

# *Rutgers University Biological Safety Guide (BSG)*

## **Table of Contents**

- I. Culture of Responsibility and Safety**
- II. Personnel Assurance**
- III. Introduction**
- IV. Responsibilities**
- V. Biohazards: Identity and Risk Assessment**
- VI. Containment and Biosafety Levels**
- VII. Engineering Controls**
- VIII. Administrative Controls**
- IX. Work Practice Controls**
- X. Personal Protective Equipment**
- XI. Decontamination/ Disinfection and Cleaning**
- XII. Waste Disposal**
- XIII. Emergency Response**
- XIV. Health, Vaccination and Medical Clearances**
- XV. Select Agents and Toxin**
- XVI. Transportation and Permits**

## **Appendices:**

- A: Genetically Modified Plants and Organisms**
- C. Biosafety Level Two Proficiency Checklist**
- C. Regulated Medical Waste Policies**
- D. Biosafety Guidelines for Teaching Laboratories**
- E. Dual Use Research of Concern**
- F. ESCRO**
- G. Biosafety for Core Facilities: Cell Sorting, Confocal**

## **I. CULTURE OF RESPONSIBILITY and SAFETY**

All scientists are accountable for the establishment of a culture of responsibility in their labs and at their institutions. Fundamental to this culture of responsibility are scientific integrity and adherence to ethical codes of conduct. For the individual scientist, an ethical code of conduct centers on personal integrity. It embodies, above all, a commitment to intellectual honesty and personal liability for one's actions and to a range of practices that characterize the responsible conduct of research, including:

- Intellectual honesty, accuracy, fairness, collegiality, transparency in conflicts of interest or potential conflicts of interest, protection of human subjects in the conduct of research, humane care of animals in the conduct of research, and adherence to the mutual responsibilities between investigators and their research teams.

In the realm of research involving pathogens and toxins, additional responsibilities include:

- Awareness of and adherence to all safety protocols.
  - Knowledge and awareness of spill and exposure protocols.
  - Knowledge of and adherence to reporting requirements related to spills, exposures, or potential releases.
  - Knowledge and awareness of all emergency response protocols (e.g., fire, tornado, inclement weather).
  - Completion of all classroom training requirements.
  - Completion of all proficiency training requirements.
  - Completion of all Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations.
  - Immediate reporting to the Principal Investigator of any situation that compromises an individual's ability to perform as required in a BSL-2 or ABSL-2 laboratory, including physical or psychological issues.
  - Immediate reporting to the Principal Investigator and the IBC, where appropriate, of behavior or activities that are inconsistent with safety and security plans.
- Awareness of and adherence to security protocols.

The establishment of support systems is essential for the individual scientist in developing an institutional culture of responsibility. At the individual level, Rutgers University has several mechanisms to support this culture. The first mechanism is the Employee Assistance program for faculty, staff and students. The EAP is a confidential service that provides support, counseling, referrals and resources for issues that impact your life and potentially compromise your ability to



The Rutgers Biological Safety Guide (BSG) is intended to be a resource for information, guidelines, and required policies and procedures that will enable and encourage those working in the laboratory environment to work safely, and to reduce or eliminate the potential for exposure to biological hazards. The information contained in this BSG should enable researchers to conduct their activities in a manner that:

- Complies with all Federal and Local regulations for the use of biohazards,
- Prevents contamination of the environment,
- Protects their specimens and keeps other research material free of contamination,
- Conforms to prudent biosafety practices, and
- Prevents employees and their families from acquiring laboratory-associated infectious diseases.

This BSG was developed by Rutgers Environmental Health and Safety (REHS), with the input and approval of the Rutgers Institutional Biosafety Committee (IBC). Guidelines developed by the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) form the basis for the safe work practices included in this BSG. The guidelines from the CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories (BMBL) <http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf>, addresses the appropriate measures and facilities for work with all microbial agents, including bacterial, viral, fungal, parasitic, and rickettsial agents. It is important to note that these guidelines must be followed to ensure the continuation of grant funds from federal agencies.

#### **IV. RESPONSIBILITIES**

**Student/ Employee-** You are responsible for your own safety!!

- Follows safe work practices,
- Attends required training and demonstrates proficiency, and
- Is familiar with, and follows the guidelines set forth in, the University's Biological Safety Guide

**Principal Investigator / Unit Supervisor –**

- Identifies employees with occupational exposure to potentially infectious materials.
- Develops, within the framework of this guide, an IBC protocol which includes a laboratory-specific Exposure Control Plan to minimize or eliminate occupational exposure to potentially infectious materials.
- Ensures that employees follow the safety practices described in this BSG and the laboratory's ECP.
- Interacts with REHS to schedule employee training and to meet other regulatory requirements.

- Ensures that laboratory workers have acquired the technical proficiency in the use of microbiological practices and safety equipment required for the safe handling of biological materials, and have developed good habits that sustain excellence in the performance of those practices. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and Biosafety cabinets (BSC), ability to respond to emergencies, and willingness to accept responsibility for protecting one's self and others is important insurance that a laboratory worker is capable of working safely.
- Registers all biohazard and recombinant/synthetic nucleic acid experiments, including transgenic plant protocols and associated field tests, with the Institutional Biosafety Committee using the online Biosafety Protocol Management System found at <http://myrehs.rutgers.edu>.

#### **University Biological Safety Officer-**

- Reviews, updates, and audits the Rutgers University Biological Safety Guide on as needed, as well as interprets applicable federal, state, and local Biosafety regulations.
- Interacts with Principal Investigators, unit supervisors, and others to schedule training and to assist them in meeting necessary requirements.
- Reviews and administers the Institutional Biosafety Committee, and performs laboratory inspections with emphasis on various aspects of biological safety.

**Institutional Biological Safety Committee (IBC)-** Approves the Rutgers University Biological Safety Guide as needed and may set additional requirements to ensure the protection of Rutgers University employees, students, and the general public. The policies of this committee are outlined in the IBC handbook.

**Occupational/Employee Health Services, Student Health Services Department:** are responsible for ensuring that all medical actions required by the Bloodborne Pathogens Standard and other applicable Standards are performed and that appropriate medical records are maintained.

## **V. BIOHAZARDS: IDENTITY AND RISK ASSESSMENT**

### **Identification of Biohazards**

Laboratory-acquired infections have been documented since microbiology's emergence as a distinct discipline in the 19<sup>th</sup> century. The knowledge, techniques, and the equipment to prevent most laboratory infections are, however, available. The practices and tools outlined in the BMBL publication have become the basis for the safe handling of *potentially infectious materials* in all laboratory settings.

The emergence of Hepatitis B, Hepatitis C, and HIV obligate clinicians and researchers to handle clinical materials, with their undefined microbial population, as a possible source of serious infections. Universal Precautions, (treating all human blood and body fluids as if known to be infectious for HIV, HBV, and other bloodborne pathogens) is necessary for working with clinical specimens. The requirements for working with clinical specimens carry over into activities involving the use of human and non-human primate cell lines in recognition of the ability of these materials to carry adventitious viruses and other microorganisms.

## HAZARDOUS CHARACTERISTICS OF AN AGENT

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease.

Any research with recombinant or synthetic nucleic acids, biological toxins, select agents, microorganisms requiring BSL2 or higher, and human cell lines and blood products must be reviewed by the IBC prior to beginning work. The PI is responsible for ensuring compliance with the IBC before work begins. The registration form for these types of experiments is available at the REHS website, <http://myrehs.rutgers.edu>

### Risk Groups

The World Health Organization (WHO) has recommended an agent risk group (<http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf>, or <http://www.absa.org/riskgroups/index.html>) classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease. The four groups address the risk to both the laboratory worker and the community. The *NIH Guidelines* established a comparable classification and assigned human etiological agents into four risk groups on the basis of hazard. The descriptions of the WHO and NIH risk group classifications are presented in the Table below.

Agent risk groups and biosafety levels are not the same. An agent risk group is one of many different factors that contributes to assigning the biosafety level for a biological agent or toxin. Using the BMBL and other authoritative references, the IBC evaluates the hazards presented by the proposed work and then determines the appropriate biosafety level.

**Table 1: Microorganism Risk Groups**

Risk Group Classification	NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA	WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004
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MOLECULES 2009		
Risk Group 1	Agents that are not associated with disease in healthy adult humans.	(No or low community risk) A microorganism that is unlikely to cause human or animal disease.
Risk Group 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions <i>may be</i> available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are <i>not usually</i> available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive

Source: [BMBL, 5<sup>th</sup> Edition](#)

## Risk Assessment

Risk implies the probability that harm, injury or disease will occur. In the biomedical laboratory, the assessment of risk focuses on the prevention of lab-acquired illnesses (LAI). In other laboratories, risk focuses on the release of genetically modified organisms into the environment, or the release of an exotic pest/ plant into the ecosystem. Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a LAI, and the probable consequences of such an infection.

The information identified by risk assessment will provide a guide for the selection of appropriate BSL, microbiological practices, safety equipment, facility safeguards, medical monitoring and post exposure prophylaxis that can prevent release into the environment and LAIs.

## Performing a Risk Assessment

1. **Identify agent hazards and perform an initial assessment of risk** - Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible host, severity of disease, and the availability of preventive

measures and effective treatments. For recombinant and synthetic nucleic acids, assess the nature of the expressed protein and the nucleic acid of interest.

2. **Identify laboratory procedure hazards** - The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.
3. **Make a determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment** - The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards.

There will be situations where the intended use of an agent requires greater precautions than those found in an authoritative reference. These situations will require the careful selection of additional precautions. An obvious example would be a procedure for exposing animals to experimentally generated infectious aerosols or the planting of a transgenic plant.

4. **Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.** The protection of laboratory workers, other persons associated with the laboratory, and the public will depend ultimately on the laboratory workers themselves. In addition, the PI should ensure also ensure that the necessary safety equipment is available and operating properly. For example, a BSC that is not certified represents a potentially serious hazard to the laboratory worker using it and to others in the laboratory. The director should have all equipment deficiencies corrected before starting work with an agent.
5. **Submit the preliminary risk assessment to REHS and the IBC for review.** A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial and sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*. Review of potentially high risk protocols by the local IBC is standard practice at Rutgers.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards.

Risk assessments for biohazardous agents are not straightforward and will depend on several factors. First, an assessment of risk must start with collecting information about the agent, including Agent Hazard Class (from the NIH Recombinant and Synthetic Nucleic Acid Guidelines) and Recommended BSL from the BMBL 5<sup>th</sup> Edition. In addition, all of the other



factors below need to be evaluated before a final determination for the safe use of the agent is made.

### **Pathogenicity**

The ability of a pathogenic agent to cause disease in humans, animals or plants, including disease incidence and severity (e.g., an agent that causes mild illness versus an agent that causes high mortality or an agent that might cause an acute self-limiting disease versus one that might cause a chronic disease) must be evaluated.

### **Route of Transmission**

The predominant probable routes of transmission in the laboratory are 1) direct skin, eye or mucosal membrane exposure to an agent; parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors; 3) ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure; and 4) inhalation of infectious aerosols.

An agent capable of transmitting disease through respiratory exposure to infectious aerosols is a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. This hazard requires special caution because infectious aerosols may not be a recognized route of transmission for the natural disease. Additionally, one must consider the inhalational effective dose, viability in aerosol form, the aerosol concentration and overall particle size.

When work involves the use of laboratory animals, the hazardous characteristics of zoonotic agents require careful consideration in risk assessment. Evidence that experimental animals can shed zoonotic agents and other infectious agents under study in saliva, urine, or feces is an important indicator of hazard.

Dissemination of seeds is important to consider when working with transgenic plants. Consideration of capable interbreeding and nearby crops is important to the risk assessment.

### **Agent Stability**

The agent's ability to survive over time in the environment is defined as agent stability. Factors such as effects of sunlight (UV rays), chemical disinfectants, drying, buffering effects (pH) and the ability of the agent to form spores can all affect its stability and the infectivity and transmissibility of the agent.

## **Infectious Dose**

The infectious dose (ID) is defined as the number of individual units required to cause an infection or illness. The ID should be considered for each route of possible infection by the specific microorganism, as these doses can differ.

## **Origin**

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 composition and nature of local plant and ecological species is important to consider due to concerns regarding the release of a noxious weed or invasive species into the environment.

## **Genetically Modified Microorganism**

Microorganisms, plants and animals may be genetically modified, either through the deletion or addition of genes. It is important to determine if the genetic modifications alter the pathogenicity, stability, route of transmission, or infectious dose of the microorganism and GM plants and animals are also highly regulated to protect the environment. The genetic modifications, if applicable, should be included in the risk assessment, and differences from the wild type strain should be delineated.

**All of the above factors are inherent to a particular microbe; external factors to be considered in a risk assessment include:**

### **Concentration**

Number of infectious organisms per unit volume. Consider the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the lab activity (e.g., agent amplification, sonication, or centrifugation).

### **Host Factors**

In order for a microbiological agent to be a pathogen it requires a susceptible host. The agent must be able to infect a suitable host. There are many microbial agents that are pathogenic for animals or plants but not for humans. There are some microbial agents that are species specific and will only infect a particular species of animal. A laboratory worker's *immune status* is also directly related to his/her susceptibility to disease when working with an infectious agent. Any personal health information will be kept confidential and protected in accordance with federal law.

### **Health Status of at-risk Employees:**

The health status of the host is also critical for determining the risk of an agent. Many pathogenic agents pose a higher risk to pregnant women, young children, the elderly and individuals who are immuno-compromised. Workers with a possible immune-compromising condition are encouraged to self-report to their Occupational/ Employee/ Student Medicine Services. Self-reporting will allow for proper monitoring during work to ensure protection from LAI.

### **Availability of an effective prophylaxis or therapeutic intervention**

In some instances, immunization may affect the BSL required. Immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Work with a pathogenic agent may need to be carried out at a higher level because there are no effective treatments available for the pathogenic agent or the agent may be resistant to normal treatment regimens (e.g., multi-drug resistant *Mycobacterium tuberculosis*).

For persons working with animals and human blood lines or blood products, the Hepatitis B vaccination or proof of immunity is required prior to beginning work or contact with these materials. The Hepatitis B vaccine is important in preventing laboratory/ clinically acquired Hepatitis B infection.

Additionally, serum banking should be considered as a way to have a baseline in case of exposure to an infectious agent in the laboratory. Laboratories should have a Standard Operating Procedure (SOP) for post exposure prophylaxis (PEP) in case of an accidental exposure. Vaccine availability should also be considered during the risk assessment, and it should be determined by the PI in conjunction with REHS and Occupational Health as to whether these vaccines would be mandatory or optional before beginning work.

### **Evaluation of Skill Level of Employees**

The PI should determine the educational and skill level of the employee/student and their ability to implement safe work practices with the pathogenic agent. If there are additional trainings needed the PI must ensure the employee/student receives those trainings before performing laboratory work. Those working in animal facilities or in a BSL3 or ABSL3 are required to undergo specific trainings for those facilities in addition to those listed earlier in the BSG. An individual must demonstrate proficiency at the lower biosafety level(s) during the training process prior to “graduating” and being permitted to work at the next higher level (e.g., demonstrate ABSL2 proficiency before working at ABSL3). Unless personnel have met all

training requirements in a proficient manner, they should not be performing experiments at that BSL.

### **Animal studies**

Laboratory studies involving animals may present many different kinds of physical, environmental, and biological hazards. The specific hazards present in any particular animal facility are unique, varying according to the species involved and the nature of the research activity. The BMBL outlines a set of four biosafety levels for work with vertebrate animals infected with agents that may infect humans. These Animal Biosafety Levels (ABSL) 1 thru 4, provide for practices, equipment, and facilities that are comparable to the laboratory biosafety levels. There are unique hazards associated with infected animals that must be understood by those personnel with animal contact and must be addressed by the animal facility.

### **Greenhouse or Field Studies**

The growing of plants or use of genetically modified crops in a greenhouse or field application has potential risk to the surrounding plant community and the environment. Pollen and seeds from these plants have the potential to be spread by wind, insects and other factors that must be controlled. These applications require permits but also a detailed risk assessment for the genetically modified material, the risk to the surrounding environment and appropriate containment for plant pathogens and pests to avoid release into the environment. Risk to workers is low, but the risk to the environment is high.

### **Facility and Safety Equipment**

The laboratory BSL and the presence of secondary containment is important to consider when making a risk assessment. If the research requires BSL2 containment, then a BSC is required and must be certified within the past year to ensure proper operation. Additional measures to consider would be the type of experiments being performed. This would include the use of sharps and whether safer sharp devices are needed. Additionally, the type and method of disinfection/ sterilization of liquid and solid wastes is important to be sure all potentially infectious materials/ pathogens are properly inactivated. In addition to the BSC, fume hoods and centrifuges with safety cups are common secondary containment devices. The centrifuges with safety cups function to prevent aerosolization. All work resulting in possible aerosolization must be performed in the BSC, e.g. sonication. Carrying out experiments at the appropriate BSL will contribute to decreasing the overall risk by the appropriate measures.

Personal Protective Equipment (PPE) is important when considering risk, and the proper PPE should be worn. PPE at BSL2 includes lab coats, and the appropriate gloves, either nitrile or powder free latex and protective eyewear.

## VI. CONTAINMENT AND BIOSAFETY LEVELS

### Containment

The goal of biosafety engineering is to prevent laboratory worker exposure to infection or injury and also to prevent exposure of biological materials to the environment. This is achieved by utilizing primary and secondary barriers. "**Containment**" describes safe methods for managing infectious materials in the laboratory environment where they are handled or maintained. The overall purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The elements of containment include **laboratory practice and technique, safety equipment, and facility design**. A risk assessment of the work to be performed with a specific agent will determine the appropriate combination of these elements:

The three elements of containment include:

**Laboratory practice and technique** are considered the most important element of containment. The basis is strict adherence to standard microbiology practices and procedures. Awareness of potential hazards and related information gained from training or academic experience is an equal partner with hands-on proficiency in empowering an individual to work safely.

**Safety equipment** includes Biological Safety Cabinets (BSCs); "sharps" containers; centrifuge safety cups or sealed rotors; and, other devices designed to remove or minimize exposures to hazardous materials. BSCs also provide protection from contamination for research materials used in them.

**Facility design and construction** (secondary barriers) refers to building features that, for research personnel, enhance the protection provided by safety equipment and provide a barrier to protect persons outside of the laboratory and the environment from infection. Examples include separation of work areas from general access, availability of autoclaves, and handwashing facilities.

Containment can be categorized as "Primary" or "Secondary":

**Primary containment** refers to protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment (e.g., engineering controls such as biosafety cabinets, other enclosure devices, and centrifuge safety cups). The use of vaccines may provide an increased level of personal protection.

**Secondary containment** refers to protection of the environment from exposure to infectious materials and is provided by a combination of facility design and operational practices.

## Biosafety Levels

Microorganisms and clinical materials are assigned to one of four Biological Safety Levels (BSLs). Each BSL consists of combinations of safety equipment, facility design features, and laboratory practices and techniques that will reduce the risk of laboratory-acquired infections and prevent release of the agent to the environment. The recommended Biosafety level(s) for some organisms can be found in [Section VII](#) (Agent Summary Statements) of the BMBL. These BSLs represent the conditions under which the agent ordinarily can be handled safely. The primary and secondary features for each BSL are outlined in Table H.1 (below).

Generally, work with known agents should be conducted at the biosafety level recommended in the BMBL. When the BMBL does not contain agent-specific guidance for a particular organism, please consult other sources for the appropriate containment level and risk classification, such as the American Biological Safety Association (ABSA) Risk Group Database (<http://www.absa.org/riskgroups/index.html>) and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). The IBC may specify practices that are more (or less) stringent than the BMBL or other guidance documents when specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered.

It is the responsibility of the PI or laboratory director to ensure that all lab personnel adhere to the biosafety level and containment requirements approved by the IBC.

**TABLE 2 - Biosafety Levels and Appropriate Protective Measures**

<b>BSL</b>	<b>Agents</b>	<b>Practices</b>	<b>Safety Equipment (Primary Barriers)</b>	<b>Facilities (Secondary Barriers)</b>
<b>1</b>	Not known to consistently cause disease in healthy adults	- Standard Microbiological Practices	None required	- Open bench tops - Sink required for hand-washing
<b>2</b>	Associated with human disease, hazard =	BSL-1 practice plus:	Primary barriers = Class I or II BSCs or other physical	BSL-1 plus:

	percutaneous injury, ingestion, mucous membrane exposure	<ul style="list-style-type: none"> <li>- Limited access</li> <li>- Biohazard warning signs &amp; labels.</li> <li>- "Sharps" precautions</li> <li>- Biosafety manual defining any needed waste decontamination or medical surveillance policies</li> </ul>	containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	- Autoclave available
<b>3</b>	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: <ul style="list-style-type: none"> <li>- Controlled access</li> <li>- Decontamination of all waste</li> <li>- Decontamination of lab clothing before laundering</li> <li>- Baseline serum</li> </ul>	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> <li>- Physical separation from access corridors</li> <li>- Self-closing, double-door access</li> <li>- Exhausted air not recirculated</li> <li>- Negative airflow into laboratory</li> </ul>
<b>4</b>	Dangerous/ exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: <ul style="list-style-type: none"> <li>- Clothing change before entering</li> <li>- Shower on exit</li> <li>- All material decontaminated on exit from facility</li> </ul>	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs <u>in combination with</u> full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: <ul style="list-style-type: none"> <li>- Separate building or isolated zone</li> <li>- Dedicated supply and exhaust, vacuum, and decon systems</li> </ul>

## VII. ENGINEERING CONTROLS

"Engineering Controls" refers to devices or processes that isolate or contain a hazard. The best engineering controls function in an automatic manner with limited user input. When available, they are given a higher priority than personal protective equipment or work practices because these two control methods are subject to human error or material defects.

### Biological Safety Cabinets (BSCs)

Biological Safety Cabinets (BSCs) are one type of engineering control. BSCs are designed to provide personnel, environmental and product protection, from aerosolized microorganisms, when appropriate practices and procedures are followed. High efficiency particulate air (HEPA) filters are used in the exhaust and/or supply systems of biological safety cabinets. HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases.

Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research, clinical, and safety needs, and care should be exercised in choosing the appropriate BSC for the intended use of the materials. Tables 10.I.2 and 10.I.3 describe the differences between the various types of cabinets. REHS should be consulted if any questions arise in the selection of appropriate containment equipment.

**Table 3 - Selection of a Safety Cabinet Using Risk Assessment**

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL 1-3	YES	NO	YES	I
BSL 1-3	YES	YES	YES	II (A, B1, B2, B3)
BSL 4	YES	YES	YES	III B1, B2

**Table 4 - Comparison of Biosafety Cabinet Characteristics**

BSC	Face Velocity	Airflow Pattern	Applications	
			Nonvolatile	Volatile Toxic
I	75	In at front; exhausted through HEPA to the outside or into the room through HEPA	Yes	Yes <sup>1</sup>



II, A	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a thimble unit.	Yes	No
II, B	100	Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter.	Yes	Yes <sup>2</sup> (minute amounts)
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter.	Yes	Yes (small amounts)
II, B3	100	Same as II, A, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter	Yes	Yes <sup>2</sup> (small amounts)
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series	Yes	Yes (small amounts)

(1) Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I cabinet in to a room should not occur if volatile chemicals are used.

(2) In no circumstances should the chemical concentration approach the lower explosion limits of the compound.

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**CAUTION:** Be aware that 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 the need for use of the biological safety cabinet or other physical containment device. Aerosols can be generated by manipulation of liquids, tissue fragmentation, preparation of bacterial plates or the improper use of laboratory equipment including centrifuges, or breakage of containers with cell cultures. Procedures with a potential for **creating infectious aerosols** or splashes may include:

Centrifuging  
Grinding  
Blending

Sonic disruption  
Opening containers of infectious materials  
Inoculating animals intranasally

Vigorous shaking or mixing  
Pipetting  
Pouring of cultures

Harvesting infected tissues from animals or  
embryonated eggs  
Cell sorting of infectious materials

Such materials may be centrifuged in an 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.

**Laminar Flow Cabinets** are not biological safety cabinets. They are devices that use a HEPA to filter air going into the cabinet. It is used to protect the product from contamination and **offers no protection to the worker** from the product.

There are two types of laminar flow cabinets: Horizontal Laminar Flow and Vertical Laminar Flow Cabinets. Since these cabinets blow air into the breathing zone of the worker, these cabinets must **never** be used when handling hazardous chemicals, potentially infectious materials including human cell lines, or during cage changes in the vivarium.

### **Effective and Safe Use of Biological Safety Cabinets**

1. Check the certification due date. Each BSC must be certified upon installation, yearly, after a cabinet is moved, the HEPA filter is changed, or when there is a possibility that servicing may have affected the containment ability. Semi-annual certification is recommended when cabinets are routinely used for work with airborne-transmitted organisms or other high-risk pathogens.
2. Turn the Biosafety Cabinet “on”. Regularly check the Magnehelic gauge or the digital display indicating the air pressure drop across the HEPA filter; a sharp change in pressure may indicate a malfunction. If a significant change is noted, contact the company that does the annual certifications to service the unit.
2. Allow cabinet to run for at least 4 minutes prior to starting work; Turn off UV lights when the cabinet is in use. These lights usually contain mercury and they must be disposed through REHS's hazardous waste program.
3. Close the room door when working in a BSC located near the laboratory entrance because drafts may interrupt and compromise the cabinet's airflow pattern.
4. Do not block front air intake grill and minimize the amount of material inside the cabinet; this compromises proper air flow through the unit. Place equipment and supplies on the side of the cabinet.

5. Protect vacuum lines: Aspirator bottles should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter.
6. Cover work surface with absorbent pad (moistened with disinfectant for high risk activities), making sure not block front or rear grilles. Conduct activities 4-6 inches from the front of the cabinet; avoid rapid arm movements.
7. Locate BSCs in low-traffic areas and away from supply ventilation grilles and doors (e.g., locate more than 10 ft from a doorway); drafts produced by these situations may disrupt the protective airflow.
10. Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag.

Volatile Chemicals - Only BSCs that are exhausted to the exterior of the building should be used when working with volatile toxic chemicals. Class I cabinets may be connected to the building exhaust system or have air recirculated back into the room depending on use. Varying quantities of these materials may be used in Class II (Type B) cabinets because these devices are ducted to building exhaust systems (see Tables 10.2 and 10.3). When using large amounts of volatile chemicals, a chemical hood should be used. Chemical hoods are connected to an independent exhaust system and operate with single pass air which is ducted directly outside the building.

### **Decontamination of Biological Safety Cabinets**

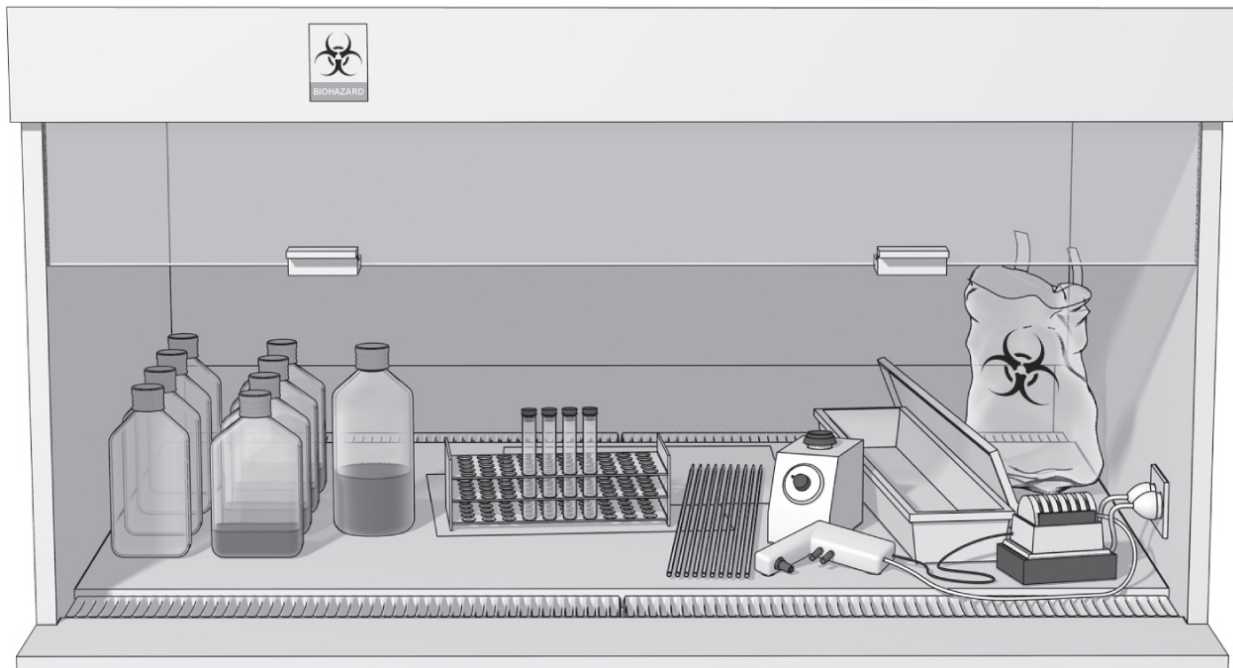
Biological safety cabinets must be decontaminated by an outside vendor before being moved. BSCs must also be decontaminated before HEPA filters are changed or internal repair work is done. The outside vendor uses procedures as required by National Sanitation Foundation Standard 49.

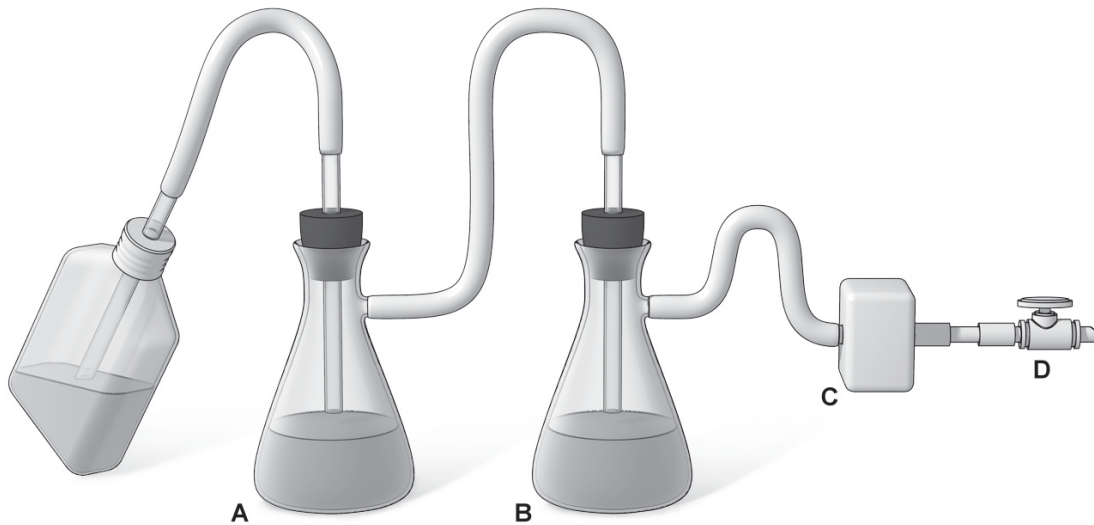
The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Note: The use of 95% alcohol as a general disinfectant should be discouraged because of flammability issues.

The surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of culture.

**Figure 1 - Working “Clean to Dirty” Within a Class II BSC**

In order to minimize arm movement in and out of the cabinet, place all needed materials in BSC at the start of procedures, arranging them to so that 'dirty' items do not pass over 'clean' ones. Clean cultures (left) can be inoculated (center); contaminated pipets can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right).





**Figure 2. Vacuum Line Protection:** (A) Collection Flask With Disinfectant; (B) Back-Up Flask (optional); (C) In-line HEPA filter; and (D) Connection to Building Vacuum.

### **Eliminate Use of Gas Bunsen Burners within a BSC**

1. The use of disposable inoculating supplies combined with the sterile atmosphere of the BSC should eliminate the need for heat decontamination throughout the procedure.
2. Open flames are not necessary in a biological safety cabinet. An open flame in a BSC creates turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface. Heat from bunsen burners may also damage HEPA filters. When absolutely necessary, touch-plate microburners may be used. Use extreme caution to ensure that the gas is not on when the burner is not lit. If this occurs turn off the gas and wait at least 10 minutes for the gas to dissipate before trying to light the burner.
3. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Be sure to check the melting temperature of the material and the temperature of the unit before use.
4. Flammable chemicals should not be used in Class II, Type A1 or A2 biosafety cabinets since vapor buildup inside the biosafety cabinet presents a fire hazard and their motors are not spark-proof. Check the manufacturers recommendations

on the use of flammable materials in your biosafety cabinet. The picture below is an example of what happens when there is a fire in a biosafety cabinet.

## VIII. ADMINISTRATIVE CONTROLS

### Signage, Posting and Labeling Requirements

Biological hazard signs/labels must be posted where work with biohazardous materials is performed or where biohazardous materials or wastes are stored. The signs/labels are used as a means to prevent accidental exposure, injury or illness.

- The caution sign on the front door of the room must have labels bearing the signal word "**BIOHAZARD**" or "**BIOLOGICAL HAZARD**", the universal "**BIOHAZARD**" symbol, the **BIOSAFETY LEVEL** of the room as well as any special precautions or requirements for entering the area, and the name and telephone number of the responsible person (Principal Investigator or Director).
- Biological hazard warning tags or labels must be used to identify containers, equipment and other items which may be contaminated through normal use of biohazards
- These labels or tags should be affixed as close as safely possible to the container or equipment by a positive means such as string, wire or adhesive that prevents their loss or unintentional removal.

The following lists examples of equipment and places where biohazard warning labels are to be affixed:

- At entrances to areas where biohazards are used;
- At entrances to areas where biohazards are stored;
- On refrigerators or freezers where biohazards are stored;
- To containers of infectious waste;
- To containers of biohazardous material;
- To the outside of packages in which biohazards are shipped;
- To containers used to store or transport biohazards;
- To cages of animals containing biohazards;
- On equipment which may be potentially contaminated with biohazardous material (e.g., centrifuges, incubators, biosafety cabinets, homogenizers, vortices, etc.); and

- To any item which may be potentially infectious (e.g., animal cages if moved outside of biohazard areas, used sharps, used containers which may have contained biohazards).

## IX. WORK PRACTICE CONTROLS

### Hand Hygiene

Handwashing is the single most important means of preventing the spread of infection, and in laboratory settings many documented laboratory-acquired infections (LAIs) have been attributed to touching mucous membranes (e.g., eyes, nose, mouth) with contaminated hands or gloves. Hands must be washed, even after gloves are used, because microscopic imperfections or tears in the glove may exist and result in contaminated hands. Additional examples of when handwashing should occur include, but are not limited to, the following:

- After completing a procedure
- After handling any blood, body fluid, tissue, or other potentially infected material
- Between all research material contacts
- Immediately following the removal of gloves or other protective equipment
- After handling animals.
- Whenever hands are soiled
- After the use of the toilet
- After coughing or blowing your nose
- Before eating, drinking, applying cosmetics, handling contact lenses, or smoking.
- Before leaving the lab

Handwashing tips:

- Routine handwashing for visibly soiled hands consists of the use of soap, running water and friction for at least 15 seconds.
- Handwashing facilities are readily available in all areas where exposures may occur.
- Handwashing facilities must be provided at a reasonable proximity to employees normal work area.
- Fingernails should be kept to less than ¼ of an inch long.
- Hand jewelry should be kept at a minimum (e.g., wedding band) in laboratory areas to enhance hand hygiene and to avoid puncturing gloves. Bracelets and other hand/ arm jewelry are not recommended.
- Hand sanitizers (such as alcohol gels) are not a substitute for handwashing. Running water and soap are needed to properly clean and decontaminate the hands and skin. Hand sanitizers may be used in certain remote field settings, but employees must wash their hands with soap and water as soon as possible after returning from the field.

## **X. PERSONAL PROTECTIVE EQUIPMENT**

It has long been recognized that working with biohazardous materials may be inherently dangerous. OSHA requires that personal protective equipment be provided to employees to control their exposure to hazardous materials.

### **Selection of Appropriate PPE**

It is the responsibility of the employer to determine, based upon a risk assessment, the appropriate PPE required to perform the work safely, to provide equipment that is in good working order and to ensure that training is conducted. Once these hazards have been determined and the appropriate PPE chosen, the employee is required by law to wear the appropriate PPE. The employee must:

- Understand the biohazard procedure or process, the nature of the biohazard and the need for the equipment;
- Be trained in the use of the PPE, be familiar with the limitations of the equipment and be fit tested for respirators (if respiratory protection is required) to ensure a proper fit;
- Understand that it is his/her responsibility to correctly use the PPE provided in the function of his/her work.

### **Types of PPE**

The types of Personal Protective Equipment may include but are not limited to:

Protective Outerwear:

- Laboratory coats, disposable gowns, Tyvek coveralls, or other type of uniform must be worn when in the laboratory area to protect individuals from contact with infectious, toxic materials and/or physical hazards.
- Long sleeves are required on all lab clothing; Laboratory coats and gowns must be fully fastened (closed). Lab coats must not be worn outside of the laboratory if they were used during work with infectious materials.
- Wear coats that are resistant to liquid penetration for activities with splash potential or use a plasticized apron. For high risk activities, use a rear-fastening lab coat/gown/tyvek coverall.

Eye Protection:



- At a minimum, safety glasses with permanently affixed side-shields should be worn in the laboratory area, if potential for aerosols is present. Face shields or goggles may be required as additional protection based on the task being performed (with a potential for aerosol, splashes sprays or aerosols) as well as any additional hazards which might also be present (e.g., corrosives).
- Safety glasses with side shields: the minimum level of protection of handling any hazardous material;
- Goggles: for activities with any splash hazard or when working with organisms transmissible through mucous membrane exposure; and
- Goggles with a face shield: when an elevated risk of large quantity splashes exists.

#### Hand Protection:

- Gloves must be worn whenever handling clinical specimens, human blood or body fluids, culture dishes or other equipment potentially contaminated with BSL-2 or BSL-3 pathogens, infected animals or infectious waste.
- The type of glove that can be selected ranges from rubber gloves for minimum protection to other types of gloves (e.g., nitrile gloves) for maximum protection against bloodborne pathogens, animals or other types of physical hazard. It should be noted that for those individuals with latex allergies, nitrile gloves may be used for protection against biohazards. Those who prefer latex should use only powder-free gloves that are designated "low protein" by the manufacturer. Corrosives and solvents may penetrate latex or nitrile gloves or diminish their protective ability; it may be necessary to stock more than one type of glove for the full range of a laboratory's activities.
  - Natural Latex or Rubber Gloves (No powder): Provide protection from most water solutions of acids, alkalis, salts, and ketones. These gloves have excellent wearing qualities, pliability, and comfort and are a good general-purpose glove. Nitrile gloves are recommended over latex gloves due to the increasing number of latex allergies.
  - Nitrile Rubber Gloves: Provide protection from chlorinated solvents (trichloroethylene, perchloroethylene). They are intended for jobs requiring dexterity and sensitivity, yet they stand up under mechanical use even after prolonged exposure to substances that cause other glove materials to deteriorate. They also resist abrasion, puncturing, snagging, and tearing.
- When using any glove with infectious materials:

- Check for visible tears and other defects.
- Do not allow rings or other jewelry to rip gloves.
- Protective ability diminishes as gloves are worn due to stretching and abrasion; change gloves regularly or as soon as possible if they are overtly contaminated.
- Wash hands immediately after removing gloves.
- Remove gloves when leaving the laboratory; even if they are "clean". The presence of gloves outside the clinic or laboratory setting justifiably worries other building occupants.
- Proper packaging and transport of infectious materials should eliminate the perceived need to wear gloves when transporting infectious materials on campus.

Respiratory Protection (mucous membranes and lungs):

- Surgical masks will help prevent ingestion and protect the mucous membrane of the nose and mouth from splashes. They do not provide protection against inhalation of organisms transmitted by aerosols.
- Respirators are used when there is the risk of airborne exposure to infectious organisms, specifically BSL3 organisms (ex. *Mycobacterium tuberculosis*) that can be transmitted by inhalation. Respirators may only be worn after the employee has been medically certified, trained, and fit tested. These services can be arranged through the campus Employee Health/Occupational Medicine Services and REHS.

## **X. DECONTAMINATION/CLEANING/DISINFECTION**

The Principal Investigator and the laboratory staff are required to develop and implement a written schedule for cleaning and decontaminating work surfaces. All work surfaces and equipment that come into contact with blood, body fluids, and any infectious agent or materials must be disinfected daily, upon completion of work, with an appropriate disinfectant. Additionally, work surfaces and equipment must be disinfected after any overt spill. Work surfaces should be covered with plastic-backed absorbent toweling to facilitate clean up and reduce production of aerosols as a result of the spill. Spills within work areas are to be cleaned up by laboratory or research personnel.

### **Disinfection**

Disinfection encompasses a continuum of outcomes in terms of the types of microorganisms destroyed. Microorganisms can be grouped as following in terms of decreasing resistance to disinfectants: bacterial endospores (*Bacillus spp.*, *Clostridium spp.*); mycobacteria; nonlipid or small viruses (poliovirus, rhinovirus); fungi; vegetative bacteria; and, lipid or medium sized virus (herpes simplex, HIV, HBV).

Table 5 is a framework for the selection of the appropriate disinfectant. The label on commercial products will note the types of action of the disinfectant, (e.g., ‘tuberculocidal’, ‘sterilant’). These claims may not appear on the label unless the manufacturer has submitted data to the EPA supporting such claims. The lists of EPA registered disinfectants can be obtained from your campus REHS office or found at. <http://www.epa.gov/oppad001/chemregindex.htm>.

Note that the EPA does not independently audit such results and research indicates that in real life situations some products do not perform as claimed. This results from manufacturers testing their products in best-case situations, e.g., on a smooth surface, at an optimal pH, in a buffer solution instead of a solution containing organic material which partially inactivates some disinfectants. For high risk pathogens, investigators may wish devise their own test to confirm a product’s claim.

- Follow label instructions regarding dilution and contact time necessary to achieve the desired level of disinfection.
- Disinfectants that require pre-use dilution should be treated as hazardous chemicals during mixing.
- Wear a lab coat and goggles, not glasses.
- Select a glove that provides protection against permeation by the disinfectant (e.g., glutaraldehyde rapidly penetrates some latex gloves).
- Decontaminate work surfaces with an appropriate disinfectant after completion of procedures, immediately when overtly contaminated, after any spill of blood or other potentially infectious materials, and at the end of the work shift when surfaces have become contaminated since the last cleaning.
- Remove and replace protective coverings such as plastic wrap and aluminum foil when contaminated.
- Inspect and decontaminate, on a regular basis, reusable receptacles such as bins, pails, and cans that have a likelihood for becoming contaminated. When contamination is visible, clean and decontaminate receptacles immediately, or as soon as feasible.
- Discard all regulated waste according to the REHS Biological and Medical Waste Disposal Policy.
- Contact REHS at 848-445-2550 with any questions.

### **Considerations for selecting and using disinfectants**

**Nature of surface** - Rough surfaces will require a longer contact time for effective treatment.

**Surface compatibility** - Bleach will corrode many metals, rinse with water after use; instruments vary in their ability to withstand disinfectants based on their composition.

**Organic matter** will inactivate some disinfectants; a second application may be necessary once visible contamination (and hence, most organic debris) has been removed.

**The removal of visible soil may be the single most critical factor in assuring effective decontamination.**

**Resistance of microorganisms**, e.g. bacterial endospore vs. vegetative bacteria.

**Contact time** necessary for desired level of decontamination.

Select the disinfectant with the **lowest toxicity** possible.

**Number of microorganisms** present need to be considered. Is it an overnight culture vs. a recently inoculated one? A 1/10 dilution of household bleach, prepared fresh daily, will suit most disinfectant needs. These solutions lose potency over time and should be prepared fresh daily.

Table 5 - Summary of Disinfectant Activities

Disinfectant	Disinfection Level	Bacteria	Lipophil. Viruses	Hydrophylic Viruses	M.tuberculosis	Fungi	Comments
Alcohols(ethyl and isopropyl) 60-85% e.g. Rubbing Alcohol (Isopropanol), Ethanol (diiuted to 70%)	Intermediate	+++	+		+++		Not sporicidal; evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish efficacy; flammable
Phenolics (0.4-5%) e.g. Vesphene, Matar, Hil-Phene, Midro-Bac	Intermediate	+++	+	+	+	+++	Not sporicidal; phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue
Glutaraldehyde (2.5%)	High	+++			+++	+++	Used to sterilize surgical instruments that can not be autoclaved; strong odor; sensitizer; use with adequate ventilation. Not for use on environmental surfaces.
Quaternary Ammonium (0.5-1.5%) e.g. A-33, Mikro-Quat, Rocaal-D	Low	+	+	=	=	+/-	May be ineffective against <i>Psuedomonas</i> ; recommendation limited to environmental sanitation (floors, walls). Low odor, irritation.
Iodophors (30-1000 ppm iodine) e.g. Tincture/ Povodine, Wescodyne, Mikroklene, Isoprep	Intermediate	+	+	+	+/-	+/-	Inactivated by organic matter.
Chlorine (100-1000ppm) e.g. Clorox, Clidox, Chloramine T	Intermediate	+	+	+	+/-	+	Not sporicidal; inactivated by organic matter; fresh solutions of hypochlorite (chlorox) should be prepared weekly; corrosive; irritating to eyes and skin.

## **XII. Waste Disposal**

**Disposal of Biological Waste and Medical Waste** – The Rutgers Biological and Medical Waste Disposal Policy (see Appendix C) provides guidance for proper collection, disinfection and disposal of all biohazardous and potentially infectious materials, ranging from clinical waste to laboratory pathogens. In addition to this policy, laboratories must follow the disinfection methods written in their approved Institutional Biosafety Committee (IBC) protocol. Any deviations from the policy or IBC protocol must be approved by REHS and/or the IBC.

Locations covered by the policy:

- Clinical Areas
- Animal Facilities
- Biological Safety Level 1 (BSL1) Laboratories
- Biological Safety Level 2 (BSL2) Laboratories
- Biological Safety Level 3 (BSL3) Laboratories
- Non-Biological Laboratories

Materials covered in the policy:

- Recombinant or synthetic nucleic acid molecules,
- Genetically engineered organisms,
- Non-human primates (NHPs) or NHP tissue/cells,
- Human cell culture (including established human cell lines),
- Human materials, tissues/organs,
- Pathogenic microorganisms (including BSL1),
- Human blood or blood products,
- Other potentially infectious material (OPIM) from humans or NHPs,
- Sharps (including glass slides, coverslips, Pasteur pipettes, unused sharps, and syringes without needles),
- Regulated medical waste, and
- Over-classified medical waste.

**Disposal of Recombinant and Synthetic Nucleic Acid Waste** – these wastes are to be treated as biohazardous and require decontamination prior to disposal. The same techniques and equipment shall be used when decontaminating recombinant and synthetic nucleic acid waste as when decontaminating biohazardous waste (Please refer to the section on biohazardous waste disposal, above). Although generally non-infectious, biosafety level 1 recombinant DNA waste requires adequate decontamination prior to disposal. Similarly, transgenic plant materials require adequate “decontamination” prior to disposal.

**Mixed Wastes** – Certain research protocols may generate hybrid waste materials that are mixtures of two or more categories of waste: biological, chemical and/or radioactive wastes. Please contact REHS at 848-445-2550 to discuss the requirements for collection and decontamination of mixed wastes prior to their generation.

Please contact the University Biological Safety Officer with any specific questions concerning biohazardous waste management and/or to arrange a sterility challenge for your autoclave.

### **XIII. Emergency Response**

Laboratories and clinics that are handling biohazardous or infectious materials should assume that accidents will occur and must become familiar with biological hazard spill response procedures found within the Rutgers University Emergency Action Plan (EAP) which can be viewed at: <http://emergency.rutgers.edu>. Certain groups may be required to develop unit-specific or agent-specific spill response plans for safely managing spill events.

#### **General Procedures**

- Alert people in immediate vicinity to leave the area and restrict access to the spill area.
- Notify supervisor(s) and post warning.
- Laboratories should allow aerosols to settle for 30 minutes before re-entering.
- Put on protective equipment.
- Cover an area twice the size of the spill with disinfectant soaked-paper towels, or surround spill with dry disinfectant as per label directions.
- Pour additional disinfectant solution onto the spill, starting at the perimeter and working inward working inward from the edges of the towels. Avoid splashing.
- Allow at least 20 minutes contact time.
- Use forceps, tongs, or broom to remove broken glass and other sharps; place in sharps container.
- Remove towels and re-clean area with disinfectant solution.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant-soaked paper towels.
- Decontaminate (autoclave, chemical disinfectant) reusable clean-up items and other equipment as appropriate.
- Inform laboratory personnel when the clean-up is complete.

#### **Spill Clean-up Materials**

Each laboratory and clinic should build their own biohazard spill kit, and everyone in the lab should know the location of this spill kit (or these supplies) in the event of a biohazard spill:

- **Forceps, Tongs, Broom and Dust Pan**
- **Goggles or Face Shield**
- **Utility Gloves**
- **Lab Coat**

- **Shoe Covers** (optional)
- **Disinfectant Solution** – For example, a 1/10 dilution of household bleach, prepared fresh weekly is effective in most situations.  
*\* Use only disinfectants or sterilants with proven efficacy against the specific biohazardous agent(s) handled in your lab or clinic. Contact REHS for disinfectant guidance.*
- **‘Biohazard’ Bag**
- **Sharps Container**
- **Paper Towels or Other Absorbent**

## Laboratory Spill Clean-up Procedures

### Spills inside a centrifuge

If a tube breaks inside a centrifuge, allow thirty minutes for any aerosols inside the chamber to settle before opening the lid. Don personal protective equipment as described above. Apply a disinfectant solution to all potentially contaminated surfaces, taking care to minimize splashing and aerosol formation. Allow 20 minutes contact time, remove buckets and rotors to nearest Biological Safety Cabinet, aspirate residual disinfectant, and wipe down surfaces with clean water. Place debris in red bags. Re-clean rotors and buckets in a BSC, follow manufacturer’s directions for selection of disinfectants to use on rotors and buckets.

Spills involving a microorganism requiring BL1 containment (organisms not known to cause disease in healthy adult humans)

Wear disposable gloves.

- Soak paper towels in disinfectant and place over spill area.
- Place towels in plastic bag for disposal.
- Clean spill area with fresh towels soaked in a disinfectant.

### Spills involving microorganisms requiring BSL-2 containment.

- Alert people in immediate vicinity to leave the area. Put on protective equipment.
- Cover an area twice the size of the spill with disinfectant soaked-paper towels. Or, surround spill with disinfectant as per label directions.
- Pour additional disinfectant solution onto the spill, starting at the perimeter and working inward from the edges of the towels. Avoid splashing.
- Allow 20 minute contact period.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant. If any walls are contaminated, those should be wiped down as well.



- Use forceps, tongs, or broom to remove broken glass and other items; place in sharps container or red bag.
- Remove towels and re-clean area with disinfectant solution.
- Decontaminate (autoclave or chemically disinfectant) reusable clean-up items and other equipment as appropriate.
- Inform laboratory personnel when the clean-up is complete.

Procedures for BSL-1 and BSL-2 laboratories should incorporate a degree of flexibility. One could safely abridge the procedures above if 1 ml. were spilled over a small bench top area. However, dropping 50 ml. of culture on the floor clearly necessitates the more detailed procedure.

### **Spills inside a Biological Safety Cabinet**

Keep the cabinet running. A Biosafety Cabinet is designed to contain microorganisms that are released during work within the cabinet. Provided that the Biosafety Cabinet is operating properly and has been inspected and certified, aerosols produced by a spill within the cabinet should be contained.

Clean-up the spill as per the directions in BSL-2 section above, making sure to wipe down back and side walls of cabinet. If material has spilled into the catch basin beneath the work surface, add a volume of disinfectant equal to the quantity in the basin, wait 20 minutes, and absorb with paper towels. After completion, allow cabinet to run for ten minutes before resuming work.

Decontaminate Biosafety Cabinet and HEPA filters. If the spill was significant the Biosafety Cabinet and filters may need to be decontaminated with paraformaldehyde gas, gaseous chlorine dioxide or vaporized hydrogen peroxide. Because of the potential for exposure to chemical and biohazardous agents, this type of decontamination should only be done by trained personnel or a qualified vendor.

### **Spills Outside the Biosafety Cabinet**

Biohazardous materials should be transported in sturdy, well sealed primary and secondary containers with a closable top, similar to the container systems required for proper shipment of such materials. This will ensure containment except in catastrophic circumstances. A test-tube rack inside a shallow tray is not acceptable.

If a spill occurs, isolate the area and wait 15-30 minutes for aerosols to settle and cover spill with paper towels, but do not attempt a clean-up without appropriate disinfectant and personal protective equipment. Notify people in the immediate area and contact RUPD to restrict traffic in the area. Obtain spill clean-up supplies and proceed with clean-up.

## **Reporting of Spills and Exposures:**

In the event of an accidental exposure to a biohazard:

1. Remove contaminated clothing
2. Vigorously wash exposed area with germicidal soap and water for one minute.
3. If exposed to aerosols of infectious microorganisms, leave the immediate area
4. In the event of exposure to the eyes, immediately flush eyeball and inner surface of eyelid with water for at least 15 minutes. Hold eye open to ensure effective wash behind eyelid
5. Obtain medical attention

After appropriate decontamination procedures and clean-up have been performed, report to Occupational Health or follow the appropriate site specific procedures if there is no on-site Medical Department immediately after the emergency situation is stabilized.

Save any offending sample(s) for further testing (e.g., unknown tissue or blood sample, monkey blood or tissue, etc.). Follow the site procedure regarding sample testing. If physical exposure has occurred, personnel **may need** to be medically monitored and quarantined until released by the attending physician or medical staff.

## **Spills involving microorganisms requiring BSL3 containment**

- Each BSL3 facility has approved spill clean-up procedures.
- Follow laboratory-specific or agent-specific procedures as outlined in the biosafety plan for your BSL3 facility.

## **All spills and exposures:**

- Must be reported to your supervisor, documented and investigated.
- Supervisors must complete an incident/accident report online at <http://myrehs.rutgers.edu>. When the report is complete and submitted, the online system automatically and simultaneously notifies the following departments: REHS, Occupational Health and Risk Management.
- Corrective actions implemented (including re-training) must be documented.

## **Emergency Response to Blood, OPIMs, or Infectious Agent Exposure**

In the event of a needle stick or mucous membrane exposure the following procedures shall be followed:

### **Exposure to Blood or OPIM/Source Individual**

- In the event of exposure to human blood or OPIM, every effort should be made to collect and keep a sample.

- In case a worker handling specimens has an exposure incident, then the specimen will be tested for HIV, Hepatitis B and Hepatitis C viruses.
- Initiation of post-exposure prophylaxis, if elected by the exposed individual, shall begin as soon as possible following exposure regardless of the availability of information about the source person's HIV, HBV and HCV status. However, the results of source-person testing and/or information about the source person's symptoms and risk factors may contribute to the decision to continue post-exposure prophylaxis.

### **Injuries Involving a Sharp**

If the injury involved a sharp, information about the sharp which caused the injury should be obtained and documented.

### **Needlestick/Sharps Injury/Bite or Scratch from Infected Animal**

- Vigorously wash exposed area with germicidal soap and water for one minute at the nearest sink, eyewash, safety shower, or other source of potable water.
- Seek medical attention immediately.

### **Mucous Membranes**

- If blood/body fluid has splashed in eye, immediately flush eyeball and inner surface of eyelid with water for at least 15 minutes. Hold eye open to ensure effective flushing behind lid.
- If in mouth, rinse out mouth with large quantity of tap water.
- Seek medical attention immediately.

### **Skin Contact**

- Remove contaminated clothing.
- If a garment(s) is contaminated by blood or other potentially infectious materials, it must be removed immediately or as soon as feasible.
- If a garment becomes minimally contaminated, employees should remove it in such a way as to avoid contact with the outer surface; e.g., rolling up the garment as it is pulled toward the head for removal. However, if the amount of exposure is such that the spill penetrates the garment and contaminates the inner surface, not only is it impossible to remove the garment without exposure to the spill, but the penetration itself constitutes exposure. Employees shall remove these items by cutting so as to prevent exposure to the face. See section below for instructions concerning contaminated clothing and PPE.
- Vigorously wash exposed area with germicidal soap and water for one minute at the nearest sink, eyewash, safety shower, or other source of potable water.
- Seek medical attention immediately.

### **Clothing Contamination**

In the event of contamination of clothing or PPE:

- A garment(s) that is contaminated by blood or other potentially infectious materials must be removed immediately or as soon as feasible.
- Contaminated laundry will be handled as little as possible with a minimum of agitation.
- Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.
- Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior.
- Contaminated laundry shall be placed and transported in red biohazard bags, or biomedical waste containers that are either red or bear the universal biohazard symbol.

### **Laundry Services for Contaminated Clothing**

- Rutgers is responsible for the cost of providing personal protective equipment (lab coats and clinic coats), and cleaning, laundering, or disposal of same.
- Disposable articles may be used whenever feasible to reduce the generation of contaminated laundry.
- Home laundering of personal protective equipment or contaminated clothing is not permitted. Should employee owned clothing be contaminated, laundry services will also be provided.

## **XIV. HEALTH, VACCINATION, AND MEDICAL CLEARANCE PROGRAMS**

### **1. Health, Vaccination and Medical Clearance**

**General:** Laboratory personnel will be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

**Health Hazard Evaluations:** REHS is available to perform a health-hazard evaluation upon request.

**Providers of Medical Care:** Medical clearance, medical surveillance, vaccinations, and other occupational health-related services shall be provided by the following:

<b>Campus</b>	<b>For Employees</b>	<b>For Students</b>
<b>Newark Campus</b>	Occupational Medical Services Stanley S. Bergen Building 65 Bergen Street Room GA-167 Newark NJ 07101-1709	Student Health Services Doctors Office Center 90 Bergen Street Suite 1750

	973-972-2900	Newark NJ 07101-1709 973-972-8219
<b>New Brunswick</b>	Occupational Health Department Hurtado Health Center (Lower Level) 11 Bishop Place New Brunswick, NJ 08901 848-932-8254	Student Health Services Hurtado Health Center (Upper Level) 11 Bishop Place New Brunswick, NJ 08901 848-932-7402
<b>Piscataway</b>	RBHS Occupational Health Services EOHSI Building 170 Frelinghuysen Road Piscataway, NJ 08854 848-445-0123	RBHS Occupational Health Services EOHSI Building 170 Frelinghuysen Road Piscataway, NJ 08854 848-445-0123
<b>Camden</b>	Student Health 326 Penn Street, Room 234 Camden, NJ 08102-1410 856-225-6005	Student Health 326 Penn Street, Room 234 Camden, NJ 08102-1410 856-225-6005

\* If your campus Occupational Health office is closed, or if you are at an off-campus location report to the nearest hospital emergency room or private physician for immediate medical care. Upon returning to campus, the employee should report to an Occupational Health office as soon as possible for medical evaluation and follow up by the Occupational Health Physician.

**Personal Health Conditions:** Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Many pathogenic agents pose a higher risk to pregnant women, young children, the elderly and individuals who are immuno-compromised.

Therefore, all laboratory personnel, and particularly women of child-bearing age, will be provided with information regarding immune competence and conditions that may predispose them to infection.

Individuals having a health condition that places them at greater risk for infection, or who are cannot be vaccinated or receive prophylactic interventions, are strongly encouraged to self-identify to Employee Health Services or Student Health Services for appropriate counseling and guidance.

**Serum Banking:** In some instances, the IBC, in consultation with Employee Health Services, may recommend or require that laboratory staff provide baseline serum samples prior to

initiating work with an agent. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility. Administration of the program, including all costs, is the responsibility of the Principal Investigator.

**Medical Evaluation for use of Respiratory Protection:** Using a respirator places a physiological burden on employees that varies with the type of respirator worn, the job and workplace conditions in which the respirator is used, and the medical status of the employee. As such, it will be necessary to medically evaluate each employee using a respirator to ensure he/she is medically able to use the designated respirator. Contact the appropriate medical provider, as listed above.

**Vaccinations:** Immunization serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Also, in some instances, immunization may affect the biosafety level required.

For example, the IBC may require work with a pathogenic agent to be carried out at a higher level because there are no effective treatments available for the pathogenic agent or the agent may be resistant to normal treatment regimens (e.g., multi-drug resistant *Mycobacterium tuberculosis*).

1. **Hepatitis B Vaccination:** In keeping with Rutgers Policy on HIV, HBV, and HCV (00-01-40-40:10, [http://www.umdnj.edu/oppmweb/university\\_policies/health\\_services/PDF/00-01-40-40\\_10.pdf](http://www.umdnj.edu/oppmweb/university_policies/health_services/PDF/00-01-40-40_10.pdf)) all faculty and staff who have contact with potentially infectious body fluids or laboratory materials, including human cell lines, **should** be immunized against hepatitis B or be able to demonstrate immunity. Non-immunized staff and faculty must be offered the vaccination annually. Similarly, students are required to be immunized against hepatitis B or be able to demonstrate immunity prior to or with 9 months of enrollment in compliance with the Rutgers Policy on Student Immunization and Health Requirements (00-01-25-40:00).

The immunization series involves three intramuscular injections over a six month period. The vaccine is effective > 95% of the time when all three doses are given and immunity is thought to last at least fifteen years after proof of immunity. It may be contraindicated for those with yeast allergy (the immunogenic antigen is cultivated in cells of *S. cerevisiae*); pregnant women should consult their physician before receiving the vaccine.

OSHA requires use of the Centers for Disease Control (CDC) guidelines current at the time of the evaluation or procedure. Employees who do not develop immunity in response to the primary vaccination series (non-responders) must be revaccinated with a second three-dose series and retested. Non-responders must be medically evaluated.

The Hepatitis B vaccination series should be started, at no cost to employees within 10 days of initial assignment for those with occupational exposure to blood or other potentially infectious materials unless:

- the employee documents previous completion of the series;
- antibody testing reveals immunity; or,
- the vaccine is contraindicated for medical reasons.

Hepatitis B vaccination booster doses must be made available if recommended by the United States Public Health Service.

To schedule and arrange for Hepatitis B vaccinations and/or antibody testing contact the Occupational Health office for your campus.

2. **Other Vaccinations:** The IBC, in consultation with Occupational Health, may recommend or require that laboratory personnel undergo vaccination prior to initiating work with an agent or toxin.

**Post-Exposure Medical Procedures:** Should an exposure incident occur, decontamination should immediately be performed at the nearest sink, eyewash, or safety shower. Exposed individuals should then notify their supervisor and obtain medical attention immediately. During regular hours, employees obtain medical follow-up from the appropriate provider as listed above.

During non-working hours (nights, weekends), individuals should go directly to the Emergency Department of the nearest hospital. Notify the appropriate health care provider (listed above) of the exposure on the next business day.

An occupational exposure should be regarded as an urgent medical circumstance and guidelines for post-exposure prophylaxis call for treatment initiation within hours rather than days. Treatment with chemo-prophylactic drugs is voluntary; they will be available according to Rutgers policy at no expense to those exposed in the course of their activities at the University. The Rutgers Policy entitled "Management of Occupational/Educational Exposures to HIV, HBV, and HCV", (00-01- 40-40:10) outlines the procedures under which post-exposure prophylaxis will be made available. This policy was developed based on the current recommendations of the Public Health Service. The policy is available at the website of the University's Office of Policy and Project Management.

For those working with BSL2 or BSL3 agents other than HIV, HBV, or HCV, an additional post exposure plan will be created and shared with all employees. The drugs will be available according to Rutgers policy at no expense to those exposed in the course of their activities at the University.

If indicated, testing on a voluntary basis, for anti-HIV and anti-HCV antibodies will be conducted at: baseline, six weeks, 12 weeks, six months, and twelve months. HBV antibody testing, or vaccine, booster, or immunoglobulin will be administered as appropriate.

The OSHA Bloodborne Pathogens Standard requires employers to maintain a log for all needlestick and sharps injuries. REHS is responsible for ensuring compliance with this

requirement. The information in the sharps injury log is recorded and maintained in such manner as to protect the confidentiality of the injured employee. The sharps injury log includes the following information:

- The type and brand of device involved in the incident,
- The department or work area where the exposure incident occurred, and
- An explanation of how the incident occurred.

(Suggested replacement for above) The injured employee's supervisor must submit an online incident report (<http://myrehs.rutgers.edu>) by the end of the work shift or as soon as possible if the incident is reported after the work shift has ended. By submitting the online incident report, the following departments are notified automatically: Risk Management, REHS, and Occupational Health.

### **Information provided to Healthcare Professionals**

The Rutgers health care professionals responsible for employees' Hepatitis B vaccination, other work required vaccinations and monitoring, post-exposure evaluation and follow-up will have a copy of the OSHA Bloodborne Pathogens Standard.

The Principal Investigator or designee in conjunction with the exposed person will also ensure that the health care professional evaluating an employee after an exposure incident receives the following:

- a description of the employee's job duties relevant to the exposure incident;
- route(s) and circumstances of exposure; and,
- if possible and applicable, the results of the source individual's blood test.

### **Healthcare Professional's Written Opinion**

Persons suffering an exposure incident are to be provided with a copy of the evaluating healthcare professional's written opinion within 15 days after completion of the evaluation.

The written opinion for post-exposure evaluation and follow-up will be limited to whether or not the employee has been informed of the results of the medical evaluation and any medical conditions which may require further evaluation and treatment. All other diagnoses must remain confidential and not be included in the written report to the employee's supervisor.

For hepatitis B vaccinations, the healthcare professional's written opinion will be limited to whether the employee requires or has received the hepatitis B vaccination.

### **Medical Records**



Medical records are maintained for each employee with occupational exposure in accordance with 29 CFR 1910.20. Occupational or Student Health Services are responsible for maintenance of the required medical records.

In addition to the requirements of 29CFR 1910.20, the medical record will include:

- The name and social security number of employee;
- A copy of the employee's hepatitis B vaccinations and any medical record relative to the employee's ability to receive vaccination;
- A copy of all results of examinations, medical testing, and follow-up procedures as required by the standard;
- A copy of all healthcare professional's written opinion(s) as required by the standard
- All employee medical records will be kept confidential and will not be disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by the standard or as may be required by law.
- Employee medical records shall be maintained for at least the duration of employment plus 30 years in accordance with 29 CFR 1910.20.
- Employee medical record shall be provided upon request of the employee or to anyone having written consent of the employee within 15 working days.

## **XV. SELECT AGENTS AND TOXINS**

### **Select Agent Registry**

The Centers for Disease Control and Prevention regulates the possession of biological agents and toxins that have the potential to pose a severe threat to public health and safety. CDC's Select Agent Program ([www.selectagents.gov](http://www.selectagents.gov)) oversees these activities. Any entity that possesses, uses, or will receive or transfer any select agent or toxin to or from entities within the US or outside the US (see Table 10.8 for a select agent list) is regulated by the New Select Agent Regulation, 42 CFR 73.0, "Possession, Use, and Transfer of Select Agents and Toxins," administered by the Centers for Disease Control and 7 CFR 331, 9 CFR 121, "Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins, Interim Final Rule" administered by the United States Department of Agriculture ([www.selectagents.gov](http://www.selectagents.gov))

CDC/ USDA prepared the select agent list for 42 CFR 73, 7 CFR 331, 9 CFR 121 after receiving extensive input from scientists representing 21 Federal government entities. The proposed list was published in the Federal Register for public comment on August 23, 2002. The HHS Secretary considered the following criteria for establishing the list as directed in 42 U.S.C. 262a (a)(1)(B):

- The effect on human health of exposure to the agent or toxin;
- The degree of contagiousness of the agent or toxin and the methods by which the agent or toxin is transferred to humans;

- The availability and effectiveness of pharmacotherapies and immunizations to treat and prevent any illness resulting from infection by the agent or toxin.

The current Select Agent Program requires facilities to register with CDC/USDA prior to transfer/receipt of select agents. The current registration process also requires submission of an application that certifies that the facility is in compliance with specific safety and security standards set forth in the regulation. All entities (except for Federal, State, or local governmental agencies), their Responsible Official (RO), alternate RO, and all individuals working with or having access to select agents or toxins must have an approved security risk assessment. An entity may not provide an individual access to a select agent or toxin unless the individual has been approved by the HHS Secretary or USDA Secretary based on this security risk assessment. Additional requirements of the "USA Patriot Act" and the "Public Health Security, Bioterrorism and Response Act of 2002" must also be satisfied. The specific components to include in the security plan as required by 42 CFR 73 are located in the regulation at § 73.11 and can be accessed at [http://www.cdc.gov/od/sap/docs/73\\_11.pdf](http://www.cdc.gov/od/sap/docs/73_11.pdf). Contact REHS for additional information and assistance.

Federal law requires that the CDC must be notified five (5) working days in advance of the **destruction or depletion of a select agent/toxin**. This notification must be coordinated through the Responsible Official and REHS (Environmental Health and Safety).

## **XVI. Transportation and Permits**

All persons wishing to ship biological samples must complete the REHS training for Category A, Category B, exempt human specimens, dry ice, etc. before shipping any materials from Rutgers University. Additionally, no biological materials may be hand carried or transported by car using city streets unless approved by REHS. A special training for this may be required.

All training dates for shipping are included in the online training calendar at <http://myrehs.rutgers.edu>. For any questions regarding shipment or transport of biological materials, please contact the REHS biosafety group at [biosafety@aps.rutgers.edu](mailto:biosafety@aps.rutgers.edu).

The importation or exportation of infectious materials, recombinant nucleic acids, transgenic plants, seeds, soils, animal specimens may require permits from the Centers for Disease Control and Prevention or the United States Department of Agriculture. For permitting questions, please contact REHS at [biosafety@aps.rutgers.edu](mailto:biosafety@aps.rutgers.edu). A copy of all permits should be forwarded to REHS for recordkeeping purposes. Permit holders are subject to regulatory inspections by the permit awarding agency. REHS must be immediately informed of any inspector that arrives to inspect any Rutgers University facility.

## Appendix A: Genetically Modified Plants and Organisms

**Confinement of Genetically Modified Plants and Organisms** - Confinement refers to the practice of confining experimental plants, animals, and microorganisms within a designated outdoor experimental zone of control with designated borders or limits. In the context of Rutgers University Biological Safety Guide, confinement refers those practices necessary to confine a field test of a transgenic plant, organism, or product with a designated test plot. Principal Investigators are required to submit all protocols involving field tests of transgenic plants, organisms, or products to the University Biological safety Committee for approval **prior** to initiation of the field test. **All field tests of transgenic plants, organisms, or products performed at Rutgers University shall be conducted in accordance with applicable sections of 7 CFR Parts 330 and 340** entitled Introduction of Organisms and products Altered or Produced Through Genetic Engineering Which are Plant Pests or Which There is Reason to Believe are Plant Pests.

There are five classes of confinement, as follows:

- 1. Physical Confinement** – Physical Confinement refers to physical barriers that may limit the survival and dissemination of organisms or product outside the test plot. Physical barriers may include, but are not limited to, border rows, isolation of test plot, dams, fences, screens, and plastic barriers.
- 2. Biological Confinement** - Biological confinement refers to biological barriers that may limit the survival and dissemination of organisms or products outside the test plot and to limit the transfer of genetic information from the test organism to other organism. Biological barriers may include, but are not limited to, genetic modifications that disable the organism, produce sterility, and reduce the ability of the organism to survive or escape predators.
- 3. Environmental Confinement** – Environmental confinement refers to environmental conditions that can be used to limit the survival and dissemination of organisms and products outside the test plot. Environmental conditions may include, but are not limited to, temperature, water supply, humidity, and length of day.
- 4. Chemical Confinement** – Chemical confinement refers to chemical treatments that can be used to limit survival and reproduction of organisms or their products outside the test plot and to limit the transfer of genetic information from the test organism to other organisms.

Chemical treatments include the application of herbicides, fungicides, insecticides, disinfectants and other materials toxic to the test organism.

**5. Scale** – Decreasing the number of test organisms used in the protocol or the size of the test plot may reduce the possibility of dissemination of the organism outside the test plot.

**Field Tests / Test Plots** -- Principal Investigators are required to use an appropriate combination of confinement strategies to ensure that transgenic plants, organisms, or products are not disseminated beyond the test plot. Usually, a USDA APHIS permit or courtesy permit under 7 CFR 340 is required to facilitate a field test of a transgenic plant, organism, or product.

**Growth Chamber and Greenhouse Practices** – All research or other activities involving recombinant DNA technology conducted at Rutgers University must conform to the NIH Guidelines, referenced above. Laboratory portions of research protocols involving transgenic plants are conducted in accordance with Appendix G of the NIH Guidelines, entitled, Physical Containment.

These guidelines are intended to prevent the dissemination of transgenic materials beyond the experimental facility into the surrounding ecosystem. Similar to laboratory biosafety levels, whole plant biosafety levels range from biosafety level 1-plant (BL1-P) to biosafety level 4-plant (BL4-P) with BL1-P being the least stringent and BL4-P being the most stringent. **Principal Investigators are required to use an appropriate combination of confinement strategies to ensure that transgenic plants, organisms, or products are not disseminated beyond the greenhouse or growth chamber.**

The level of confinement used to prevent the dissemination of genetic material beyond an outdoor test plot or a growth chamber or greenhouse should be designed for the specific characteristics of the organism in use. Organisms that are **generally recognized as compatible with the environment (GRACE)** may be handled at **BL1-P**. GRACE organisms have virtually no potential for adverse effects on human health or natural ecosystems and have virtually no potential for the transfer of genetic material to other organisms for rapid reproduction.

Organisms that may cause adverse effects on human health or natural or managed ecosystems, **the consequences of which are predictably low**, considering the potential of the organism to transfer genetic material to other organisms, widespread dissemination in the environment, and rapid reproduction, may be handled at **BL2-P**.

Organisms that may cause adverse effects on human health or natural or managed ecosystems, **the consequences of which are predictably moderate**, considering the potential of the organism, to transfer genetic material to other organisms, widespread dissemination in the environment, and rapid reproduction, may be handled at **BL3-P**.

Organisms that may cause adverse effects on human health or natural or managed ecosystems, **the consequences of which are predictably high**, considering the potential of the organism to transfer genetic material to other organisms, widespread dissemination in the environment, and

rapid reproduction may be handled at **BL4-P**. Currently, there is no maximum containment greenhouse (BL4-P) facility at Rutgers University, nor is one planned for the future.

## **Transgenic Plants**

**Standard Containment Practices for Transgenic Plants** – The following basic procedures are applicable for all containment levels and categories:

**Whole Plants** – Transgenic whole plants should be clearly labeled to prevent accidental release. Transgenic whole plants should be destroyed at the end of the experimental protocol.

**Pollen** – Pollen is viable only if it comes into contact with a female flower of the same species. The dispersal of pollen of entomophilous species, i.e., insect pollinated, may be prevented by an effective insect control program. Individuals working with transgenic plants that are producing pollen should be considered as potential vectors for pollen dispersal. This route of dispersal can be effectively blocked by restricting access to transgenic plants, proper labeling of transgenic plants, handling transgenic plants carefully, and proper hygiene.

**Seeds** – Since seeds of most species remain viable for long periods of time and have minimal requirements for growth and reproduction, the dispersal of seed should be the prime focus of containment. Seeds may be destroyed by physical means, e.g., autoclaving, or by chemical means, e.g., immersion in alcohol. Pollination bags may be placed over inflorescence to contain accidental seed dispersal. Accidental spills of transgenic seeds should be contained, the seeds collected, and the area chemically treated to inactivate any unrecovered seeds. Individuals handling transgenic plants which have set seeds should be considered potential vectors for seed dispersal. This route of dispersal can be effectively blocked by restricting access to transgenic plants and seed, proper labeling of transgenic plants and seeds, handling transgenic plants and seeds carefully, and proper hygiene.

**Restricted Access to Transgenic Plants and Seeds** – The Principal Investigator should limit access to transgenic material to those with appropriate training and who are directly involved in the experiments or facility maintenance. Laboratories, growth chambers, and/or greenhouses containing transgenic materials should be appropriately labeled and secured. Doors shall remain closed at all times and locked when unattended.

**Transport of Transgenic Plants and Seeds** – Transgenic plants and seeds may not be left unattended outside of restricted areas, e.g., growth chambers and greenhouses. Transgenic seeds and transgenic plants with mature or nearly mature seed pods shall not be transported between restricted areas except in unbreakable, leak-proof containers. Transport of transgenic plants and seeds that are considered regulated articles (7 CFR 330 and 340) between facility sites may only be accomplished with appropriate federal and state permits.

**Recordkeeping** – Principal Investigators shall maintain records of all experiments and field trials involving recombinant DNA in growth chambers, greenhouses, and test sites.

**Disposal of Transgenic Plants and Seeds** – Transgenic plants and seeds must be rendered biologically inactive by a proven method prior to disposal from the restricted area (growth chamber or green house). The principal means by which transgenic plants and seeds are rendered biologically inactive are by autoclaving, chemical treatment, and incorporation may also be acceptable.

**Maintenance of Greenhouse Facilities** – Greenhouse supervisors shall utilize a program to control undesired species such as weeds, rodents, arthropod pests, and pathogens. All methods used to control undesired species shall conform to all applicable federal, state, and local regulations. Principal Investigators are responsible for maintaining greenhouse work areas in a neat and clean manner.

**Labeling of Plants** – All transgenic plants maintained within a restricted area shall be labeled “Transgenic Plant”. Transgenic plants may be labeled individually or by flat.

**Containment Levels** – The containment level in a specific greenhouse is determined by the highest level of containment required by any individual plant in that greenhouse. The following tables summarize the standard practices and facility requirements for plant biosafety levels 1 through 3.

<b>Comparison of Standard Practices for Transgenic Plants</b>		
<b>BSL1- P</b>	<b>BSL2-P</b>	<b>BSL3-P</b>
Discretionary Access	Access limited only to those directly involved with experiments	Access restricted to required persons only
Personnel must read and follow instructions	Personnel must read and follow instructions	Personnel must read and follow instructions
Procedures followed are appropriate for organisms	Greenhouse manual adopted, advises of consequences of release, contingency plan in place	Greenhouse manual adopted, advises of consequences of release, contingency plan in place
Records kept of experiments in facility	Records kept of experiments in facility and movement of materials into/out of facility	Records kept of experiments in facility and movement of materials into/out of facility
	Containment required for movement into/out of facility	Containment required for movement into/out of facility, external decontamination required
Biologically inactive experimental organisms	Biologically inactivate experimental organisms, gravel decontamination periodically	Biologically inactivate experimental organisms (including water runoff), decontaminate equipment and supplies
Pest control program in place	Pest control program in place	Pest control program in place
Appropriate caging and precautions for escape of motile organisms	Appropriate caging and precautions for escape of motile organisms	Appropriate caging and precautions for escape of motile organisms

	Door posted for restricted experiment in progress with plant names, responsible person, and special requirements	Door posted for restricted experiment in progress with plant names, responsible person, and special requirements, biohazard symbol if risk to humans
		Minimize aerosol creation to reduce contamination
		Protective clothing worn to minimize dissemination, hands washed thoroughly before leaving
Concurrent experiments use same practices	Concurrent experiments use same practices	Concurrent experiments use same practices

<b>Comparison of Facilities for Transgenic Plant Containment</b>		
<b>BSL1-P</b>	<b>BSL2-P</b>	<b>BSL3-P</b>
Facility/Greenhouse	Facility/Greenhouse	Facility/Greenhouse
Porous floor material, impervious walkway	Gravel floor unless soil dissemination occurs	Impervious floor with water runoff collection
Windows with optional screens	Windows with fly screens	Windows closed/sealed, negative pressure optional
		Self-contained structure with restricted traffic flow, entrance through double self closing/locking doors
		Security fence or equivalent protection
		Surfaces impervious/sealed from chemicals/liquids, all surface penetrations sealed
		Work surfaces impervious, resistant to chemicals
		Hand washing facilities with "hands-free" controls
	Autoclave available	Autoclave in facility, double door autoclave recommended
		Filtered vacuum lines, disinfectant traps for liquid lines
	Air intake fan open during operation only	Individual air supply direction/pressure controlled as required
		Exhaust air HEPA filtered, zero inward air flow
	Growth chamber suitable alternative for containment if intent of guidelines are met	Growth chamber suitable alternative for containment if intent of guidelines are met

**Appendix B:**

*Rutgers, The State University of New Jersey*

**Checklist for BSL-1 and BSL-2 Knowledge and Proficiency**

**Trainee Name:** \_\_\_\_\_

**Position:** \_\_\_\_\_

**Name of Supervisor Completing This Form:** \_\_\_\_\_

**Date:** \_\_\_\_\_

Directions: Supervisor to mark "Y" (yes) or "N" (no) in the second column; mark "N/A" if not applicable.

<b>Tasks: Biosafety Level 1</b>	<b>Knowledgeable and Proficient?</b>
Perform hand washing after working with potentially hazardous agents & before leaving the lab	
Refrain from eating or drinking in the lab	
Refrain from mouth pipetting; use only mechanical pipetters	



Perform all procedures to minimize splashes and aerosols	
Handle and store sharp items such as razor blades and needles only in a safe manner	
Dispose sharp items only in sharps containers	
Demonstrate proper use of biosafety cabinet ( <i>see second page for breakout section</i> )	
Understand basics of chemical disinfection, understand need to follow manufacturer's concentration and contact time, as well as expiration, and demonstrate proper use of disinfectants	
Decontaminate work surfaces properly	
Decontaminate cultures and other liquid wastes prior to disposal down the drain	
Demonstrate awareness of basic lab and biohazard signage	
Properly use protective equipment (lab coat, eyewear, gloves)	

<b>Tasks: Biosafety Level 2 (in addition to BSL-1 tasks)</b>	<b>Knowledgeable and Proficient?</b>
Be aware of hazards and meet specific entry requirements	
Be aware of and participate in medical surveillance and immunizations	
Read and understand biosafety manual	
Transport potentially infectious materials only in leak-proof labeled containers	
Be aware of post-exposure procedures in case of infectious agent exposure	
Perform all aerosol-producing procedures only inside BSC	
Use centrifuge safety cups properly	

Remove and dispose of protective garb in the lab	
Understand principals of steam sterilization and understand how to operate an autoclave	
Understand emergency response procedures in the lab	

<b>Tasks: Use of a biological safety cabinet (BSC)</b>	<b>Knowledgeable and Proficient?</b>
Understand the principles of a Class II BSC (HEPA-filtered air over the work surface, room air drawn in through the front grille, and HEPA-filtering of exhaust air)	
Understand what the magnehelic gauge represents and how to verify proper directional airflow	
Verify that the BSC is current for annual certification and is safe to use	
Demonstrate how to turn the blower on and off, and where to place the sash	
Turn the blower on and allow it to run for 5 minutes prior to starting work	
Demonstrate good control and movement of hands and arms to minimize disruption of the air curtain	
Keep the front and rear grilles clear from supplies and equipment	
<b>Tasks: Use of a biological safety cabinet (BSC)</b>	<b>Knowledgeable and Proficient?</b>
Maintain a safe distance from anyone working at the biosafety cabinet	
Place aerosol-producing equipment such as vortex equipment inside the BSC and toward the rear to prevent aerosols from reentering the worker's environment	
Load centrifuge safety cups and rotors inside the BSC	
Use protective equipment when working inside the BSC	

Work from “clean” to “dirty” and avoid contaminating clean/sterile materials	
Disinfect used plastic ware by placing in disinfectant reservoirs inside BSC	
Disinfect tools and equipment used in the BSC prior to removal	
Bag disposable items inside the BSC, and seal waste bags, prior to removal	
Understand why the use of flames and gas burners should be avoided	
Demonstrate proper use of vacuum systems including use of traps containing disinfectant and HEPA filters	
Demonstrate proper spill control and cleanup, including cleanup inside the drain pan; awareness of drain valve	
Disinfect the BSC at the end of the work session or the end of the day	
Turn off the blower at the end of the day and close the sash as applicable	
Understand the limitations of UV light and safe operation of UV light in the presence of workers in the lab	
Perform appropriate procedures in case of an emergency such as a power failure or failure of the blower	

<b>Tasks: Understanding of general practices for cell and tissue culture</b>	<b>Knowledgeable and Proficient?</b>
Understand the nature of the materials being handled; i.e. awareness if materials are non-infectious or infectious, if they are of human origin, if they are of animal origin and if so which species	
Understand how infectious agents are transmitted in the research setting Direct skin, eye, or mucosal membrane exposure Sharp injury (needle stick, scalpel cut) Animal bite or scratch Inhalation of droplets or aerosols Ingestion due to accidental contamination of hands/food/drink	

Understand the basics of recombinant DNA and if materials being handled are recombinant in nature and the subsequent biological hazards present	
Demonstrate familiarity with biological hazards: Natural mode of infection Means of transmission in the lab Susceptibility to disinfectants and treatment methods Recommended lab safety practices Occupational health requirements	

Tasks: Animal Biosafety Level 1 (in addition to BSL-1 tasks)	Knowledgeable and Proficient?
Understand that all animal procedures must be on an IACUC protocol and be familiar with the IACUC protocol in use	
Demonstrate safe handling of animals (grip techniques, positioning animal)  <u>List animal species the trainee has worked with:</u>	
Demonstrate competence in applicable veterinary procedures such as blood collection, injection, other surgical procedures, and euthanasia and necropsy  <u>List veterinary procedures the trainee has performed:</u>	
Demonstrate competence in applicable husbandry procedures such as cage change, cage cleaning, handling bedding, and room and equipment disinfection  <u>List husbandry procedures the trainee has performed:</u>	
Enroll in and understand occupational health program that considers individuals' health status and immune competence and addresses naturally-occurring infections in animals and animal allergens	
Demonstrate understanding and proper use of protective equipment particularly mucous membrane protection when handling non-human primates	

Tasks: Animal Biosafety Level 2 (in addition to all above tasks)	Knowledgeable and Proficient?
<p>Demonstrate proper use of physical and chemical restraints to handle animals                      Physical restraints may include engineering controls such as squeeze mechanisms</p> <p><u>List physical and chemical restraints with which the trainee has experience:</u></p>	
<p>Demonstrate proper use of additional protective equipment required to handle infectious animals</p>	
<p>Demonstrate understanding of how to capture an escaped animal</p>	
<p>Handle and disinfect bedding, carcasses, tissues, and cages</p>	
<p>Demonstrate understanding and proper use of biocontainment caging</p>	
<p>Understand emergency response procedures in the animal facility</p>	

References:

- U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Biosafety in microbiological and biomedical laboratories. 5<sup>th</sup> ed. U.S. Public Health Service, 2009.
- Department of Environmental Health and Safety, University of Pittsburgh. Biological safety cabinets- the basics. <http://www.ehs.pitt.edu/assets/docs/BiologicalSafetyCabinets-Basics.pdf>.
- Department of Environmental Health and Safety, University of Pittsburgh. Biological safety cabinets. EHS Policy #05-004, January 1, 2007. <http://www.ehs.pitt.edu/assets/docs/bio-cabinets.pdf>.
- U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guidelines for Biosafety Laboratory Competency. MMWR 60, April 15, 2011.

## **Appendix D: BIOSECURITY and DUAL USE RESEARCH OF CONCERN**

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. While the objectives are different, biosafety and biosecurity measures are usually complementary.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements, material transfer documentation, training, emergency planning, and program management. However, biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

Dual Use Research of Concern (DURC) is research that can provide knowledge, information, products, or technologies that could be directly misused to pose a significant threat to public health and safety, agricultural crops, animals, the environment or national security. Currently, in the United States the focus is on research in the life sciences, although DURC can be found in many areas of study.

In 2012, the United States Government (USG) published a policy applying to grant funding agencies in regards to the requirements for oversight and reporting of research determined to fall into the categories of DURC. In September 2014, the USG published the policy for Institutional Oversight of Life Sciences DURC. This policy will be implemented by September 2015, and requires a committee for DURC oversight at each institution. At Rutgers, the IBC will provide this oversight. The policy covers only certain pathogens, and areas of research, but ALL research reviewed by the IBC will be, and has been, reviewed for DURC potential.

The 15 agents and toxins listed in this Policy are subject to the select agent regulations (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121), which set forth the requirements for possession, use, and transfer of select agents and toxins, and have the potential to pose a severe threat to human, animal, or plant health, or to animal or plant products. It is important to note, however, that the Federal Select Agent Program does not oversee the implementation of this Policy or the March 2012 DURC Policy.

- a) Avian influenza virus (highly pathogenic)
- b) *Bacillus anthracis*
- c) Botulinum neurotoxin

6 For the purposes of this Policy, there are no exempt quantities of botulinum neurotoxin. Research involving any quantity of botulinum neurotoxin should be evaluated for DURC potential.

- d) *Burkholderia mallei*
- e) *Burkholderia pseudomallei*
- f) Ebola virus
- g) Foot-and-mouth disease virus
- h) *Francisella tularensis*

- i) Marburg virus
- j) Reconstructed 1918 Influenza virus
- k) Rinderpest virus
- l) Toxin-producing strains of *Clostridium botulinum*
- m) Variola major virus
- n) Variola minor virus
- o) *Yersinia pestis*

The categories of experiments included in this review are:

- a) Enhances the harmful consequences of the agent or toxin
- b) Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification
- c) Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
- d) Increases the stability, transmissibility, or the ability to disseminate the agent or toxin
- e) Alters the host range or tropism of the agent or toxin
- f) Enhances the susceptibility of a host population to the agent or toxin
- g) Generates or reconstitutes an eradicated or extinct agent or toxin listed in 6.2.1, above

### **Reporting of Incidents**

The following shall be promptly reported to RUPD, REHS and the Department Chairperson:

- 1. Theft or loss of keys, access cards, pincodes, and/or other means of access to laboratory areas or sensitive laboratory information.
- 2. Theft or loss of biological agents, specimens, and/or other any other research related materials. This includes the theft or loss of laboratory chemicals, drugs, supplies, equipment, computers, information storage devices, log books and/or other written records.
- 3. Escape or loss of animal that have been dosed with an infectious agent or material.
- 4. Incidents of vandalism or suspicion of tampering in laboratory areas.
- 5. Suspicious persons in laboratory areas.
- 6. Threatening phone calls or threats received in writing.

### **Routine Biosecurity Practices**

- 1. Control access to areas where materials are used or stored.
- 2. The laboratory door should be closed when work with BSL1 or BSL2 materials is in progress. Close and lock the laboratory door when no one is present.
- 3. Know what materials are being brought into the lab
- 4. Keep an inventory of biological materials stored in the lab
- 5. Document when biological materials are removed from the lab.



December 2014

6. Close and lock lab doors when no lab staff is present in the lab.
7. Do not leave biological materials unattended or unsecured at any time. Promptly dispose of unneeded agents and specimens.
8. Lock refrigerators and freezers where agents are stored.