Biosafety Manual & Standard Operating Procedures

Emergency Contact Information

Fire and Medical Emergencies	911
RUPD/After Hours Notification	831-5500
RU Environmental Health & Safety	831-7790
Office of Emergency Preparedness	831-7155
Principal Investigator (*office*)	_____
Principal Investigator (*home*)	_____
Principal Investigator (*mobile*)	_____

Radford University
Office of Environmental Health & Safety
Current Revision November 2012

I. Acknowledgement Form

Principal Investigators (PIs) and all personal active in research within laboratories under their charge must sign and date the following statement to demonstrate acceptance of the policies and conditions set forth in this document.

1. **Principal Investigator**: I am familiar with and agree to comply with the provisions of the Radford University Biosafety Manual and have added information where required to address hazardous conditions which are specific to the laboratory spaces under my charge. I have thoroughly discussed the content of this manual with the personnel listed below and have given them proper training and 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, 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.

The Office of Environmental Health and Safety has developed this model biosafety manual to assist principal investigators and laboratory directors in limiting exposure of RU faculty, staff, students, and the community to bio-hazardous agents and to better ensure university compliance with all applicable regulations promulgated by the federal, state, and local regulatory agencies. This biosafety manual serves as the university model; through adding requested information and applicable attachments (appendices) a document which meets the NIH 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 RU EHS radiation and chemical hygiene plans that can be accessed at RU EHS programs. This biosafety manual must be available and accessible to all laboratory personnel.

This biosafety manual is applicable to all laboratory, research, service, and support activities that may involve exposure to bio-hazardous agents or materials and research that comes under the purview of the Radford University Environmental Health & Safety office or the Institutional Biosafety Committee (IBC). Activities which are specifically addressed are those involving:

- Bacterial, viral, fungal, and parasitic agents
- Recombinant DNA, including experiments that may be exempt under the NIH Guidelines
- Human blood, body fluid, tissues, and cell cultures
- Listed select agents and toxins
- Exposure to research animals
- Infectious waste

B. Regulatory Requirements.

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) form the basis for the practices included in this biosafety manual. It should be noted that there may be additional guidance documents and regulations imposed by various funding agencies that individual PIs must be aware of and incorporate into their laboratory-specific biosafety SOPs. Guidelines must be followed to ensure the continuation of grant funds from federal agencies and for health and safety purposes.
Compliance with the minimum conditions set forth in this biosafety manual is mandatory for all university personnel and facilities. All university research laboratories are strongly advised to fully review the following information offered by regulatory agency websites:

NIH: Guidelines for Research Involving Recombinant DNA (rDNA) Molecules
CDC-NIH: Biosafety in Microbiological and Biomedical Laboratories 5th ed. (BMBL)
OSHA: Bloodborne Pathogens Standard: CFR 29 1910.1030
VDEQ: Regulated Medical Waste Regulations

The requirements for packaging and shipment of etiological agents are provided in the Public Health Service regulation 42 CFR Part 72 Interstate Shipment of Etiologic Agents and parts of the Department of Transportation Hazardous Materials regulation 49 CFR, Parts 171 – 180.

C. **Responsibilities**.

Development of a complete biosafety program requires cooperation and interaction between the following university entities:

- **Principal Investigator:**

  (PI): A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials, and are ultimately responsible for ensuring implementation of a comprehensive biological safety program for all laboratories under their charge.

  - Completes registration forms for all research protocols involving the use of recombinant DNA, biological materials, or select agents and toxins;
  - Develops specific biosafety standard operating procedures for each bio-hazardous and select agent used in his/her laboratory;
  - Accepts direct responsibility for the health and safety of those working with biological materials and/or select agents and toxins in his/her laboratory;
  - Ensures proper training and instruction for laboratory personnel in safe practices and protocols, including, at a minimum, training in aseptic techniques and biology of the organism(s) used;
  - Ensures that laboratory personnel receive any necessary medical surveillance;
  - Ensures that biosafety cabinets are certified as needed;
  - Ensures that personal protective equipment is provided and used; and
  - Ensures compliance by laboratory personnel with relevant regulations, guidelines, and policies.

This individual should also consult with RU EHS and other health and safety professionals with regard to risk assessment.

- **Laboratory Employees**:

  - Participate in appropriate training and instruction and ensure that they are adequately trained and fully understand the instructions;
  - Fully comprehend all biological agents and select agents and toxins being used in the lab and the potential risks associated with exposure, as well as fully understanding the associated emergency response procedures;
  - Follow all laboratory practices and protocols and comply with all applicable guidelines and policies;
  - Complete any necessary medical surveillance; and
  - Report all thefts, security incidents, accidents, spills, or contamination incidents to supervisor.

- **Deans, Directors, and Chairs**:

  - Ensure that registration forms are completed by each Principal Investigator conducting research involving potentially bio-hazardous materials such as recombinant DNA; infectious agents; human blood, body fluids, tissue, or cell culture; or select agents and toxins.

- **Office of Environmental Health & Safety**:

  Administers the University's Safety Program, coordinates the university Laboratory Safety Program, the university Respiratory Protection Program, and appoints a Biosafety Officer (BO) who is ultimately responsible for establishing/interpreting university biosafety policies.

  - Develops, implements, and maintains the university's biosafety program;
  - Reviews all registration forms for research proposals submitted by Principal Investigators;
  - Consults with researchers on issues of biosafety and the safe use of biological materials in the laboratory;
  - Develops protocols and procedures to address issues of biosafety;
  - Provides training in safe use and practices for those working with potentially bio-hazardous materials and activities;
  - Conducts annual laboratory biosafety audits to determine compliance status;
  - Promotes regulatory compliance and a safe laboratory environment;
  - Advises researchers on proper waste disposal methods based on federal and state regulations;
  - Provides oversight of the Radford University Bloodborne Pathogen Program and conducts training for laboratory personnel with such exposure.

- **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 Research Involving Recombinant DNA Molecules (NIH Guidelines). Failure to comply with the requirements mandated during the IBC review process may result in the suspension of research involving chemical, biological, *r*DNA, and/or institutional privilege to use animals.

  - Facilitates the registration of biological research by providing materials and information to Principal Investigators;
  - Reviews research involving recombinant DNA and other potentially bio-hazardous agents, and approves those that comply with NIH and CDC guidelines;
  - Reviews and approves research involving the use of select agents and toxins;
  - Adopts policies supporting the safe use of biological materials and the elimination or reduction of exposure to potentially bio-hazardous materials and agents; and
  - Addresses biosafety issues related to experimentally infected laboratory animals.

- **Visitors, Vendors, and Contractors**:

  - Comply with all safety requirements and procedures;
  - Be accompanied by university personnel at all times while in BSL-2 areas; and
  - Use personal protective equipment provided for them by the laboratory. **NOTE**: Contractors must ensure that appropriate personal protective equipment is available for their own workers.

III. 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:

- **Primary Containment**
    - **Personal Protective Equipment**
        - **Gloves**. Gloves must be worn whenever manipulations involving potentially bio-hazardous agents or hazardous chemicals are performed. Select glove type based on specific bio-hazardous agents and chemical compound(s) to be handled.
        - **Safety Glasses/Goggles**. Required for all procedures involving potentially bio-hazardous 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 bio-hazardous agents shall require the use of a face shield in addition to safety glasses or chemical splash goggles.
        - **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. Shorts and other clothing exposing feet or legs, sandals, and other open-toed shoes are prohibited laboratory attire. Non-disposable personal protective equipment items may not be taken home for laundering or laundered in public facilities (e.g., laundromats).
        - **Disposable gowns/scrubs** must be utilized whenever potential for splashing of hazardous materials exists.
        - **Respirators** shall be utilized whenever potential for aerosolization or other airborne biohazard exposure threat exists and cannot adequately be controlled through engineering controls. Utilization of respiratory protection devices is subject to the review and approval of RU EHS under the university Respiratory Protection Program. All staff participating in the Respiratory Protection Program must be identified.
    - **Engineering Controls**
        - **Biological Safety Cabinets** (BSCs). Manipulations having the potential of generating biological aerosols must be performed within certified BSCs. Complete information regarding

BSC certification requirements, maintenance procedures, and classifications may be viewed at the CDC website.

- **Centrifuge safety cups** must be utilized when centrifuging materials that have the potential of producing bio-hazardous aerosols.

- **Secondary Containment**.

  The secondary barriers required will depend on the risk of transmission of specific agents. For example, in working with agents at Biosafety Level 2 (see Laboratory Biosafety Levels below), the exposure risks involve direct contact with the agents or inadvertent contact through contaminated work environments. Recommended secondary barriers in these laboratories include separation of the laboratory work area from public access, hand washing facilities, and availability of a decontamination facility such as an autoclave.

  When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional airflow, HEPA filtration to remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate modules to isolate the laboratory.

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

  - **Waste Disposal**. All hazardous waste generated within university research facilities must be disposed of through the Radford University Environmental Health & Safety Office, 831-7790, and must be packaged and labeled in accordance with federal and state requirements.

B. 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 bio-hazardous agents/infected research animals. These biosafety levels range from BSL/ABSL-1 (low individual risk, low community risk), involving agents with minimal risk to normal, immunocompetent individuals and to the environment through BSL/ABSL-4 (high individual risk, high community risk), which involves bio-hazardous agents that are extremely dangerous to humans and/or the environment (note that research involving BSL-2 is permitted at RU with the proper approval while BSL/ABSL 3 and 4 work is not permitted at RU). Persons working with infectious agents or potentially infected materials must be aware of potential hazards, recommended biosafety level for the agents being manipulated, and must be trained and proficient 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. A brief outline of the requirements of BSL-1 through BSL-3 is provided below. For complete listing of the requirements of CDC BSL/ABSL refer to the BMBL.

Table 2 – Biosafety levels

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top, sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: • Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences. **Currently not permitted at RU**	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	**Not permitted at RU**			

From BMBL section III, table 1

C. Guidelines for Good Laboratory Practices at BSL-2

BSL-2 applies to work with a broad spectrum of moderate-risk agents that are generally present in the environment at large and are associated with human disease of varying severity. Microorganisms assigned to this containment level include Salmonella spp., Toxoplasma spp., and Hepatitis B. With the use of good microbiological techniques, much of this work can be done on open bench tops as long as there is limited potential for splashes and aerosol creation.

1. Laboratory employees must immediately notify the laboratory supervisor or PI; and they should in turn notify RU EHS, OEP, and RUPD, in case of an accident, injury, illness, or overt exposure associated with laboratory activities. When deemed necessary, proceed to the RU Student Health Center for appropriate medical surveillance and/or treatment, after proper decontamination.

2. **Access to the laboratory must be limited or restricted by the PI when experiments or work with cultures or specimens is in progress. In addition:**

    - Only personnel advised of the special hazards and meeting any specific entry requirements, i.e., FBI clearance for work with select agents, appropriate immunizations, or serum sampling, are permitted in the laboratory. All bio-hazardous procedures, provided by the PI, must be understood and followed by laboratory occupants.

    - Lockable, self-closing doors and other security measures must be provided for facilities maintaining select agents and toxins to control access.

    - Ensure that when bio-hazardous agents and toxins are in use or stored in the laboratory, a biohazard sign is posted on the lab access door. This sign identifies the agent(s) in use, the biosafety level, any required immunizations, the PI's name and telephone number, and any PPE that must be worn in the laboratory.

    - The PI must ensure that all laboratory personnel receive appropriate training on hazards associated with the agents/toxins involved, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive training before starting work with the agents/toxins, as well as annual updates and additional training as necessary for procedural or policy changes. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 2, all personnel demonstrate proficiency in standard microbiological practices and techniques.

3. Laboratory employees must wash hands frequently and always after handling viable material or animals, after removing gloves, and before leaving the laboratory. A sink for hand washing must be present in each laboratory. Foot, knee, or automatically operated hand washing sinks should be considered. An eyewash and safety shower must be readily accessible.

4. Eating, drinking, smoking, chewing gum, handling contact lenses, or applying cosmetics is not allowed in the laboratory. Persons wearing contact lenses in the laboratory should also wear goggles or a face shield.

5. Food, medications, or cosmetics should not be brought into the laboratory for storage or later use. Food must be stored outside the work area in cabinets or refrigerators designated specifically for that purpose.

6. Mouth pipetting is forbidden; only mechanical pipetting devices are permitted.

7. All procedures must be performed carefully to minimize the creation of splashes or aerosols.

8. Establish and follow policies for safe handling of sharps, including:

    - Use a high degree of caution when handling any contaminated sharp item, such as needles and syringes, slides, pipettes, capillary tubes, and scalpels;
    - Restrict needles and syringes or other sharp instruments in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.

    - Substitute plastic-ware for glass whenever possible.

    - Handle broken glassware with a brush and dustpan, tongs or forceps, not directly with hands;

    - Use only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) for injection or aspiration of infectious material. Syringes which re-sheathe the needle, needle-less systems and other safety devices should be used whenever possible and appropriate; and
    - Do not bend, shear, break, recap, or remove used needles from disposable syringes or otherwise manipulate such units by hand before disposal. Dispose of needles and syringes in the puncture resistant sharps container provided in the laboratory for this purpose. Place full sharps containers in an autoclave bag and sterilize before disposal in biohazard containers.

9. Lab coats, gowns, smocks, or other provided protective garments must be worn while in the lab. When exiting the lab, remove lab coats and other protective clothing in the lab for disposal or laundering.

10. Gloves must be worn when manipulating bio-hazardous agents or when hands must otherwise contact contaminated surfaces. Remove and change gloves when overtly contaminated or when torn or punctured. Do not wear contaminated gloves outside the lab. Do not wash or reuse disposable gloves. Consider alternatives to latex gloves to prevent allergic response.

11. Appropriate face protection (goggles, mask, face shield or other splatter guard) must be worn for anticipated splashes or sprays of bio-hazardous agents to the face when agents must be handled outside the BSC.

12. Equipment and work surfaces must be decontaminated at completion of work, at the end of the day, and following spills of viable materials.

13. Bench tops must be impervious to water and resistant to solvents, acids, alkalis, and chemicals used for surface decontamination. Laboratory surfaces and spaces between fixtures must be designed to be easily cleaned; no carpets or rugs are allowed in the laboratory.

14. Work in the open laboratory is permitted, except for those instances where a properly maintained biological safety cabinet is required, such as:

    - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs; and
    - High concentrations or large volumes of bio-hazardous agents or toxins are used. Such materials may be centrifuged in open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

- Air sampling studies have shown that most of the common manipulations of bacterial and viral cultures in research laboratories release aerosols of viable organisms. This must be considered when evaluating need for use of the biological safety cabinet or other physical containment device.

15. Dispose of all bio-hazardous wastes and associated wastes as outlined by RU EHS Policy.

16. All containers of cultures, tissues, specimens of body fluids, or other potentially infectious waste must be covered to prevent leakage during collection, handling, processing, storage, transport, or shipping.

17. An insect and rodent control program is in place at RU. Occupants should routinely ensure screens are fitted on exterior windows that open into the lab and contact Facilities Management when pest control is needed.

18. Illumination must be adequate for all activities, avoiding reflections and glare that could impede vision.

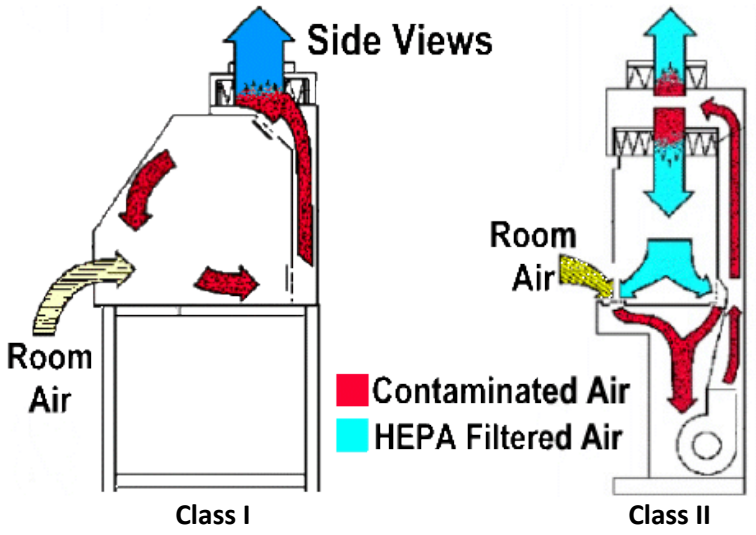
D. Biological Safety Cabinets (BSCs)

BSCs are classified as Class I, Class II, or Class III cabinets. When properly maintained and operated, they effectively contain and capture microbial contaminants and infectious agents using HEPA (High Efficiency Particulate Air) filters. Biosafety cabinets should not be confused with clean benches which only protect the material being worked with and are not suitable for work with infectious or toxic material. (Although clean benches, like BSCs, have HEPA-filtered air, in clean benches the air flows over the experimental material toward the user rather than being drawn away.) BSCs should also not be confused with conventional fume hoods that do not filter microorganisms.

Types of BSCs

Class I BSCs provide personnel and environmental protection, but not product protection.

Class II BSCs are the most commonly used BSC for bio-hazardous agents. These cabinets provide personnel, environmental, and product protection. Only those which are hard ducted to the outside and provide a face velocity of 80 to 125 feet per minute should be used when working with volatile chemicals. Additionally, cabinets are not designed to prevent ignition of volatile flammable chemicals, such as ethanol and isopropanol.



Guidelines for Working in a BSC

- Turn off the ultraviolet lamp if one is in use. Turn on the fluorescent lamp.
- Inspect the air intake grilles for obstructions and foreign material and remove if necessary.
- Adjust view screen to proper height.
- Turn the cabinet on for at least 10 minutes prior to use, if the cabinet is not left running.
- Prepare a written checklist of materials necessary for the particular activity.
- Wash hands and arms with mild soap. Put on a rear-fastening, long-sleeved gown with tight-fitting cuffs. Put on safety glasses and a pair (or two pairs) of high quality nitrile gloves.
- Disinfect work surface with a suitable disinfectant.
- Place items into the cabinet so that they can be worked with efficiently without unnecessary disruption of the airflow, working with materials from the clean to the dirty side.
- Adjust the working height of the stool so that the worker's face is above the front opening.
- Delay manipulation of materials for approximately one minute after placing hands/arms inside the cabinet.
- Minimize the frequency of moving hands in and out of the cabinet.
- Do not disturb the airflow by covering any portion of the grillwork with materials.
- Work at a moderate pace to prevent the air flow disruption that occurs with rapid movements.
- Wipe the bottom and side of the hood surfaces with disinfectant when work is completed.

NOTE: Be very careful when using small pieces of materials such as chemwipes in the hood. These can be blown into the hood and disrupt the motor operations.

Certification of the BSC

Certification is a series of performance tests on the BSC to confirm that it will provide the user and experimental material the protection for which it is designed. The airflow, filters, and cabinet integrity are checked to ensure that the cabinet meets minimum performance standards. Certification is arranged through each department and provided by an outside vendor.

BSCs intended for user protection and/or BSL2 work must be certified:

- after they are received and installed (before use with infectious materials);
- after filter changes;
- and after being moved (even a few feet); and
- annually

BSC decontamination (using the formaldehyde gas production process) must be provided by an outside vendor and needs to be done:

- before any maintenance work requiring disassembly of the air plenum, including filter replacement;
- prior to cabinet recertification;
- before moving the cabinet to a new laboratory; and
- before discarding or salvaging.

The production of formaldehyde gas is a health concern; therefore, extreme caution should be used when having the procedure performed.

E. Laboratory Emergency Postings:

- Names of responsible individuals to be contacted in case of emergencies must be posted outside of entrance doors leading into each laboratory.

- 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):
- Use/storage of bio-hazardous agents, acute carcinogens and toxic chemicals, radiological agents, and flammable materials.
- Presence of strong magnetic equipment.
- Emission of X-rays.
- Required PPE.
- A listing of all alarms in the laboratory and whom to contact if an alarm is sounding

F. Evacuation Routes:

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

- Familiarization with primary and secondary (alternate) evacuation routes.
- Awareness of alarm method(s) used to signal a building evacuation.
- Designation of post evacuation meeting areas for laboratory staff.
- For assistance in determining proper evacuation procedures contact the RU EHS.

G. Emergency Response.

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 exposure incident or spill.

Exposure to Bio-hazardous Agent
In the event of an exposure to a bio-hazardous agent or material, the following guidelines should be used:

Intact Skin
- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
- Vigorously wash contaminated skin for 1 minute with soap and water.
- Call 911 or seek immediate medical attention, if necessary.
- Inform the laboratory's PI and RU EHS.

Broken, Cut or Damaged Skin or Puncture Wound
- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
- Vigorously wash contaminated skin for 5 minutes with soap and water.
- Call 911 or seek immediate medical attention, if necessary.
- Inform the laboratory's PI and RU EHS.

Eye
- Immediately flush eyes for at least 15 minutes with water, using an eyewash. (Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly.)
- Remove contaminated clothing. Clothing should not be pulled over the face as contact with eyes, nose, and mouth may occur. Shirts should be cut off.
- Call 911 or seek immediate medical attention, if necessary.
- Inform the laboratory's PI and RU EHS.

Ingestion or Inhalation

- Move to fresh air immediately.
- Call 911 or seek immediate medical attention, if necessary.
- Do not induce vomiting unless advised to do so by a health care provider.
- Inform the laboratory's PI and RU EHS.

The essential elements of a bio-hazardous spill response plan suitable for addressing the two most common types of incidents encountered within university laboratories are listed below:

- **Spills inside 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 bio-hazardous spill within a BSC, the following procedures shall be followed:
    - Leave the cabinet on: while wearing gloves, spray or wipe cabinet walls, work surfaces, and equipment with suitable disinfectant as specified by the MSDS. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface with disinfectant for a contact time of at least 20 minutes.
    - 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.
    - Autoclave all clean-up materials before disposal in the biohazard waste container. Wash hands thoroughly with soap and water after the clean-up procedure.

- **Spills in the open laboratory.** Bio-hazardous 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:
    - When potentially bio-hazardous materials are spilled in open area of the laboratory evacuate the laboratory immediately to limit exposure to aerosols.
    - Upon exiting the laboratory, warn other personnel in the area of the incident.
    - 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.
    - Remove contaminated clothing with gloved hands, folding contaminated area inward. Discard clothing in a red biohazard bag or place clothing directly in an autoclave.
    - Thoroughly wash all potentially contaminated areas, arms, face, and hands with soap and warm water.
    - Avoid reentry into the laboratory for at least 30 minutes to allow for the settling of aerosols potentially generated by the spill.
    - Don appropriate PPE for cleaning operation. This must include at a minimum: gloves, eye protection, and laboratory coat. Spills involving high risk bio-hazardous agents with high potential for aerosol transmission may require additional PPE including respiratory protection.
    - 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.
    - Allow disinfectant to stand for at least 15 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.
    - If the floor and sink are affected by the spill, flush these areas with disinfectant.
    - Dispose of all liquid and solid waste generated during spill cleanup as bio-hazardous waste through Radford University Environmental Health & Safety Office, 831-7790

Spill kits: are required in all labs conducting research involving potentially bio-hazardous 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 can 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 bio-hazardous waste generated during spill response or for overpacking 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 foot covers.
    e. Protective breathing devices such as N-95 respirators.
7. Forceps, broom, heavy-duty brush, and dustpan (for spills involving sharps materials).
8. Extra clothing to replace items contaminated during spill/cleanup (scrubs, e.g.).

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 bio-hazardous spills must be available in all areas where manipulations involving potentially bio-hazardous agents are conducted.

VIII. 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 bio-hazardous 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.
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 MSDS and the CDC Disinfecting Guidelines whenever determining appropriate disinfectants. If bleach is a compatible choice, RU EHS recommends use of a bleach-water 10% solution (i.e., one part household bleach to nine parts water) prepared daily. Decontaminants are an essential component of an emergency spill response kit.

- **When to Decontaminate;** All material and equipment contaminated with or containing potentially bio-hazardous agents should be decontaminated;
- upon completion of procedures involving the use of bio-hazardous or select agents and toxins;
- in the event of spills of such materials;
- before being washed, stored, or discarded; and
- at least daily.

IV. Packaging and Shipping

The definitions below apply to the packaging and shipping instructions that follow:

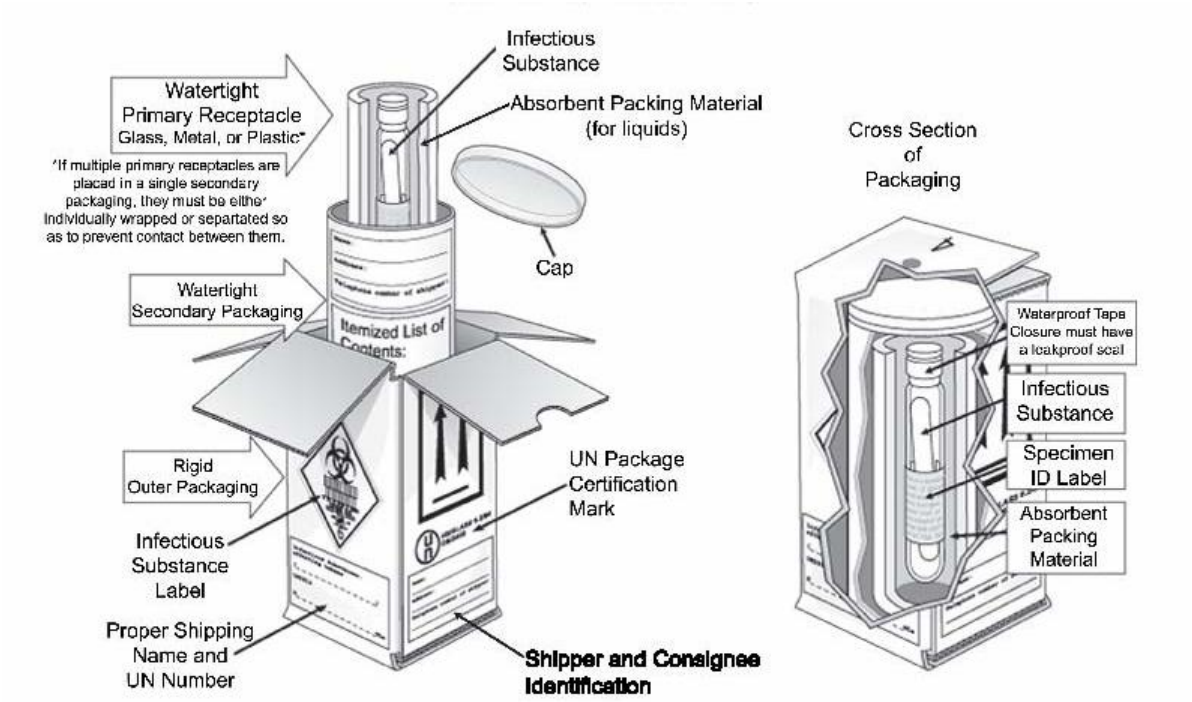
- **Etiologic agent** means a viable microorganism or its toxin which causes, or may cause, human disease.
- **Diagnostic specimen** means any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, etc., which is reasonably believed to contain an etiologic agent and is being shipped for purposes of diagnosis.
- **Biological product** means a biological prepared and manufactured in accordance with regulations that govern the manufacture of vaccines, reagents, etc.
- **Interstate shipping** means shipping across state lines within the continental United States. Intrastate shipping means shipping within the State of Virgina.

Packaging

All biological materials including diagnostic specimens and biological products that may contain an etiologic/bio-hazardous agent must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions possible with ordinary handling and transportation (passage through cancellation machines, sorters, conveyors, etc). Contents should not leak to the outside of the shipping container even if leakage of the primary container occurs.

Specific packaging requirements apply to materials that are known to contain, or reasonably believed to contain, certain etiologic agents. For such materials the following procedures apply (See Figure 1. Source: Biosafety in Microbiological and Biomedical Laboratories 5th Edition, Appendix C).

Figure 1



Volume not exceeding 50 milliliters (ml)

1. Place material in a securely enclosed, watertight primary container (test tube, vial, etc.). Enclose this primary container in a secondary, durable, watertight container. Several primary containers may be enclosed in a single secondary container as long as the total volume of material in all the primary containers enclosed does not exceed 50 ml.
2. Place absorbent nonparticulate material (e.g. paper towels, **not** sawdust or vermiculite, etc.) in the spaces at the top, bottom, and sides between the primary and secondary containers. Use enough absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage.
3. Enclose each set of primary and secondary containers in an outer shipping container constructed of corrugated fiberboard, cardboard, wood or other material of equal strength. Do not use bags, envelops, or similar materials.
4. If you package the material with dry ice, see the section below (Packaging with Dry Ice).

Volume greater than 50 ml:

1. Follow requirements for lesser volumes outlined above.
2. Place shock absorbent material at the top, bottom, and sides between the secondary container and the outer shipping container. (This material should at least equal the amount of absorbent material placed between the primary and secondary containers).
3. Ensure single primary containers contain no more than 1000 ml of material; however, two or more primary containers (combined volumes not exceeding 1000 ml) may be placed in a single secondary container. The maximum amount of etiologic agent which may be enclosed within a single outer shipping container must not exceed 4000 ml.

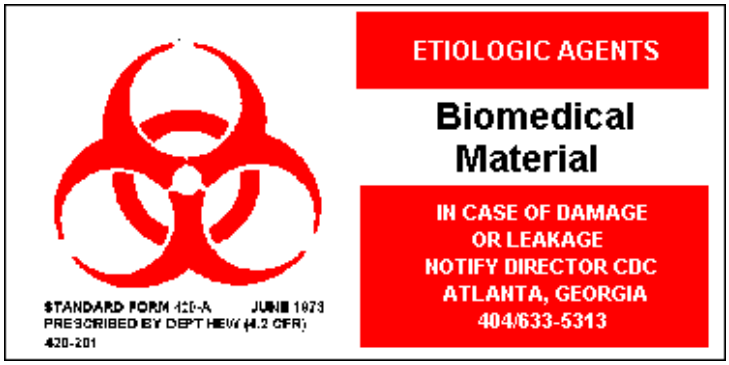
Packaging with Dry Ice

1. If used, place dry ice between the secondary and outside containers.
2. Place shock absorbent material so as to prevent the secondary container from becoming loose inside the outer container as the dry ice sublimates.
3. Use the DOT dry ice label.

Labeling

The outer shipping container of all materials containing etiologic/bio-hazardous agents which are being shipped or transported must bear a special label, as illustrated in Figure 2. These labels are available from your laboratory supply vendor.

Figure 2



Shipping and Transportation Methods and Requirements

Registered Mail or the Equivalent

For a list of etiologic agents that use registered mail or an equivalent system which provides the sender with immediate notification of receipt refer to Appendix A of the CDC Guidelines on Additional Requirements for Facilities Transferring or Receiving Select Agents and Toxins, 49 CFR Part 72.

Federal Express or UPS

1. For Federal Express/UPS shipments, internationally or domestically, follow the International Air Transport Association (IATA) Dangerous Goods Regulations. (Receipt of shipment notice is not required since the shipment is traceable through the specific carrier.)
2. Apply appropriate labels to the outer shipping container for packages containing dry ice and/or bio-hazardous substances as shown in Figures 3 and 4, respectively.
3. Contact the specific carrier's dangerous goods agent prior to shipment for any additional packaging and labeling requirements.

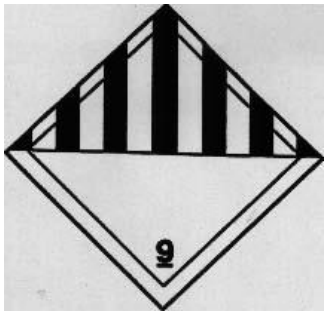


Figure 3

Figure 4

Damaged Packages

When evidence of leakage or any other damage to packages bearing an Etiological Agents/Biomedical Material label is discovered, the carrier must promptly isolate the package and notify the Director, Centers for Disease Control and Prevention (CDC), (404) 633-5313, 1600 Clifton Road NE, Atlanta, Georgia 30333.

Notice of Delivery

In the event that a package sent from RU is not received by the recipient within 5 days following the anticipated delivery of the package, the sender must notify the Director, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, Georgia 30333 or by telephone (404) 633-5313.

Importation/Exportation of Etiologic Agents

Importation of bio-hazardous agents, etiologic agents, and vectors that may contain such agents is governed by federal regulation. In general, an importation permit is required for any infectious agent known to cause disease to humans. This includes, but is not limited to, bacteria, viruses, rickettsia, parasites, yeasts, and molds. In some instances, an agent which is suspected of causing human disease also requires a permit.

Importation permits are issued by the U.S. Public Health Service (USPHS) only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

Instead of an importation permit, a Letter of Authorization may be issued by the Centers for Disease Control and Prevention after review of an "Application to Import an Etiological Agent". The letter is issued for materials that are judged to be noninfectious, but which might be construed to be infectious by U.S. Customs inspection personnel. Letters of Authorization may be issued for items such as formalin-fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine, cerebrospinal fluid, and other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent. Letters of Authorization are in effect for two years and do not require a shipping label to be issued by CDC.

Importation permits and Letters of Authorization are issued by the Biosafety Branch, Office of Health and Safety, CDC, 1600 Clifton Road, Atlanta, Georgia 30333, after review of a completed application form. Application forms

may be obtained by calling CDC at their FAX Information System. Dial 1-888-CDC-FAXX and enter document number 101000. CDC can also be contacted on the Internet at http://www.cdc.gov/od/eaipp/
Completed forms may be returned to CDC by mail or FAX at 404-639-2294. Application to CDC for the importation permit should be made 15 working days in advance of the shipment date to allow time for processing, issuance, and delivery of the permit and shipping labels to the permittee.

Other Permits

APHIS permits are required for importation or domestic shipping of infectious agents of livestock, poultry, and other animal diseases, and any materials that might contain these agents**.** Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture-grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U.S. Applications for USDA/APHIS permits may be obtained by calling the USDA/APHIS at (301) 734-3277 or through the Internet at http://www.aphis.usda.gov/permits/index.shtml
The importation or domestic transfer of plant pests is also regulated by the USDA. Such a permit is required for plant pests, plant biological agents, or any material that might contain them. Information may be obtained by calling (301) 734-3277 or through the Internet at http://www.aphis.usda.gov/plant_health/permits/index.shtml
USDA permits are required for certain live animals and all live bats. Call (800) 358-2104 for further information.
Export of infectious materials may require license from the Department of Commerce (DoC). Exporters of a wide variety of etiologic agents of human, plant, and animal diseases, including genetic material and products which might be used for culture of large amounts of agents will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at https://bxa.ntis.gov/

V. Biohazard Declaration

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, and other mammalian species and the potentially infectious agents these cells may contain. See BMBL Appendix H for specific hazards and handling recommendations.

_____

□ **Allergens**: (adjuvants, animal's dander, latex, etc.):

_____

□ **Toxins:** (bacterial, fungal, plant, etc.):

_____

□ **Recombinant DNA and related products:**

_____

□ **Clinical Specimens:**

_____

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

_____

□ **Select agents**: including and of the select agent materials on the CDC or USDA listing.

_____

VI. Recordkeeping Requirements

Standard Operating Procedures (SOPs) Manual. Laboratory SOPs for procedure involving bio-hazardous and *r*DNA 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 bio-hazardous 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.

Biological Material Safety Data Sheets. In addition to the inventories of MSDSs compiled for hazardous chemicals, all laboratories performing research with known or potential bio-hazardous agents must maintain a comprehensive collection of MSDSs for each bio-hazardous agent. Questions or concerns regarding acquisition of MSDSs of bio-hazardous materials should be directed to the Biosafety Officer.

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.

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

E. Sharps Injury Log: In accordance with the requirements of the OSHA Bloodborne Pathogens Standard, laboratories performing research involving known or suspected bloodborne pathogens must document all incidents involving potential employee exposure via sharps injury. University sharps injury logs must be obtained from Human Resources 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 on file 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 bio-hazardous agents. Medical surveillance 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 BSL-2 or greater bio-hazardous agents: annual testing/certification required.

2. Biological glove box units utilized for procedures involving BSL-2 or greater bio-hazardous agents: annual testing/certification required.

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

4. Chemical fume hoods: quarterly testing provided by RU EHS.

5. Autoclave units utilized for onsite treatment of bio-hazardous waste: monthly testing and regular documentation required.

H. Respiratory Protection Program: Research involving certain toxic chemicals and/or bio-hazardous agents 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.

APPENDIX A: Material Safety Data Sheets (MSDSs)

Attach an agent/product-specific MSDS copy for each bio-hazardous agent and/or biological toxin in use with in the laboratory. If an MSDS is not available for specific agents/toxins provide whatever hazard information is available.

APPENDIX B: **Laboratory SOPs**

Attach Laboratory Standard Operating Procedures (SOPs) for all Tasks Involving Biological Hazards and Recombinant DNA materials.

APPENDIX C: Exposure Control Plan

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

APPENDIX D: Respiratory Protection Program

Laboratory personnel who are issued respiratory protective equipment (including N-95 masks) 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 Radford University Environmental Health & Safety Office if you need assistance 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 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:_____

APPENDIX E: **Job Safety Analysis (JSAs)**

Attach Job Safety Analysis (JSAs) for all Tasks Involving Biological Hazards, Select Agents or Toxin, and or Recombinant DNA materials.