, red bags, and personal protective equipment (PPE). If the event of a spill, research personnel will leave room immediately to limit exposure to aerosols and avoid reentry into the laboratory for at least 20 minutes to allow for settling

of aerosols. Research staff will don appropriate PPE (refer to Bioraft registration) prior to entering space to conduct cleanup.

1. Work surface (benchtop, floor, etc.) spills: spills must be cleaned thoroughly with an appropriate disinfectant (70% EtOH, unless otherwise specified). Waste materials generated during spill response will be managed/disposed of by research staff as regulated medical waste via university red bag or orange bag procedures as specified on Bioraft registration.

2. Minor spills resulting in contamination of outer surface areas microscopy equipment: If spill is minor and only affects outer/accessible surface of scope, research staff will wipe down area thoroughly with tissue damp with disinfectant (70% EtOH). Waste materials generated during spill response will be managed/disposed of by research staff as regulated medical waste via university red bag or orange bag procedures as specified on Bioraft registration.

3. Major spills resulting in potential contamination of inner/inaccessible areas of microscope: research staff will leave room immediately and notify Microscopy Core staff of the incident. Due to presence of electrical shock hazard and potential for damage to sensitive components of the microscope, research staff will not attempt to disinfect or otherwise access internal surfaces of microscope.

K. Hands will be thoroughly washed with soap and water immediately upon completing above tasks.

L. Following completion of imaging tasks, all samples must be transferred (per line III.E) back to the host laboratory for disposal per conditions of Bioraft registration.

III. Additional Training Requirements: Research staff will be trained per the conditions of this SOP and the corresponding Bioraft registration prior to conducting live imaging procedures involving BSL-1 and BSL-2 specimens. Research staff will also be required to complete any training as required by the Microscopy Core prior to use of the facility.

SOP for Vacuum System Operation Involving Biohazardous Materials

List lab spaces where vacuum systems will be utilized for applications involving biohazardous and rDNA materials will be performed: _____

1. The PI will ensure that vacuum system operation will be conducted responsibly in order to prevent contamination of central and local systems and to prevent potential harmful exposure to maintenance workers, laboratory staff, and other university personnel.

2. Prior to vacuum systems operation, staff will verify that the primary trap flask has been charged with bleach sufficiently to maintain a minimum 10% bleach, content concentration, and that a second overflow flask is in place downstream from primary flask.

3. Prior to operation, staff will also verify that an in-line HEPA filter is in place immediately downstream form the overflow flask to protect vacuum system from contamination – the HEPA filter will be replaced as needed to ensure proper filtration and exhaust air flow.

4. If contamination of a central ("house") vacuum system is suspected, vacuum use will be ceased immediately and the PI will notify Facilities Management Zone Manager and the Biosafety Office (mtelliot@vcu.edu)

5. If contamination of local pump(s) is suspected, vacuum operations will be immediately ceased and the affected space will be evacuated and decontaminated following the "Biohazardous Spill Cleanup SOP" prior to returning lab to routine lab operation. The contaminated pump(s) will be red-bagged and discarded properly (contact Biosafety Office for assistance).

SOP for Exposure/Incident Reporting and Reporting Procedures

All laboratories performing applications involving Biological and/or rDNA materials must comply with the conditions set forth by this SOP.

1. Personnel Exposure:

a. Exposure Examples:

• Needle sticks or other percutaneous injuries from a contaminated sharp item.

• Splashes to mucous membranes (eyes, nose, or mouth).

• Bites/scratches from animals that have been exposed to any recombinant or synthetic nucleic acid material, whether or not the exposure leads to illness.

b. Immediate Response:

• SKIN exposure: Immediately remove contaminated personal protective equipment or clothing and wash the contaminated area with an iodine solution or antibacterial soap and copious water for 15 minutes.

• EYE exposure: Flush the eye with water for at least 15 minutes at an eyewash station.

c. Notify PI or supervisor. If PI/supervisor is not available, immediately proceed to next step.

d. Medical Treatment

• During work hours (8:00 AM to 4:00 PM), report to VCU Employee Health Services.

• After hours and weekends: report to VCU Health Systems Emergency Room.

• If emergency, call 911.

e. Reporting:

i. Notify EHS/SRM (828-9834) immediately.

ii. The BSO will investigate the incident and notify the IBC Chair and EHS Director.

iii. The PI will complete an Internal Incident Report Form and submit it to the BSO and Safety and Risk Management within 24 hours of incident.

iv. If the IBC Chair and BSO determine that the incident involves rDNA/synthetic nucleic acid molecules, the BSO will submit an NIH incident report to the NIH Office of Biotechnology Activitieswithin 30 days. Incidents occurring in BSL2 laboratories resulting in an overt exposure will be immediately reported to NIH OBA.

v. The Office of Safety and Risk Management and the Vice President for Research will also receive a copy of the incident report

SOP for Percutaneous Hazards

Percutaneous Hazards: If needles/other sharps devices are being utilized for delivery of viral vectors/rDNA material, laboratories will follow this SOP to limit exposure potential and verify that staff have completed training per the SOP. Minimum elements covered in SOP include:

1. Personal Protective Equipment: staff conducting injections will don lab coat, examination gloves, and eye protection (safety glasses or goggles) and proper laboratory attire.

2. Engineering Controls: All injections will be conducted within a certified BSC (unless otherwise specified and IBC-approved under biological registration).

3. Safety engineered sharps equipment must be used where feasible. The PI will document that a review has been conducted to determine whether safety engineered alternative options are available, and will substitute for safety engineered equipment if feasible. If the safety engineered equipment substitution is not feasible the PI will provide written justification for utilization of standard equipment (below).

4. Access to space must be restricted during procedures involving biological/rDNA hazards, proper hazard signage must be posted on entry door during procedures.

5. Work Methods: bending, shearing, and recapping of needles is prohibited, staff must be provided with hand's on training regarding proper use of syringes other sharps devices used in association with biological/rDNA hazards, animals must be properly secured during procedures (*in vivo* applications).

6 Verification that all staff involved in injection/other sharps delivery of biological/rDNA hazards have completed training per this SOP (below).

*The laboratory will utilize the following potential sharps hazards for procedures involving biological/rDNA hazards:*_____

*Are the sharps devices utilized safety-engineered?*_____

*If sharps devices are not safety engineered, provide justification:*_____

*Identify staff who have received full training and will conduct related procedures:*_____

SOP for handling infectious/human samples-FACS Aria

1. All sorting is to be done with the Aria that is contained in the Baker BioProtectIII hood. Tubes to be used are uncapped under this hood.
2. Cover keyboard, mouse, and other instrument control surfaces w/ cellophane; clear surfaces of clutter, use absorbent pads for samples.
3. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols!
4. Prepare sort collection chamber. Make sure the O-ring is installed in the groove on the top of the collection tube holder. Close sort collection chamber door.
5. Make sure sheath tank is filled and standard waste tank contains enough bleach to give a final 10% solution when filled. Fill a spray bottle with a freshly made 10% bleach solution for work area decontamination.
6. Wear gloves and lab coat before handling samples. Lab door must be closed.
7. Have two previously cam adjusted nozzles, with new O-rings installed, available in case of a clog.
8. Procedures during sorting/analysis:
   a) Filter samples prior to sort to avoid clogs.
   b) Fill sample tube with as much sample as possible to minimize loading and unloading sample. DO NOT fill higher than ¼ inch from the top of the tube.
   c) Make sure the "Sweet Spot" is enabled.
   d) Close sort collection chamber door before starting sample.
   e) If during the sort the stream is deflected (due in part to a clogged flow cell tip), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:
      - If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door.
      - Wait at least 60 seconds.
      - With the sort block chamber door, aspirator drawer, and collection chamber door all closed, turn the stream on and off several times or perform a 'Clean flow Cell' with DI H_20 to see if the clog will clear itself.
      - If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% bleach for 30 minutes. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring.
      - Install prepared nozzle with O-ring, ensure that the sort block chamber and collection chamber doors are closed before turning the stream on.
      - Turn stream off.
      - Open the sort block chamber door and dry plates and surfaces as needed.
      - Do not remove any samples from the sort collection chamber until the sample acquisition has been stopped for at least 30 seconds. This procedure will clear aerosols for the sort chamber. After this time sorted samples can be removed from the sort chamber safely.

f) When changing collection tubes:

- Stop the sample flow and close the aspirator drawer by clicking the Acquire button.
- Wait at least 60 seconds before opening sort collection chamber door.
- When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

9. Procedures after sort/analysis:
    a. Lift the cover to turn off the lasers. Disengage "Sweet Spot" and turn the stream off.
    b. Disinfect sample lines using a freshly made 10% bleach solution as follows:
        i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted.
        ii. Select from the menu - Instrument >Cleaning Modes>Clean Flow Cell
        iii. Repeat two more times, or until fluid is seen in tubing exiting the flow cell.
        iv. Wait 30 or more minutes with 10% bleach in flow cell.
        v. Clean Flow Cell two times with DI water.
    c. Select from the menu – Instrument>Cleaning Modes>Long Clean>Clean Bulk Injection Chamber. Verify that chamber fills, in not repeat until successful.
    d. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a Hype-Wipe 10% bleach towel and/or 10% bleach from a spray bottle. Clean keyboard cover, remove any cellophane and discard in MPW box. Let bleach air dry.
    e. When leaving the lab:
        i. make sure all samples are capped.
        ii. Remove gloves, respirator & lab coat (remember outside of gloves are contaminated!).
        iii. WASH HANDS!

APPENDIX D: Respiratory Protection Program

Laboratory personnel who are issued respiratory protective equipment (including N-95 filtering face pieces) are required to participate in a respiratory protection program which includes medical evaluation, proper use training, and fit-testing. All staff who are issued a respirator must receive medical clearance and fit-testing annually. Contact the Biosafety Office (mtelliot@vcu.edu) if assistance is needed with the development of this program. Laboratory personnel who have completed respiratory protection program requirements should be listed below, along with training/fit-testing date, and type/size of respirator(s) approved to wear:

Name _____

Initial fit-testing/training date _____

Annual fit-testing renewal _____

Respirator type(s) approved to utilize: _____

Name _____

Initial fit-testing/training date _____

Annual fit-testing renewal _____

Respirator type(s) approved to utilize: _____

Name _____

Initial fit-testing/training date _____

Annual fit-testing renewal _____

Respirator type(s) approved to utilize: _____

Name _____

Initial fit-testing/training date _____

Annual fit-testing renewal _____

Respirator type(s) approved to utilize: _____

Name _____

Initial fit-testing/training date _____

Annual fit-testing renewal _____

Respirator type(s) approved to utilize: _____

APPENDIX E: Exposure Control Plan

In accordance with the OSHA Bloodborne Pathogens Standard (CFR 29 1910.1030), personnel with reasonable potential for occupational exposure to blood borne pathogens (BBP) must be included in an Exposure Control Plan (ECP). Principal investigators performing research involving blood borne pathogens should attach a completed Exposure Control Plan which includes all personnel with reasonable potential for exposure to BBP here: