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Rutgers Biosafety Guide
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 perform safely in the laboratory, such as child/ elder care, family or marriage counseling, stress, illness, alcohol and/ or drug abuse, etc. Another important mechanism is formal, confidential reporting mechanisms for instances of non-compliance with established safety and/ or security policies established for Rutgers University. At Rutgers University, multiple avenues of reporting exist depending on the issue. These options include: 1) reporting to your PI/ supervisor; 2) Reporting to your Department Administrator and/ or Chair; 3) Reporting to the Rutgers University’s Compliance hotline at 800-215-9664; 4) Reporting to the biosafety group in Rutgers Environmental Health and Safety (REHS); 5) Reporting to the Department of Environmental Health and Safety or the Department of Risk Management. Depending on the nature of the reports, notification to the Rutgers Institutional Biosafety Committee may occur.
II. PERSONNEL ASSURANCE

Please complete the table below with the names of the individuals in the laboratory working with biohazards including cell culture, human material, and/or toxins of biological origin. The signature of these persons indicates that they have read, understand and will comply with the contents of the BSG. This information should be updated **annually**.

<table>
<thead>
<tr>
<th>Name</th>
<th>Job Title</th>
<th>Signature</th>
<th>Date</th>
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<tbody>
<tr>
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</tbody>
</table>
III. INTRODUCTION

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](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.
• Follows reporting requirements for incidents as outlined in the Emergency Response Section of this Guide.
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.
- Reports incidents as required in the NIH Guidelines for Recombinant and Synthetic Nucleic Acids on behalf of the IBC.

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

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: IDENTIFICATION AND RISK ASSESSMENT

Identification of Biohazards
Laboratory-acquired infections have been documented since microbiology's emergence as a distinct discipline in the 19th 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

<table>
<thead>
<tr>
<th>Risk Group Classification</th>
<th>NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES 2009</th>
<th>WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 1</td>
<td>Agents that are not associated with disease in healthy adult humans.</td>
<td>No or low community risk) A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
<tr>
<td>Risk Group 2</td>
<td>Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.</td>
<td>(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.</td>
</tr>
<tr>
<td>Risk Group 3</td>
<td>Agents that are associated with serious or lethal human disease for</td>
<td>(High individual risk; low community risk) A pathogen that usually causes serious human or</td>
</tr>
</tbody>
</table>

Rutgers Biosafety Guide
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

Source: BMBL, 5th Edition
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

Rutgers Biosafety Guide
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 5th 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 immuno-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 regiments (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>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Safety Equipment (Primary Barriers)</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
</table>
| 1   | Not known to consistently cause disease in healthy adults | - Standard Microbiological Practices | None required | - Open bench tops  
- Sink required for hand-washing |
| 2   | Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure | BSL-1 practice plus:  
- Limited access  
- Biohazard warning signs & labels.  
- "Sharps" precautions  
- Biosafety manual defining any needed waste decontamination or medical surveillance policies | Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed | BSL-1 plus:  
- Autoclave available |
| 3   | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences | BSL-2 practice plus:  
- Controlled access  
- Decontamination of all waste  
- Decontamination of lab clothing before laundering  
- Baseline serum | 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:  
- Physical separation from access corridors  
- Self-closing, double-door access  
- Exhausted air not recirculated  
- Negative airflow into laboratory |
| 4   | 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:  
- Clothing change before entering  
- Shower on exit  
- All material decontaminated on exit from facility | Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit | BSL-3 plus:  
- Separate building or isolated zone  
- Dedicated supply and exhaust, vacuum, and decon systems |
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

<table>
<thead>
<tr>
<th>Biological Risk Assessed</th>
<th>Protection Provided</th>
<th>BSC Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Personnel</td>
<td>Product</td>
</tr>
<tr>
<td>BSL 1-3</td>
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<tr>
<td>BSL 1-3</td>
<td>Yes</td>
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</tr>
<tr>
<td>BSL 4</td>
<td>Yes</td>
<td>Yes</td>
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</tbody>
</table>
Table 4 - Comparison of Biosafety Cabinet Characteristics

<table>
<thead>
<tr>
<th>BSC</th>
<th>Face Velocity</th>
<th>Airflow Pattern</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonvolatile</td>
</tr>
<tr>
<td>I</td>
<td>75</td>
<td>In at front; exhausted through HEPA to the outside or into the room through HEPA</td>
<td>Yes</td>
</tr>
<tr>
<td>II, A</td>
<td>75</td>
<td>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.</td>
<td>Yes</td>
</tr>
<tr>
<td>II, B</td>
<td>100</td>
<td>Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter.</td>
<td>Yes</td>
</tr>
<tr>
<td>II, B2</td>
<td>100</td>
<td>No recirculation; total exhaust to the outside through hard-duct and a HEPA filter.</td>
<td>Yes</td>
</tr>
<tr>
<td>II, B3</td>
<td>100</td>
<td>Same as II, A, but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter</td>
<td>Yes</td>
</tr>
<tr>
<td>III</td>
<td>N/A</td>
<td>Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series</td>
<td>Yes</td>
</tr>
</tbody>
</table>

(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 into 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.
CAUTION: Be aware that air-sampling studies have shown that most of the common manipulations of bacterial and viral cultures in research laboratories release \textit{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 \textit{creating infectious aerosols} or splashes may include:

- Centrifuging
- Grinding
- Blending
- Vigorous shaking or mixing
- Pipetting
- Pouring of cultures
- Sonic disruption
- Opening containers of infectious materials
- Inoculating animals intranasally
- 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.

\textbf{Laminar Flow Cabinets (LFCs)}

\textbf{Laminar Flow Cabinets} are \textbf{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 \textbf{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 \textbf{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**
Consult with REHS at biosafety@aps.rutgers.edu to determine whether biological safety cabinets (BSCs) will require decontamination by an outside vendor prior to be moved. Decontamination by an outside vendor will be required before HEPA filters are changed and/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 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 recombinant and synthetic nucleic acids and 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 and recombinant/synthetic nucleic acid 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:
  o Check for visible tears and other defects.
  o Do not allow rings or other jewelry to rip gloves.
  o 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.
  o Wash hands immediately after removing gloves.
  o 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.
  o 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 likelihood for becoming contaminated. When contamination is visible, clean and decontaminates 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

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Disinfection Level</th>
<th>Bacteria</th>
<th>Lipophil. Viruses</th>
<th>Hydrophylic Viruses</th>
<th>M.tuberculosis</th>
<th>Fungi</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols (ethyl and isopropyl) 60-85%</td>
<td>Intermediate</td>
<td>+++</td>
<td>+</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>Not sporicidal; evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish efficacy; flammable</td>
</tr>
<tr>
<td>e.g. Rubbing Alcohol (Isopropanol), Ethanol (diuted to 70%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolics (0.4-5%)</td>
<td>Intermediate</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>Not sporicidal; phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue</td>
</tr>
<tr>
<td>e.g. Vesphene, Matar, Hil-Phene, Midro-Bac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde (2.5%)</td>
<td>High</td>
<td>+++</td>
<td></td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>Used to sterilize surgical instruments that can not be autoclaved; strong odor; sensitizer; use with adequate ventilation. Not for use on environmental surfaces</td>
</tr>
<tr>
<td>Quaternary Ammonium (0.5-1.5%)</td>
<td>Low</td>
<td>+</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>+/-</td>
<td>May be ineffective against Psuedomonas; recommendation limited to environmental sanitation (floors, walls). Low odor, irritation.</td>
</tr>
<tr>
<td>e.g. A-33, Mikro-Quat, Rocaal-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodophhors (30-1000 ppm iodine)</td>
<td>Intermediate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>Inactivated by organic matter.</td>
</tr>
<tr>
<td>e.g. Tincture/ Povodine, Wescodyne, Mikroklene, Isoprep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine (100-1000ppm)</td>
<td>Intermediate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>Not sporicidal; inactivated by organic matter; fresh solutions of hypochlorite (chlorox) should be prepared weekly; corrosive; irritating to eyes and skin.</td>
</tr>
</tbody>
</table>
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 recombinant and synthetic nucleic acid material, biohazardous and potentially infectious materials, ranging from clinical waste, transgenic plants and animals 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 recombinant and synthetic nucleic acids, 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. The biological hazard spill response policy applies to all recombinant and synthetic nucleic acid materials as well as BSL1 and higher pathogens/other potentially infectious material. 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**

The below procedures apply for all BSL1 materials and higher, including recombinant and synthetic nucleic acid material.

**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:**

The reporting requirements and procedures listed below apply to all biohazardous materials, recombinant and synthetic materials, blood, and OPIM:

In the event of an accidental exposure to a biohazard or to recombinant/ synthetic nucleic acid material:

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.
All overt exposures to BSL2 recombinant materials/agents must be immediately reported to REHS. REHS will in report these to the NIH as required in the NIH Guidelines for recombinant and synthetic nucleic acids.

**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.
- Report to REHS immediately.

**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](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, Recombinant/Synthetic Nucleic Acids 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 Membrane**

- 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 of 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

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 (Table 6).

<table>
<thead>
<tr>
<th>Campus</th>
<th>For Employees</th>
<th>For Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newark Campus</td>
<td>Occupational Medical Services</td>
<td>Student Health Services</td>
</tr>
<tr>
<td></td>
<td>Stanley S. Bergen Building</td>
<td>Doctors Office Center</td>
</tr>
<tr>
<td></td>
<td>65 Bergen Street</td>
<td>90 Bergen Street</td>
</tr>
<tr>
<td></td>
<td>Room GA-167</td>
<td>Suite 1750</td>
</tr>
<tr>
<td></td>
<td>Newark NJ 07101-1709</td>
<td>Newark NJ 07101-1709</td>
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<tr>
<td></td>
<td>973-972-2900</td>
<td>973-972-8219</td>
</tr>
<tr>
<td>New Brunswick</td>
<td>Occupational Health Department</td>
<td>Student Health Services</td>
</tr>
<tr>
<td></td>
<td>Hurtado Health Center (Lower Level)</td>
<td>Hurtado Health Center (Upper Level)</td>
</tr>
<tr>
<td></td>
<td>11 Bishop Place</td>
<td>11 Bishop Place</td>
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<td></td>
<td>New Brunswick, NJ 08901</td>
<td>New Brunswick, NJ 08901</td>
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<tr>
<td></td>
<td>848-932-8254</td>
<td>848-932-7402</td>
</tr>
<tr>
<td>Piscataway</td>
<td>RBHS Occupational Health Services</td>
<td>RBHS Occupational Health Services</td>
</tr>
</tbody>
</table>
* 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 regiments (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. cerevesiae*); 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) 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)

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.

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. 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 to 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.

Any potential release of a genetically modified plant, seed, or organism that is not intentional, or loss of material, must be immediately reported to REHS at biosafety@aps.rutgers.edu and to the applicable regulatory agency.

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 or 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 entomophious species, i.e., insect pollinated, may be prevent 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 or 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 greenhouse). 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.
### Table A.1: Comparison of standard practices for transgenic plants

<table>
<thead>
<tr>
<th>Comparison of Standard Practices for Transgenic Plants</th>
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<tbody>
<tr>
<td><strong>BSL1-P</strong></td>
</tr>
<tr>
<td>Discretionary Access</td>
</tr>
<tr>
<td>Personnel must read and follow instructions</td>
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<tr>
<td>Procedures followed are appropriate for organisms</td>
</tr>
<tr>
<td>Records kept of experiments in facility</td>
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<tr>
<td>Biologically inactive experimental organisms</td>
</tr>
<tr>
<td>Pest control program in place</td>
</tr>
<tr>
<td>Appropriate caging and precautions for escape of motile organisms</td>
</tr>
<tr>
<td>Concurrent experiments use same practices</td>
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</table>

*Rutgers Biosafety Guide*
### Comparison of Facilities for Transgenic Plant Containment

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

**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.

<table>
<thead>
<tr>
<th>Tasks: Biosafety Level 1</th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perform hand washing after working with potentially hazardous agents &amp; before leaving the lab</td>
<td></td>
</tr>
<tr>
<td>Refrain from eating or drinking in the lab</td>
<td></td>
</tr>
<tr>
<td>Refrain from mouth pipetting; use only mechanical pipetters</td>
<td></td>
</tr>
<tr>
<td>Perform all procedures to minimize splashes and aerosols</td>
<td></td>
</tr>
<tr>
<td>Handle and store sharp items such as razor blades and needles only in a safe manner</td>
<td></td>
</tr>
<tr>
<td>Dispose sharp items only in sharps containers</td>
<td></td>
</tr>
<tr>
<td>Demonstrate proper use of biosafety cabinet <em>(see second page for breakout section)</em></td>
<td></td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Decontaminate work surfaces properly</td>
<td></td>
</tr>
<tr>
<td>Decontaminate cultures and other liquid wastes prior to disposal down the drain</td>
<td></td>
</tr>
</tbody>
</table>

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Demonstrate awareness of basic lab and biohazard signage

Properly use protective equipment (lab coat, eyewear, gloves)

<table>
<thead>
<tr>
<th>Tasks: Biosafety Level 2 (in addition to BSL-1 tasks)</th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be aware of hazards and meet specific entry requirements</td>
<td></td>
</tr>
<tr>
<td>Be aware of and participate in medical surveillance and immunizations</td>
<td></td>
</tr>
<tr>
<td>Read and understand biosafety manual</td>
<td></td>
</tr>
<tr>
<td>Transport potentially infectious materials only in leak-proof labeled containers</td>
<td></td>
</tr>
<tr>
<td>Be aware of post-exposure procedures in case of infectious agent exposure</td>
<td></td>
</tr>
<tr>
<td>Perform all aerosol-producing procedures only inside BSC</td>
<td></td>
</tr>
<tr>
<td>Use centrifuge safety cups properly</td>
<td></td>
</tr>
<tr>
<td>Remove and dispose of protective garb in the lab</td>
<td></td>
</tr>
<tr>
<td>Understand principals of steam sterilization and understand how to operate an autoclave</td>
<td></td>
</tr>
<tr>
<td>Understand emergency response procedures in the lab</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Use of a biological safety cabinet (BSC)</th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>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)</td>
<td></td>
</tr>
<tr>
<td>Understand what the magnehelic gauge represents and how to verify proper directional airflow</td>
<td></td>
</tr>
<tr>
<td>Verify that the BSC is current for annual certification and is safe to use</td>
<td></td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Tasks: Use of a biological safety cabinet (BSC)</th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintain a safe distance from anyone working at the biosafety cabinet</td>
<td></td>
</tr>
<tr>
<td>Place aerosol-producing equipment such as vortex equipment inside the BSC and toward the rear to prevent aerosols from reentering the worker’s environment</td>
<td></td>
</tr>
<tr>
<td>Load centrifuge safety cups and rotors inside the BSC</td>
<td></td>
</tr>
<tr>
<td>Use protective equipment when working inside the BSC</td>
<td></td>
</tr>
<tr>
<td>Work from “clean” to “dirty” and avoid contaminating clean/sterile materials</td>
<td></td>
</tr>
<tr>
<td>Disinfect used plastic ware by placing in disinfectant reservoirs inside BSC</td>
<td></td>
</tr>
<tr>
<td>Disinfect tools and equipment used in the BSC prior to removal</td>
<td></td>
</tr>
<tr>
<td>Bag disposable items inside the BSC, and seal waste bags, prior to removal</td>
<td></td>
</tr>
<tr>
<td>Understand why the use of flames and gas burners should be avoided</td>
<td></td>
</tr>
<tr>
<td>Demonstrate proper use of vacuum systems including use of traps containing disinfectant and HEPA filters</td>
<td></td>
</tr>
<tr>
<td>Demonstrate proper spill control and cleanup, including cleanup inside the drain pan; awareness of drain valve</td>
<td></td>
</tr>
<tr>
<td>Disinfect the BSC at the end of the work session or the end of the day</td>
<td></td>
</tr>
<tr>
<td>Turn off the blower at the end of the day and close the sash as applicable</td>
<td></td>
</tr>
<tr>
<td>Understand the limitations of UV light and safe operation of UV light in</td>
<td></td>
</tr>
</tbody>
</table>

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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

<table>
<thead>
<tr>
<th><strong>Tasks: Understanding of general practices for cell and tissue culture</strong></th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>
| 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 | |

<table>
<thead>
<tr>
<th><strong>Tasks: Animal Biosafety Level 1 (in addition to BSL-1 tasks)</strong></th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Understand that all animal procedures must be on an IACUC protocol and be familiar with the IACUC protocol in use</td>
<td></td>
</tr>
<tr>
<td>Demonstrate safe handling of animals (grip techniques, positioning animal)</td>
<td></td>
</tr>
<tr>
<td>List animal species the trainee has worked with:</td>
<td></td>
</tr>
<tr>
<td>Demonstrate competence in applicable veterinary procedures such as blood</td>
<td></td>
</tr>
<tr>
<td>Collection, injection, other surgical procedures, and euthanasia and necropsy</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>List veterinary procedures the trainee has performed:</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Demonstrate competence in applicable husbandry procedures such as cage change, cage cleaning, handling bedding, and room and equipment disinfection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>List husbandry procedures the trainee has performed:</strong></td>
<td></td>
</tr>
</tbody>
</table>

| 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 |  |

<table>
<thead>
<tr>
<th><strong>Tasks: Animal Biosafety Level 2 (in addition to all above tasks)</strong></th>
<th>Knowledgeable and Proficient?</th>
</tr>
</thead>
</table>
| Demonstrate proper use of physical and chemical restraints to handle animals  
   Physical restraints may include engineering controls such as squeeze mechanisms |  |
| **List physical and chemical restraints with which the trainee has experience:** |  |

| Demonstrate proper use of additional protective equipment required to handle infectious animals |  |

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<table>
<thead>
<tr>
<th>Demonstrate understanding of how to capture an escaped animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handle and disinfect bedding, carcasses, tissues, and cages</td>
</tr>
<tr>
<td>Demonstrate understanding and proper use of biocontainment caging</td>
</tr>
<tr>
<td>Understand emergency response procedures in the animal facility</td>
</tr>
</tbody>
</table>

**References:**


APPENDIX C: Biological and Medical Waste Disposal Policy

I. Biological Waste

Waste generated from processes related to the basic sciences (e.g., Biology, Chemistry, Physics) and that do NOT involve research pertaining directly to the diagnosis, treatment or immunization of humans or animals (i.e., biomedical research).

Biological waste can be classified into 2 categories:

- **Items generated from processes involving infectious agents**, including pathogenic bacteria, viruses, biological toxins, human cell lines, blood and body fluids, all human and animal cell cultures.
  
  All of these materials will be collected and disposed of as regulated medical waste (see definition in Section II).

- **Items generated from processes involving non-infectious materials**, including non-infectious human, animal, plant and insect agents and environmental organisms. This includes work with recombinant DNA/synthetic nucleic acid work at Biosafety Level 1.
  
  Some of these materials are NOT required to be collected and disposed of as regulated medical waste (see Section I.A.).

All Laboratories

*Registration Reminder:* Experiments involving ANY of the following materials must be registered with, reviewed and approved by the Rutgers Institutional Biological Safety Committee (IBC) prior to starting work.

- (a) recombinant or synthetic nucleic acid molecules,
- (b) genetically engineered organisms,
- (c) genetically engineered plants,
- (b) non-human primates (NHPs) or NHP tissue/cells,
- (c) human cell culture (including established human cell lines),
- (d) human materials, tissue/organs,
(e) pathogenic microorganisms (including BSL1),
(f) human blood or blood products, or
(g) other potentially infectious material (OPIM) from humans or NHPs

All registration submissions for these experiments must be made electronically by utilizing the Biosafety Protocol Management System accessed through logging into [http://myrehs.rutgers.edu](http://myrehs.rutgers.edu). Paper registration and renewal/amendment documents are no longer accepted.

A. Biosafety Level 1 (BSL1) Laboratories

BSL1 applies to work with agents that pose a minimal potential threat to laboratory workers and the environment and do not consistently cause disease in healthy adults. For plant research, BSL1 refers to work with transgenic plants in which there is no evidence that the modified organism would be able to spread in the environment and, if accidentally released, would not pose an environmental risk.

BSL 1: Solids

All solid waste items which are potentially contaminated with microorganisms, tissue culture, cell culture, recombinant or synthetic nucleic acid molecules, genetically engineered organisms, or genetically engineered plants regulated by the CDC/NIH or USDA/APHIS at Biosafety Level 1 (BSL1) must first be chemically disinfected or autoclaved in accordance to their approved biosafety protocol if disposing in regular solid waste (trash). If disposing in regular trash, only clear autoclave bags are permitted to be used (no biohazard symbol). If untreated, BSL1 waste must be collected in the RMW box - untreated BSL1 waste must NOT be disposed of in the regular trash!

**Remember:** Red, orange and even clear autoclave bags with biohazard symbols (regardless of claim that symbol will ‘disappear when autoclaved) must NOT be used for autoclaving BSL1 waste that will be disposed in regular trash receptacles or dumpsters. These bags or any
biohazard labeled items found in regular trash must be reported immediately to biosafety@aps.rutgers.edu.

For laboratories performing both BSL1 and BSL2 activities, all BSL1 solid waste must be overclassified and disposed of as RMW in the RMW box. BSL1 waste does not have to be autoclaved prior to being placed in the RMW box for disposal. BSL2 items must be handled as outlined in the BSL2 section below.

**Autoclave Procedures for BSL1 Waste**

(h) The clear autoclave bag should be filled to two-thirds of its capacity. Autoclaves must be validated according to the REHS policy on autoclave validation (see Appendix G).

(i) After the bag is 2/3 full, it should be loosely taped closed and labeled with the investigator’s name.

(j) Autoclave tape should be affixed to the exterior of the bag to ensure the waste has reached the proper temperature. Waste is autoclaved using appropriate cycle parameters.

(k) After autoclaving, waste is disposed in building dumpster by laboratory staff or by arrangement with housekeeping staff.

**BSL-1: Liquids**

BSL1 contaminated liquid waste must be autoclaved or chemically disinfected (with appropriate disinfectant) prior to drain disposal of the liquid. Any use of chemical disinfectant must allow for the appropriate contact time of the disinfectant before drain disposal. Contact REHS at biosafety@aps.rutgers.edu for information regarding which disinfectants are appropriate for your BSL1 materials.

**BSL-1: Sharps**

BSL1 contaminated sharps, such as syringes (with and without needles), scalpels and blades, including glass Pasteur must be disposed of in an appropriate Sharps Container.
Note: Glass Pasteur pipets, slides and coverslips used in processes that do not fit the regulated medical waste definition, or are not in contact with infectious materials/ recombinant and/ or synthetic nucleic acids may be disposed in the broken glassware box (e.g. preparation of solutions for HPLC, work with BSL1 wet mounts for teaching labs).

B. Biosafety Level 2 (BSL2) Laboratories

Applies to work, with infectious organisms that pose a moderate hazard to healthy adults. For plant researchers, BSL2 is assigned to work with transgenic plants which, if released outside the greenhouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed. BSL2 also applies to transgenic research where the entire genome of an indigenous infectious agent or plant pathogen, which is either indigenous to the area and potentially harmful to the environment, but are manageable, or are exotic but have no potential for causing serious harm to managed or natural ecosystem.

BSL 2: Solids

All solid waste items which are potentially contaminated with microorganisms, tissue culture, cell culture, recombinant or synthetic nucleic acid molecules, genetically engineered organisms or genetically engineered plants which are regulated by the CDC/NIH or USDA/APHIS at Biosafety Level 2 (BSL2) must be autoclaved or chemically disinfected in accordance with their approved protocol and placed into the Regulated Medical Waste stream as Overclassified Medical Waste as outlined below. The following autoclave procedures should be followed when processing biological waste generated in BSL2 laboratories. Note: The color of the autoclave bags used for BSL2 waste is unimportant since the waste is packaged in the Regulated Medical Waste (RMW) boxes for ultimate disposal.

Autoclave Procedures for BSL2 Waste

(a) The orange, red or clear autoclave bag should be filled to two-thirds of its capacity.

(b) After the bag is 2/3 full, it should be loosely taped closed and labeled with the investigator’s name.
(c) Autoclave tape should be affixed to the exterior of the bag to ensure the waste has reached the proper temperature.

(d) Laboratory staff must periodically challenge autoclaves using biological indicators such as spore strips to ensure that biological waste is being appropriately disinfected. See RU Biosafety Guidelines, Appendix G.

(e) Autoclave waste using appropriate cycle parameters for waste.

(f) After autoclaving, waste is labeled with an inner container label (supplied by REHS) and disposed in cardboard RMW box located by the autoclave(s). When the RMW box is full, seal the liner bag, close the bin or seal the box with tape, and affix an outer container label to the outer box.

BSL2: Liquids

All BSL2 liquid waste must be autoclaved or chemically disinfected (with appropriate disinfectant and contact time) prior to drain disposal of the liquid. Contact REHS at biosafety@aps.rutgers.edu for information regarding which disinfectants are appropriate for your BSL2 materials.

BSL2: Sharps

BSL2 contaminated sharps, such as syringes (with and without needles), scalpels and blades, including glass Pasteur pipettes, microscope slides and cover slips must be disposed of in an appropriate Sharps Container. If these materials are used in a mixed BSL1/ BSL2 laboratory, they need to be over-classified into a sharps container.

C. Biosafety Level 3 (BSL3) Laboratories

Applies to work with indigenous or exotic agents that may cause serious or lethal disease via aerosol transmission. For plant research, this applies to plant pathogens, or other organisms that have a recognized potential for significant detrimental impact on the environment. This category also applies to non-genetically engineered plant research that involves exotic infectious agents capable of causing serious environmental harm.
BSL3: Solids, Liquids, Sharps

BSL3 laboratories treat all liquid and solid waste as outlined in a reviewed and approved Standard Operating Procedures (SOPs). These SOPs are in accordance with approved IBC protocols and outline specific procedures for waste disposal. All waste (liquid, solid and sharps) is autoclaved out of the facility in autoclave bags. After autoclaving, inner container labels are affixed to the bag, and the bag is then placed in cardboard regulated medical waste containers. Laboratory staff are to then seal the container and affix an outer container label to the outside. All BSL3 autoclaves are challenged with biological indicators monthly. Select agent facilities must challenge their autoclaves weekly with biological indicators. All autoclave challenges with biological indicators are to be performed as outlined in approved standard operating procedures and/or IBC protocols.

II. Regulated Medical Waste (RMW)

The following instructions apply to generators of Regulated Medial Waste (RMW). At Rutgers University, RMW generators may be engaged in health care delivery, athletics or biomedical research. Rutgers University employees who are reasonably anticipated to come into contact with human blood or blood products must adhere to the Rutgers University Bloodborne Pathogen (BBP) program. Contact REHS at 848-445-2550 or visit [http://rehs.rutgers.edu](http://rehs.rutgers.edu) for BBP program details.

All laboratories and clinical areas generating RMW must attend a RMW Training Session provided by REHS. The requirements for RMW disposal are included in REHS laboratory, biosafety and clinical health and safety trainings. If you plan to generate RMW please contact REHS at biosafety@aps.rutgers.edu or log into [http://myrehs.rutgers.edu](http://myrehs.rutgers.edu) to register for a course or complete an online training module, as applicable. Sessions may also be scheduled and provided as needed to individuals, groups, departments, clinical personnel or laboratories.

The following procedures for the proper processing, transportation, and ultimate disposal of RMW are taken from the Comprehensive Regulated Medical Waste Management Act

Rutgers Biosafety Guide

A) Definition of RMW

The Regulated Medical Wastes subchapter 3A (N.J.A.C. 7:26-3A.6) defines RMW as solid waste that meets both the process definition and the classification definitions listed below. It is defined as any solid waste generated from one of the following processes: the diagnosis, treatment or immunization of humans or animals; research pertaining to the diagnosis, treatment or immunization of humans or animals (i.e., biomedical research); or the production or testing of biologicals.

1) Process Definition: RMW is any solid waste generated from one of the following processes: the diagnosis, treatment or immunization of humans or animals; research pertaining to the diagnosis, treatment or immunization of humans or animals (i.e., biomedical research); or the production or testing of biologicals.

2) Classification Definition: To be considered as RMW, items that are included in the above process definition must also belong to one of the following classes of regulated medical waste.
Table C.1: Classes of Regulated Medical Waste

<table>
<thead>
<tr>
<th>Class</th>
<th>Waste Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>Cultures and Stocks</td>
<td>Cultures and stocks of infectious agents and associated biologicals: cultures from medical or pathological labs; cultures and stocks of infectious agents from research labs; wastes from the production of biologicals; discarded live and attenuated vaccines; culture dishes and devices used to transfer, mix, or inoculate cultures</td>
</tr>
<tr>
<td>Class 2</td>
<td>Pathological Wastes</td>
<td>Human pathological wastes including tissues, organs, and other body parts and fluids that are removed during surgery or autopsy or other medical procedures; specimens of body fluids and their containers</td>
</tr>
<tr>
<td>Class 3</td>
<td>Human Blood &amp; Body Products</td>
<td>Liquid waste human blood; items saturated, dripping or caked with human blood (including serum, plasma and other blood components) which were used or intended for use in either patient care, testing and laboratory analysis, or the development of pharmaceuticals. Intravenous bags, soft plastic pipettes and plastic blood vials are also included in this category.</td>
</tr>
<tr>
<td>Class 4</td>
<td>Sharps</td>
<td>Sharps that were used in animal or human patient care or treatment in medical research or industrial laboratories. Includes hypodermic needles, all syringes to which a needle can be attached (with or without the needle), Pasteur pipettes, scalpel blades, blood vials, carpules, needles with attached tubing, and broken or unbroken glassware (slides and coverslips) that were in contact with infectious agents.</td>
</tr>
<tr>
<td>Class 5</td>
<td>Animal Waste</td>
<td>Contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research, production of biologicals, or testing of pharmaceuticals.</td>
</tr>
<tr>
<td>Class 6</td>
<td>Isolation Waste</td>
<td>Biological waste and discarded materials contaminated with blood, excretions, exudates, or secretions from humans or animals that are isolated to protect others from certain highly</td>
</tr>
<tr>
<td>Class 7</td>
<td>Unused Sharps</td>
<td>Unused, discarded sharps that were intended to be used. Includes hypodermic needles, suture needles, syringes and scalpel blades.</td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>

Communicable diseases.
B) Overclassified Regulated Medical Waste (RMW)

Overclassified RMW is material that does not meet the strict definition of RMW listed in part A, above. Overclassified RMW materials resemble RMW but are generated from activities that do not meet both the “process” definition and the “classification” definition of RMW. Examples of overclassified RMW include materials generated in teaching laboratories (while research laboratories are covered in the process definition, teaching laboratories are not). Overclassified RMW must be packaged and labeled in the same manner as RMW and is collected by the RMW vendor.

Some activities may meet the process definition but generate wastes that do not belong in any of the seven specific classes. In such cases, contact REHS at biosafety@aps.rutgers.edu for guidance on whether the waste should be packaged and labeled as overclassified RMW.

C) RMW In Process

All solid waste containers for RMW collection must be closed when not in use. Laboratories and clinics may have small rigid, leakproof, bag-lined containers with lids and biohazard labels near work areas or on bench tops to collect RMW as it is being generated.

For laboratories, the preferred method of collection for solid RMW at the lab bench is a small, red bag or a rigid container lined with a small red bag that can be closed (either by tying the bag or lid covering the container). Small bench top containers must be emptied into the larger RMW box either when full or not in use. Remember that BSL2 and higher solid RMW must be autoclaved or chemically disinfected prior to being placed in the large RMW box, unless otherwise approved by REHS.

For clinical areas, the solid RMW does not need to be autoclaved or chemically disinfected. Small/temporary containers may be used in areas where the RMW
box would not be appropriate (e.g., patient exam room). When temporary containers are full, the bags must be pulled out by lab or clinic personnel, closed/sealed, labeled with an inner container label and placed in a RMW bin/box. The RMW box is then closed/sealed and labeled with an outer container label.

D) Segregation of RMW

As RMW is generated it must be segregated into the following three categories: sharps (both class 4 and class 7), fluids (greater than 20cc), and other RMW(solid). Collect solid and sharps RMW in separate inner containers appropriate for that waste stream. These inner containers will ultimately be closed and placed into the outer container which is the RMW box/bin. Liquid RMW greater than 20cc must be chemically disinfected for the appropriate contact time, and then drain disposed, unless otherwise approved by REHS. Needles, Pasteur pipettes, glass cover slips, scalpel blades and syringes must be collected in a sharps container; culture transfer devices, blood soaked items, and other paper or cloth related items must be collected in autoclave bags or red RMW liner bags. Do not chop, bend, break or otherwise destroy hypodermic needles or syringes before discarding them into the sharps container.

E) Treatment of RMW

Generally, it is not necessary to treat RMW or overclassified RMW before placing it in the outer container (RMW box) for ultimate disposal. However, Rutgers University policy requires that laboratories working with materials regulated by the CDC or NIH at Biosafety Level 2 or higher autoclave or chemically disinfect their waste prior to placing the waste into RMW boxes for collection by the RMW vendor. After autoclaving or chemical disinfection, this waste material is considered overclassified RMW.
F) **Storage of RMW**

Outer containers must be stored in a secure area protected from the elements, high temperatures, vandalism, insects and rodents. Unauthorized personnel must be denied access to this designated storage area. REHS recommends that RMW boxes/bins are not stored in common areas, e.g. accessible autoclave rooms, hallways. If RMW is stored in a common area the location must be secured, e.g. locked, and the door appropriately labeled. When storing containers, be sure that their labels face outward so that they can be easily seen. Containers must also be sealed securely to prevent spillage, putrescence or the leaking of vapors. Liquids (e.g. blood) must be put into containers that are packaged with a sufficient amount of surrounding absorbent material to absorbent leakage. Volumes of liquid may not exceed 20cc per individual container.

G) **Limitations on Storage of RMW**

NJDEP Solid and Hazardous Waste Rules subchapter 3A: Regulated Medical Wastes (N.J.A.C. 7:26-3A) allows RMW to be stored on site for up to one year. In order to comply with this subchapter, RMW generators must dispose of RMW containers on a yearly basis, even if RMW containers are not full. REHS recommends frequent disposal of RMW boxes/bins.

H) **Packaging, Labeling and Marking Requirements**

1) **Packaging:** The generator must package all RMW before the RMW vendor can remove it. The RMW vendor will not package your waste. The RMW bin/ box must be lined with a red bag before any waste can be placed inside. All needles, syringes, scalpels and any sharp objects must be packaged in an appropriate puncture- resistant sharps container. Unbroken as well as broken glass must be packaged to prevent puncture of the outer RMW container. All other items may be packaged in autoclave bags or other appropriate inner containers. These items must then be packaged in an appropriate medical waste box/bin before removal. Boxes/bins used for the first shipment of RMW can be obtained by contacting REHS at 848-445-2550. Replacement boxes for use with future disposal of RMW will be available from the waste vendor upon arrival or subsequent
pickups. If bins are used, new bins will be supplied to the laboratory. Only the outer containers supplied by REHS or the vendor may be used to package RMW.

2) **Labeling and Marking**: Generators shall mark each package of RMW according to the following labeling and marking requirements before it can be transported off-site by the RMW vendor. The outermost surface of each RMW box/bin prepared for shipment shall be labeled with a biohazard label (most times preprinted on the outer container) and also with a special water resistant identification label called “Medical Waste Outer Container Label.” The Medical Waste Outer Container Label is available from REHS and provides the following information: campus, building and room where waste was generated. If these labels are unavailable, the required information may be written directly on the outside of the box/bin. Only indelible or waterproof ink or permanent marker may be used to complete this label, or to label the box. If there is not a biohazard label preprinted on the container, a label must be affixed prior to disposal. In addition to the requirements above, the generator must label inner containers including sharps containers and fluid containers. Each inner container shall be labeled only with a special water resistant identification label called “Medical Waste Inner Container Label.” The Medical Waste Inner Container Label is available from REHS and provides the following information: campus, building, room, phone number and contact person name for the location where the waste was generated.

Note that all containers, both inner and outer, must be marked with the required information. Labels may be obtained by contacting REHS at biosafety@aps.rutgers.edu.

I) **Tracking Form for RMW**

The NJ Medical Waste Tracking Form (Section C.1) is used to ensure proper transportation of RMW to an appropriate disposal site. Rutgers University has arranged with the RMW vendor to supply the four-copy RMW Tracking Form. The RMW vendor will fill out the tracking form. The generator must check Items 1 through 14 on the tracking form for purposes of verifying the accuracy of the information listed. After a thorough review of items 1 through 14, the generator must then sign Item 15 of the tracking form. After the
RMW transporter has also signed in Item 16, Copy 4 (goldenrod sheet) of the tracking form will be given to the generator.

After the RMW is received by the disposal facility, a disposal facility representative will sign in Item 22. Copy 1 (white sheet) will be mailed back to REHS. Copy 4 (goldenrod sheet) of the tracking form must be kept by the generator or building contact representing the generator at the generation site for at least three years from the date the waste was accepted by the RMW transporter. The destination facility will send Copy 1 to REHS within 15 days of receipt of the tracking form from the RMW hauler, if Copy 1 is sent to you inadvertently please forward Copy 1 to REHS.

J) **RMW Inspections**
Periodically, the New Jersey Department of Environmental Protection inspects RMW compliance at Rutgers University facilities. The inspector may visit health centers and other clinical areas, laboratories, or athletic training areas. If any inspector visits without a REHS representative, please contact REHS immediately and wait for the REHS representative to arrive before beginning any opening conference with the inspector. REHS is the designated university representative.

K) **Scheduling a RMW Pickup**
The RMW vendor makes regularly scheduled pick-ups of RMW boxes. Most buildings and campuses have weekly pick-ups, but the frequency varies based on the volume of waste generated at each pick-up location. Additional pick-up requests and one-time pick-ups must be requested online at http://rehs.rutgers.edu. It is important that all requirements be completed prior to a pickup (e.g. labeling of the inner and outer container and sealing the box). Note: The RMW vendor will not pick up the waste without a representative of the RMW generator (e.g., a lab member or building contact representing the generator) being present to sign the RMW tracking form.

L) **Supplies**
REHS will provide the following upon an initial request: RMW boxes, RMW liner bags
(red bags), and RMW labels (inner and outer). After the first set of supplies are delivered to the area, REHS can assist laboratories and clinical areas with acquisition of supplies from the RMW vendor. It is the responsibility of individual laboratories to purchase sharps containers, autoclave bags, autoclave indicator tape, and packing tape from appropriate laboratory supply vendors.

\textbf{M) Definitions}

1) “Biologicals” means preparations made from living organisms and their products; includes vaccines and cultures intended to be used for diagnosing, immunizing, or treating humans or animals or in research pertaining thereto.

2) Blood Products” means any product derived from human blood, including blood plasma, platelets, red or white blood corpuscles; and other derived licensed products including interferon, etc.

3) “Generator” means any person, by site, whose act or process produces regulated medical waste as defined in N.J.A.C. 7:26-3A.6, or whose act first causes a regulated medical waste to become subject to regulation.

4) “Infectious agent” means any organism (such as a virus or bacteria) that is capable of being communicated by invasion and multiplication in body tissues and capable of causing disease or adverse health impacts in humans.

5) “Inner container” means any container (sharps container, autoclave bag, 5-gallon bucket) that would collect RMW and would ultimately be placed into a properly lined outer container. This container must be labeled with the “inner container label.”

6) “Inner container label” means the label available from REHS which states the campus, building, room, phone number and contact person name for the location where the RMW was generated.

7) “Laboratory” means any research, analytical, or clinical facility that performs health care related analysis or service. This includes medical, pathological, pharmaceutical, research, commercial and industrial laboratories.

8) “Medical waste” means any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals; in research pertaining thereto; in the testing of biologicals; or in home self-care.

Rutgers Biosafety Guide
9) “Outer container” means the cardboard box that is supplied by the RMW vendor or REHS to collect inner containers of RMW. This outer container must be lined with a red RMW bag prior to placing any inner containers into the box. This box also must be labeled with the “outer container label.”

10) “Outer container label” means the label available from REHS which states the campus, building and room where RMW was generated.

11) “Regular trash” means non-regulated, non-contaminated waste. This waste will not be transferred off site to a dedicated waste facility, but will be co-mingled with regular waste streams.

12) “Transporter” means a person engaged in the off-site transportation of regulated medical waste by air, rail, highway, or water.

III. Other Facilities generating RMWs

A) Animal Facilities
Animal carcasses, body organs and bedding from animals that had been exposed to an agent that can cause disease in humans must be disposed of according to the procedures outlined in the IBC and/or IACUC protocols. All such materials must be autoclaved and then collected in regulated medical waste, unless otherwise approved by REHS. Animal carcasses, body parts and bedding from animals exposed to pharmaceutical compounds must also be collected as regulated medical waste. Exceptions to this would be animals exposed to hazardous chemicals, which materials required disposal through REHS. For any questions, contact REHS at biosafety@aps.rutgers.edu.

B) Clinical Areas
Clinical areas may generate different waste streams than research laboratories. These areas generate regular trash, dirty linens, sharps, body fluids, other potentially infectious material, and other materials generated during patient treatment. Any liquid waste generated over 20cc should be referred to REHS for disposal instructions. Solid waste that must be disposed of as regulated medical waste includes tubing, gloves, paper gowns, paper linens, and clean up debris that is visibly contaminated with blood or...
potentially infectious human fluids. Fecal smear cards must also be collected as solid regulated medical waste. Solid waste must be disposed and packaged as outlined in Section II - Regulated Medical Waste.

C) Non-Biological Laboratories

Laboratories not performing biological related research may generate different waste streams than BSL1, BSL 2, or BSL 3 research laboratories. These laboratories may generate sharps (Class 4 RMW) and Unused Sharps (Class 7 RMW). Any sharps waste generated must be referred to REHS for disposal instructions. All needles, syringes without needles, razors and scalpels must be placed in an approved Sharps Container and disposed as RMW, even if they have not been in contact with any biological or infectious material. Other materials such as glass pipets, Pasteur Pipets may be placed into broken glass if they were not used in conjunction with recombinant materials, pathogens or animals. Any sharps waste contaminated with chemicals and/or radiological material must still be placed in a separate approved Sharps Container and referred to REHS for disposal in accordance with the respective Hazardous Waste Disposal Policy and/or Radiological Waste Disposal Policy.
**Attachment C.1: Regulated Medical Waste Tracking Form**

```
<table>
<thead>
<tr>
<th>Field</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generator's Name and Mailing Address</td>
<td></td>
</tr>
<tr>
<td>Tracking Form Number</td>
<td>B 1091411</td>
</tr>
<tr>
<td>State Permit or ID No.</td>
<td></td>
</tr>
<tr>
<td>Transporter's Name and Mailing Address</td>
<td></td>
</tr>
<tr>
<td>Telephone Number</td>
<td></td>
</tr>
<tr>
<td>State Transporter Permit or ID No.</td>
<td></td>
</tr>
<tr>
<td>Destination Facility Name and Address</td>
<td></td>
</tr>
<tr>
<td>Telephone Number</td>
<td></td>
</tr>
<tr>
<td>New Jersey Waste Description</td>
<td></td>
</tr>
<tr>
<td>Total No. Cont.</td>
<td></td>
</tr>
<tr>
<td>Total Weight</td>
<td></td>
</tr>
<tr>
<td>Additional Information</td>
<td></td>
</tr>
<tr>
<td>Overclassified Material</td>
<td></td>
</tr>
<tr>
<td>Central Collection Point, Transfer Station Activity and Other Information</td>
<td></td>
</tr>
<tr>
<td>Transporter 1 (Certification of Receipt of RMW as described in Items 11, 12, &amp; 13)</td>
<td>PRINTED / TYPED NAME</td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Transporter 2 or Intermediate Handler</td>
<td></td>
</tr>
<tr>
<td>Total No. Cont.</td>
<td></td>
</tr>
<tr>
<td>Total Weight</td>
<td></td>
</tr>
<tr>
<td>Description of RMW</td>
<td></td>
</tr>
<tr>
<td>State Transporter Permit or ID No.</td>
<td></td>
</tr>
<tr>
<td>New Tracking Form Number (for consolidated or unmanifested RMW)</td>
<td>PRINTED / TYPED NAME</td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Destination Facility (Certification of Receipt of RMW as described in Items 11, 12, &amp; 13)</td>
<td>PRINTED / TYPED NAME</td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
<tr>
<td>Emergency Telephone Numbers (24 hours a day):</td>
<td></td>
</tr>
<tr>
<td>Emergency Response</td>
<td>National Response Center: 1-800-427-9602</td>
</tr>
<tr>
<td>Emergency Discharge</td>
<td>US Department of Transportation: 1-800-252-7122</td>
</tr>
<tr>
<td>Infectious Substance Spills</td>
<td>Centers for Disease Control: 1-800-539-7222</td>
</tr>
</tbody>
</table>
```
The generator must package all RMW before the RMW vendor can remove it.

- **Is RMW Generated?**
  - Yes: **Is RMW Generated Mixed with Radiological or Chemical Waste?**
    - Yes: Contact REHS for guidance.
    - No: **Is BSL-2/2+ Waste Disinfected or Inactivated?**
      - Yes: Are Containers and/or Bags Sealed?
        - Yes: Place Sharps Containers and RMW bags in lined Box provided for Disposal. Label with a “Medical Waste Inner Container Label”.
        - No: Autoclave or Chemically Disinfect Waste
      - No: Seal Bag, Place a “Medical Waste Inner Container Label” on the Outside of the Bag, Tape the Box Close, and Place a “Medical Waste Outer Container Label” on the Outside of the Box (See Picture Below)

- No: Follow Applicable Disposal Policy

Request Disposal by submitting the On-Line Biological and Medical Waste Disposal Request Form at [http://rehs.rutgers.edu/](http://rehs.rutgers.edu/) or by following your facility-specific procedure.

- **Medical Waste Inner Container Label**
- **Medical Waste Outer Container Label**
- **RMW Shipping Box**
- **RMW Red Liner Bag**
The generator must package all RMW before the RMW vendor can remove it.

- **Is RMW Generated?**
  - Yes: Place Sharps Containers and RMW bags in lined Box provided for Disposal. Label with a “Medical Waste Inner Container Label”
  - No: Are Containers and/or Bags Sealed?
    - Yes: Seal Bag, Place a “Medical Waste Inner Container Label” on the Outside of the Bag, Tape the Box Close, and Place a “Medical Waste Outer Container Label” on the Outside of the Box (See Picture Below)
    - No: Ensure Lids and Bags are closed/sealed

- **Is RMW Generated Mixed with Radiological or Chemical Waste?**
  - Yes: Contact REHS for guidance.
  - No: Follow Applicable Disposal Policy

Request Disposal by submitting the On-Line Biological and Medical Waste Disposal Request Form at [http://rehs.rutgers.edu/](http://rehs.rutgers.edu/) or by following your facility-specific procedure.
## Regulated Medical Waste Disposal Table: Research Laboratories

<table>
<thead>
<tr>
<th>Items</th>
<th>Infectious (includes human/non-human primate materials, toxins, microorganisms)</th>
<th>Recombinant or Synthetic Nucleic Acid (r/sNA) (includes plant materials)</th>
<th>Other Biological (non-biomedical research, non r/sNA, non-infectious)</th>
<th>Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sharps:</strong> Syringes with and without needles</td>
<td>Red Sharps Container into Regulated Medical Waste (RMW) box</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(For your safety do not remove needles from syringes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unused sharps</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scalpel blades</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Needles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass blood vials (empty/residual only)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glass material:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasteur pipettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serological pipettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flasks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plates</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Microscope slides/cover slips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass vials with agar slant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broken or intact glassware</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disposable Non-Sharps:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serological pipettes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micropipette tips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petri dishes with/without agar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloves, disposable gowns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bench paper and towels</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Paper materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BSL1:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Autoclave in Clear autoclave bag and dispose in regular trash, OR:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Place directly into RMW box</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>BSL2:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Autoclave or disinfect for appropriate contact time (e.g., 1:10 dilution of bleach for 15 minutes) and place into RMW</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biological/Plant toxins/pathogens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disinfect for appropriate contact time (e.g., 1:10 dilution of household bleach for 15 minutes), then drain dispose.</td>
<td></td>
<td></td>
<td>Follow REHS guidance for collection and disposal as hazardous chemical waste: (<a href="http://rehs.rutgers.edu/pdf_files/hazwaste_disposal.pdf">http://rehs.rutgers.edu/pdf_files/hazwaste_disposal.pdf</a>).</td>
<td></td>
</tr>
<tr>
<td><strong>Liquid Waste:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid media and cultures aspirated or decanted from flasks and dishes</td>
<td>Disinfect for appropriate contact time (e.g., 1:10 dilution of household bleach for 15 minutes), then drain dispose.</td>
<td></td>
<td>Follow REHS guidance for collection and disposal as hazardous chemical waste: (<a href="http://rehs.rutgers.edu/pdf_files/hazwaste_disposal.pdf">http://rehs.rutgers.edu/pdf_files/hazwaste_disposal.pdf</a>).</td>
<td></td>
</tr>
<tr>
<td><em>Note:</em> No standing liquids allowed in biomedical waste box.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mixed Wastes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazardous chemicals and/or Radioisotopes mixed with biohazardous/infectious materials</td>
<td>Contact REHS before generating such waste. 848-445-2550</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact REHS before generating such waste. 848-445-2550</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Regulated Medical Waste Disposal Guide for Laboratories

## Medical Waste Disposal:
- [http://halflife.rutgers.edu/forms/medwaste.php](http://halflife.rutgers.edu/forms/medwaste.php)

## Hazardous Waste Disposal:
- [http://halflife.rutgers.edu/forms/hazwaste.php](http://halflife.rutgers.edu/forms/hazwaste.php)

## Radioactive Waste Disposal:
- [http://halflife.rutgers.edu/forms/radwaste.php](http://halflife.rutgers.edu/forms/radwaste.php)

## General Trash
- paper and plastic packaging, wrappers
- small amounts of **non-hazardous** solid waste (i.e., sugars, salts, amino acids, enzymes, etc.)
- **non-hazardous** media and gels (i.e., No gels containing more than 0.5 µg/ml ethidium bromide!)
- other **non-contaminated** solids (e.g., paper towels, bench paper, gloves)
- alkaline batteries

### RECYCLE NON-HAZARDOUS WASTES
As Appropriate

### CLEAR BAG
- **uncontaminated** broken and intact glassware
- empty, triple rinsed* chemical reagent bottles (deface the label and remove the lid first)
- Glass boxes must be lined with a **clear**, thick plastic bag
- **When the container is ⅔ full, the entire box must be removed by Physical Plant Environmental Services.**
- The laboratory replaces with a new box and liner.

### GLASS BOX
- **uncontaminated** broken and intact glassware
- empty, triple rinsed* chemical reagent bottles (deface the label and remove the lid first)
- **Glass boxes must be lined with a clear, thick plastic bag**

### SHARPS CONTAINER
- Including, but not limited to:
  - needles (including those with attached tubing or filters)
  - syringes – **with or without** needle (don’t remove needle!)
  - razors/scalpels
  - pasteur pipettes
  - blood vials (animal or human)
  - slides/cover slips

### REGULATED MEDICAL WASTE (RMW) BIN
- *BSL2 and higher infectious, rDNA and synthetic DNA waste must be autoclaved or chemically disinfected - before disposal!*
- After disinfection, the following liquids can be drain disposed:
  - human/non-human primate cell culture
  - human/non-human blood
  - human/non-human body fluids
  - bacterial/viral/fungal cultures

### OTHER MEDICAL WASTE
- Solid waste contaminated with:
  - cultures/stocks of infectious agents or BSL2 microorganisms
  - Live and attenuated vaccines/viruses
  - human/non-human primate cells, blood, body fluid, tissues, and other source materials
  - recombinant/synthetic DNA materials

  *Includes contaminated plasticware, serological pipet, pipet tips, tubes (i.e., Eppendorf, PCR), gloves, pathological waste (without fixative), and specimen bags with biohazard labels

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**All RMW containers should have a biohazard warning label on the container and the lid.**

**Keep RMW containers CLOSED when you are not actively adding waste.**

**Do not overfill containers!**

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All hazardous waste and rinsate should be disposed of through REHS. Please go to the appropriate website to request for waste disposal.

Medical Waste Disposal: [http://halflife.rutgers.edu/forms/medwaste.php](http://halflife.rutgers.edu/forms/medwaste.php)

Hazardous Waste Disposal: [http://halflife.rutgers.edu/forms/hazwaste.php](http://halflife.rutgers.edu/forms/hazwaste.php)

Radioactive Waste Disposal: [http://halflife.rutgers.edu/forms/radwaste.php](http://halflife.rutgers.edu/forms/radwaste.php)
# Regulated Medical Waste Disposal Guide for Clinical Areas

<table>
<thead>
<tr>
<th>CLEAR BAG</th>
<th>CLEAR BAG</th>
<th>SHARPS CONTAINER</th>
<th>REGULATED MEDICAL WASTE (RMW) BIN or BOX</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Clear Bag" /></td>
<td><img src="image2" alt="Clear Bag" /></td>
<td><img src="image3" alt="Sharps Container" /></td>
<td>Regulated Medical Waste must be placed in an approved box or bin that is lined with a red plastic bag in accordance with the RU Biological and Medical Waste Policy. Other disposal methods must be arranged with REHS.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>General Trash</th>
<th>Dirty Linens</th>
<th>Used and Unused Sharps</th>
<th>Liquids</th>
<th>Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Paper and plastic packaging, wrappers</td>
<td>- Non-contaminated (NO visible blood): paper liners and gowns, disposable gloves</td>
<td>- Each clinical site is responsible for arranging used linen collection, as needed.</td>
<td>- Including, but not limited to:</td>
<td>- Including, but not limited to:</td>
</tr>
<tr>
<td>- Plastic trays/holders from sterile procedure trays (e.g., bone marrow biopsy trays, lumbar biopsy trays).</td>
<td>- All used linen, even linen contaminated with visible blood, must be placed in an appropriate collection receptacle while storing prior to laundering.</td>
<td>- Needles (including those with attached tubing or filters)</td>
<td>- Items contaminated with visible blood and/or body fluids:</td>
<td>- Items contaminated with visible blood and/or body fluids:</td>
</tr>
<tr>
<td>- REMEMBER: ALWAYS check trays for presence of sharps prior to disposal!</td>
<td></td>
<td>- Biopsy needles</td>
<td>- tubing</td>
<td>- tubing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Syringes – with or without needle (don’t remove, bend or recap needles!)</td>
<td>- gloves</td>
<td>- gloves</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Razors/scalpels</td>
<td>- paper gowns</td>
<td>- paper gowns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Scissors/tweezers</td>
<td>- paper liners</td>
<td>- paper liners</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Wires</td>
<td>- Blood/body fluid clean up debris</td>
<td>- Blood/body fluid clean up debris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Slides/cover slips</td>
<td>- Contact REHS for Inner and Outer Container Labels!</td>
<td>- Contact REHS for Inner and Outer Container Labels!</td>
</tr>
</tbody>
</table>

**All RMW containers should have a biohazard warning label on the container and the lid.**
RMW Bins or Boxes must be lined with red plastic bags and CLOSED when you are not actively adding waste. When 2/3 full, staff MUST seal the bags with tape and label bags with a REHS provided “Inner Container Label” A REHS provided “Outer Container Label” must be placed on the sealed box Do not overfill containers!

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**Biological and Medical Waste Disposal can be requested**
APPENDIX D: Biosafety Guidelines for Teaching Laboratories

The American Society for Microbiology (ASM) Education Board published Guidelines for Teaching Laboratories in 2012 (1). The ASM publication was influenced by the lack of clear safety guidelines for microbiology teaching labs and a multistate outbreak of *Salmonella typhimurium* originating in teaching and clinical laboratories in 2011 (2). Unfortunately, a similar incident occurred in 2014, thus reinforcing the need for these guidelines (3). The ASM guidelines include recommendations for working at Biosafety level 1 and Biosafety Level 2. A major finding of the epidemiological investigation of the outbreak was deficiencies in biosafety awareness and proper training of staff and students. Rutgers University has many teaching labs at the introductory, and advanced undergraduate levels as well as graduate levels. Rutgers Environmental Health and Safety (REHS) has compiled guidelines with input from the Rutgers University Institutional Biosafety Committee and instructors of these courses in order to ensure our teaching labs are safe for students and to prevent pathogen exposure to persons and the environment.

This document contains biosafety requirements for teaching laboratories operating at BSL1 and BSL2. This document is meant to supplement the detailed resources outlined in the Rutgers Biosafety Guide. Not all teaching laboratories are equipped to safely operate at BSL2. Any and all use of BSL2 organisms must be preapproved by the Rutgers Institutional Biosafety Committee (IBC). An IBC protocol must be completed in the biosafety database, located at http://myrehs.rutgers.edu before any BSL2 work is performed in the teaching laboratory. Please contact the biosafety group in REHS at biosafety@aps.rutgers.edu with any questions or clarifications regarding assigned biosafety levels.

*A word about subculturing “unknown” samples and teaching about differential and selective media:*

The procedures needed to identify unknown microorganisms can be performed safely, and with little to no risk to the students. Students are permitted to culture organisms from soil, water, food materials, and the air. Subculturing from the initial culture plate is permitted for the above samples, but IBC review and approval must be obtained if differential media used in the experiment could select for the growth of organisms listed at risk group 2 or higher. If the
samples will be used to only count and understand the types of organisms in a particular environment, and no subculturing performed, then IBC approval will not be required. If the laboratory will include subculturing and isolation from environments such as water fountains, door handles or other areas that could harbor pathogens, review and approval by the IBC must be obtained. Additionally, samples must never be cultured from the students themselves without approval from the Institutional Biosafety Committee, and possibly the Institutional Review Board, as there is the potential to grow microorganisms that require BSL2, or even BSL3 containment.

It is recommended that testing of unknowns should be performed from a mixture of known microorganisms (to the instructor), or from a culture where the contents are known to the instructor, instead of from the environment.

For recommendations on surrogate microorganisms, please contact the REHS biosafety office at biosafety@aps.rutgers.edu.

**Minors working in biological labs at Rutgers University**

All minors working in research laboratories will have their research projects approved by Rutgers Environmental Health and Safety, and the University policy on the Protection of Minors will apply. Minors in laboratories are permitted to work with well-established BSL1 materials only, unless approved by REHS.

**Biosafety Level 1**

Biosafety Level One (BSL1) includes microorganisms that are not known to cause human disease, and that can be handled safely on bench tops. The use of BSL1 is the most appropriate for most teaching laboratories.

**BSL1 Requirements**

**Laboratory Facility Requirements:**

- Non-porous floor, bench tops, chairs and stools*
- Sink for hand-washing
- Eyewash station
- Lockable door to the laboratory
- **Proper pest control practices**
- **Recommended**: Separate storage area for personal belongings
- **Recommended**: Access to a working and validated autoclave

*It is understood that some current facilities may not be able to meet these requirements due to the original design of the laboratory space. Any facility renovation or new construction would need to include these requirements.

**Please refer to the Rutgers University Biosafety Guide for details and contact REHS with questions** ([biosafety@aps.rutgers.edu](mailto:biosafety@aps.rutgers.edu)).

**Stock Culture Requirements:**
- Stock cultures must be from authorized, commercial or reputable sources. As indicated above, subculturing microbes isolated from the environment, clinical samples or other unknown locations is discouraged as BSL2 classified microbes may be isolated. Subculturing from the environment must be reviewed and approved by the IBC.
- **Recommended Microbes and ATCC numbers**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>BSL</th>
<th>ATCC number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alcaligenes faecalis</em></td>
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<td><em>Aspergillus niger</em></td>
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<td><em>Bacillus globigii</em></td>
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<td><em>Bacillus stearothermophilus</em></td>
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<td><em>Bacillus subtilis</em></td>
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<td><em>Bacillus megaterium</em></td>
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<td><em>Citrobacter freundii</em></td>
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<td><em>Enterobacter cloacae</em></td>
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<tr>
<td><em>Enterococcus casseliflavus</em></td>
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<td><em>Enterococcus durans</em></td>
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<td><em>Escherichia coli K12</em></td>
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<td><em>Geobacillus</em></td>
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<tr>
<td>--------------------------------</td>
<td>-----------</td>
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<tr>
<td>stearothermophilus</td>
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<tr>
<td>Halobacterium salinarum</td>
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<td>Klebsiella oxytoca</td>
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<td>Lactobacillus acidophilus</td>
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<td>Micrococcus luteus</td>
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<td>Penicillium chrysogenum</td>
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<td>Serratia liquefacens</td>
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<td>Serratia marcescens Bizio</td>
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<td>Staphylococcus epidermidis</td>
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<tr>
<td>Staphylococcus saprophyticus</td>
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</table>

- Laboratory instructor must maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis (at least annual) to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Protocols that can be performed easily at BSL1: anaerobic growth, Gram stain, capsule stain, endospore stain, flagella stain, carbohydrate fermentation, casein hydrolase, catalase and oxidase test, bacterial enumeration, eosin methylene blue plate, gelatin hydrolysis, hanging drop, indole methyl red Vogues-Proskauer and Citrate (IMViC), Kirby-Bauer, Luria broth, litmus milk, 4-methylumbelliferyl-β-D-glucuronide Escherichia coli broth medium (E. coli MUG), MacConkey Agar, mannitol, nitrate reduction, spread, pour and quadrant streak plate, starch hydrolysis, transformation assay,
urease, triple sugar iron, use of lambda bacteriophage, bacterial transformation, plasmid DNA isolation, restriction endonuclease digestion, and gel electrophoresis.

**Personal Protective Equipment Requirements:**

- Safety goggles or safety glasses (with side shields) must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash. If glasses are shared among students, they must be sanitized with an appropriate disinfectant after use.
- Long pants/long skirts (ankle length), or other clothing to cover exposed skin must be worn.
- Closed toe and heel shoes that cover the top of the foot must be worn.
- Gloves must be worn when the student has fresh cuts or abrasions on the hands, when staining microbes and when handling hazardous chemicals. Gloves are not required for standard microbiological work at BSL1 as long as proper hand hygiene is performed. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.
- **Recommended:** Laboratory coats should be worn when handling cultures. In rooms where classes are working with risk group 2 agents, laboratory coats must be worn by all persons at all times.

**Laboratory Work Practices:**

- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
- Lab benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Any materials that are spilled must be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. REHS can be consulted for disinfectant recommendations.
- Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
• Do not touch your face, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.

• All personal items must be stowed in a clean area while in the laboratory. The use of cell phones, tablets and other personal electronic devices is prohibited.

• Mouth pipetting is prohibited.

• All containers must be labeled clearly.

• The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor must approve all persons entering.

• Sharps usage must be minimized. Needles and scalpels are to be used according to institutional guidelines. All sharps must be disposed in a sharps container (includes coverslips, slides and Pasteur pipets).

• Test tube racks or other secondary containers must be used to move cultures in the laboratory.

• Stocks and other cultures must be stored in a leak-proof container when work is complete.

• Waste materials from the laboratory must be disposed in accordance with the Rutgers Biological and Medical Waste Disposal Policy

• Broken glass must be handled using a dustpan and broom or forceps/ tongs, not picked up by students or laboratory personnel with their hands. If contaminated, broom will need to be disposed or sterilized.

• All spills or injuries must be immediately reported to the laboratory instructor. Spills or injuries must then be documented with REHS according to established RU policies. REHS can be reached at 848-445-2550.

• Teach, practice and enforce the proper wearing and use of personal protective equipment.

• Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. (Students should not be asked to reveal if they are immunocompromised. A general announcement should be made that students with a reduced immune status should consult with Student Health Services. A note from Student Health Services is sufficient to excuse a student from laboratory work.)
• **Recommended**: Supply pens and pencils for students, and keep separate from personal items.

• **Recommended**: Keep note taking and discussions separate from work with laboratory materials.

• **Recommended**: Use micro-incinerators rather than Bunsen burners.

**Training Practices:**

• Faculty and teaching assistants must complete REHS laboratory safety, blood-borne pathogens and biosafety trainings.

• Instructors and/or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign-in sheet maintained by the instructor. This can be performed using an online system, such as Sakai, Moodle, or Blackboard.

• Students and instructors are required to handle microorganisms safely and in conjunction with requirements outlined in the Rutgers University Biosafety Guide

• Inform students of safety precautions applicable to each exercise before the procedure is performed.

**Documentation:**

• Safety Data Sheets (SDS) must be available in the laboratory for all chemicals

• Require students to sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. The laboratory instructor must maintain student signed agreements in the laboratory. Alternatively, this can be performed and maintained online within Sakai, Moodle or Blackboard.

• Prepare, maintain and post caution signs on lab doors (complete with biohazard symbol).

• Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.

• Emergency phone numbers and information must be posted in the laboratory.
• Annual submission of course manual and list of microorganisms used in the laboratory to
  the REHS Teaching Laboratory Database located in the Biosafety Protocol Management
  System at http://myrehs.rutgers.edu. Any major deviation from the material submitted
  must be updated and approved before the new semester as appropriate.

Biosafety Level Two

Biosafety Level Two (BSL2) laboratories are suitable for working with microbes posing a
  moderate risk to the individual and a low community risk for infection. There are many
  microorganisms handled at BSL2 that can cause disease in humans via ingestion or inoculation.
  The guidelines for BSL2 laboratories build upon those for BSL1 facilities, and typically include
  additional engineering controls to protect students such as biological safety cabinets, centrifuge
  safety cups and safety needle devices.

BSL2 Requirements

Laboratory Facility Requirements:
• Non-porous floor (e.g. tile or epoxy), bench tops, chairs and stools*
• Sink for hand-washing
• Eyewash station
• Lockable door to the laboratory
• Proper pest control practices
• Separate storage area for personal belongings*
• Working and validated autoclave (using biological spore indicators as outlined in
  biosafety guide)
• Biohazard signage where cultures are used and stored (e.g. incubators), on the door to the
  room and on containers used to transport cultures.
• Recommended: Biological Safety Cabinet. Please see requirements below. All biological
  safety cabinets must be certified by an approved vendor annually.
• *It is understood that some current facilities may not be able to meet these requirements
  due to the original design of the laboratory space. Any facility renovation or new
  construction would need to include these requirements.
Please refer to the Rutgers University Biosafety Guide for details and contact REHS with questions (biosafety@aps.rutgers.edu).

Stock Culture Requirements:

- Stocks must be from authorized, commercial or reputable sources. Do not subculture microbes isolated from the environment, clinical samples or other unknown locations because they may be microbes that require BSL2 practices and facilities. Samples must never be obtained from clinical sites unless a full description of strain antibiotic susceptibility and resistance is provided, and the IBC has approved the use of these strains for the laboratory.
- Strains resistant to clinically relevant antibiotics may not be handled in teaching laboratories.
- Maintain documentation for all stock organisms, sources and handling of stock cultures.
- Obtain fresh stock cultures of microorganisms on a regular basis to be certain of the source culture, minimize spontaneous mutations and to reduce contamination.
- Store stocks in a secure (locked) area.
- Substitute surrogates for common BSL2 pathogens

- Common Microbes and ordering information from ATCC

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>BSL</th>
<th>ATCC Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus mirabilis</td>
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<tr>
<td>Proteus vulgaris</td>
<td>2</td>
<td>33420,8427,6380,49132</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>2</td>
<td>700720</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>12600</td>
</tr>
</tbody>
</table>

- When choosing a test organism, many instructors want to choose organisms that are clinically relevant, i.e. pathogens. There are six microorganisms that are considered major threats, not because they cause the most devastating illnesses but because they comprise the majority of antibiotic-resistant infections observed in health care settings. These are referred to as ESKAPE pathogens and include Enterococcus faecium, Staphylococcus aureus,
Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and species of Enterobacter (ESKAPE).

- ESKAPE pathogen > Safe Relative
  Enterococcus faecium > Enterococcus raffinosus or Enterococcus casseliflavus
  Staphylococcus aureus > Staphylococcus epidermidis
  Klebsiella pneumonia > Escherichia coli
  Acinetobacter baumannii > Acinetobacter baylyi
  Pseudomonas aeruginosa > Pseudomonas putida
  Enterobacter species > Enterobacter aerogenes

Personal Protective Equipment Requirements:
- Safety goggles or safety glasses must be worn when handling liquid cultures, spread plating, or when performing procedures that may create a splash.
- Closed toe and heel shoes that cover the top of the foot must be worn.
- Long pants/long skirts (ankle length), or other clothing to cover exposed skin must be worn.
- Laboratory coats must be worn. These can be disposable or made of cloth. Disposable coats may be reused but must be replaced on any sign of damage or degradation. Lab coats must be stored within the laboratory and must be assigned to individual students, not shared.
- Gloves must be worn when handling cultures, when staining microbes and when handling hazardous chemicals. Hands must be washed immediately after handling microbial cultures and anytime accidental contact occurs with the skin. Hand cleansing must be performed with soap and water, and if none is available with ethanol based hand sanitizer. Soap and water must be used as soon as possible if hand sanitizer is used.

Laboratory Work Practices:
- Wash hands after entering and before leaving the laboratory.
- Long hair must be tied back.
- Long, dangling jewelry is not permitted in the laboratory.
• Lab benches must be disinfected upon entering the laboratory and at the end of the laboratory session. Additionally, if any materials are spilled, they will be immediately cleaned-up. Disinfectants used must be effective against microbes used in the laboratory. REHS can be consulted for disinfectant recommendations.
• Food, water bottles, gum, and drinks of any kind are prohibited in the laboratory.
• Do not touch your face, apply cosmetics, adjust contact lenses, bite nails, or chew on pens/ pencils in the laboratory.
• All personal items must be stowed while in the laboratory. The use of cell phones is prohibited.
• Mouth pipetting is prohibited.
• All containers must be labeled clearly.
• The laboratory door must remain closed at all times when the lab is in session. The laboratory instructor approves all persons entering.
• Minimize use of sharps. Needles and scalpels are to be used according to institutional guidelines. All sharps must be disposed in a sharps container.
• Test tube racks or other secondary containers must be used to move cultures in the laboratory.
• Stocks and other cultures must be stored in a leak-proof container when work is complete.
• Students must be taught proper technique to minimize production of aerosols. For example: when pipetting, place tip on side of tube and allow liquid to run down the side of the tube, and when flaming a loop to transfer culture, have a sterile agar plate used as a “sizzle” plate so students do not touch a culture with a really hot loop.
• All procedures that generate aerosols: centrifuging, grinding, blending, shaking, mixing, sonicating, etc., must be performed inside a biological safety cabinet or using appropriate engineering controls (centrifuge safety cups). Biological safety cabinets must also be used when opening a container that can become depressurized when opened, and could release aerosols of the stock culture.
• Waste materials from the laboratory must be disposed in accordance with the Rutgers Biological and Medical Waste Disposal Policy
• Broken glass must be handled using a dustpan and broom or forceps/tongs, not picked up by students or laboratory personnel with their hands.
• All spills or injuries must be immediately reported to the laboratory instructor. These must then be documented with REHS according to established RU policies
• Teach, practice and enforce the proper wearing and use of personal protective equipment
• Advise immune-compromised students and students living with or caring for an immune-compromised person to consult physicians to determine the appropriate level of laboratory participation. (Students should not be asked to reveal if they are immune-compromised. A general announcement should be made that students with a reduced immune status should consult with student health services. A note from Student Health Services is sufficient to excuse a student from laboratory work.)
• Supply pens and pencils for students, and keep separate from personal items.
• Keep note taking and discussions separate from work with laboratory materials. Note taking can be performed on a pull out desk shelf, if available, but must be taken away from the work area. If this is not available, lecture must be performed before any materials are brought to the bench areas.
• Use micro-incinerators rather than Bunsen burners. Bunsen burners are not permitted in biological safety cabinets. Micro-incinerators can also be used to heat fix bacterial smears on microscope slides and flaming the end of a test tube by passing these items over the entrance to the micro-incinerator.

Training Practices:
• Teaching assistants must complete REHS laboratory safety, BBP and biosafety trainings
• Instructors and/ or teaching assistants must review basic biosafety and microbiological practice with students on the first day of lab. The requirements listed above must be included in this training session. Training session must be documented with a sign in sheet maintained by the instructor. This can be performed using an online system, such as Sakai, Moodle, or Blackboard.
• Require students and instructors to handle microorganisms safely and in conjunction with requirements outlined in the Rutgers University Biosafety Guide.
• Inform students of safety precautions applicable to each exercise before the procedure is performed.
• Require students to demonstrate proficiency in standard aseptic technique and BSL1 practices before allowing them to work at BSL2.

Documentation:
• Safety Data Sheets (SDS) sheets must be available in the laboratory for all chemicals.
• Require students to sign safety agreements indicating that they have been informed about the safety requirements and the hazardous nature of the microbes and materials that they will handle throughout the semester. Maintain student signed agreements at the institution. Alternatively, this can be performed and maintained online within Sakai, Moodle or Blackboard.
• Prepare, maintain and post caution signs to the laboratory, complete with biohazard symbol
• Instructors must provide a detailed list of microorganisms that will be handled in the laboratory to students. This list can be included in the syllabus, laboratory manual, or online at the course website.
• Register all work at BSL2 with the Institutional Biosafety Committee.
• Follow all requirements for BSL2 as outlined in the Rutgers University Biosafety Guide.
• Emergency numbers and information must be posted in the laboratory.

References:
APPENDIX E: 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
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:

- Theft or loss of keys, access cards, pin codes, and/or other means of access to laboratory areas or sensitive laboratory information.
- 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.
- Escape or loss of animal that have been dosed with an infectious agent or material.
- Incidents of vandalism or suspicion of tampering in laboratory areas.
- Suspicious persons in laboratory areas.
- Threatening phone calls or threats received in writing.

**Routine Biosecurity Practices**

- Control access to areas where materials are used or stored.
- 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.
- Know what materials are being brought into the lab
- Keep an inventory of biological materials stored in the lab
- Document when biological materials are removed from the lab.
- Close and lock lab doors when no lab staff is present in the lab.
- Do not leave biological materials unattended or unsecured at any time. Promptly dispose of unneeded agents and specimens.
- Lock refrigerators and freezers where agents are stored
APPENDIX F: Embryonic Stem Cell Oversight

Ethical concerns surrounding research utilizing human embryonic stem cells have necessitated regulations and guidance regarding the use of these materials. The National Academy of Sciences and the State of New Jersey required the formation of an Embryonic Stem Cell Research Oversight (ESCRO) Committee.

Currently, the review of ESCRO protocols is submitted as part of the IBC protocol form, and is then sent to ESCRO for review. Since research with human materials must be reviewed by the IBC, this allows for a streamlined application process.

For questions regarding ESCRO, please contact Laszlo Szabo (laszlo.szabo@rutgers.edu) or biosafety@aps.rutgers.edu.
APPENDIX G: Biological Indicator Spore Testing Procedure

Autoclaving is one of the most dependable methods for sterilizing laboratory materials or waste. Autoclaves use saturated steam under pressure to achieve high temperature to kill microorganisms. Attaining the proper temperature for the proper length of time is essential for an effective kill. Without biological indicator testing, adequacy/efficacy of the sterilization process cannot be assumed.

Biological indicator (BI) spore testing should be performed monthly on all research autoclaves used for biological waste except for select agents, transgenic plants (seeds and other crops) which is performed weekly.

Ampules containing spores of Geobacillus stearothermophilus are used for the testing, due to its resistance to heat, to measure biological performance. REHS recommends the ampules included in this document for their ease of use. They may be purchased directly from Mesa Labs (catalog # PS1-6-100). Those ampules are safe to use (no need to crack them open for incubation), easy to read the result, and are in compliance with ISO 11138-3 and ISO 11138-1 (Sterilization of health care products-biological indicators for moist heat sterilization process). If alternate indicators are needed, please contact REHS biosafety at biosafety@aps.rutgers.edu for guidance. Indicators used must be ones designed for autoclave validation.

Special Practices, Precautions and/ or Notes before use:
1. A run cannot be started unless door is closed and gaskets charged (pressurized sealed).
2. Verify that the autoclave printer has enough tape and ink printer
3. A maximum of 1 Mesa lab ampule can be autoclaved per waste cycle.
4. Fill out the Autoclave spore test log sheet, including the cycle name (see appendix A)
5. Spore test shall NOT be left inside the autoclave overnight.
6. The user performing the BI spore test is responsible for placing it into an incubator, reading the results, documenting it on the autoclave log and notifying the Principal Investigator or designee of the results. Spore tests should be run on all waste cycles on a monthly basis (more frequent testing may be required by REHS based on biosafety protocol and risk level).
Equipment, Materials and/or Service Information

1. Autoclave
2. Printer paper tape and ink
3. Autoclavable biohazard bags (Note: bags must be clear with no pre-printed biohazard symbol if used for BSL-1 waste being disposed of in regular trash).
4. Plastic or metal autoclavable trays/bins
5. Insulated gloves.
6. Two Biological Indicators (BI): one for testing and one positive control. Mesa labs ProSpore Ampoule cat#PS1-6-100. Ampules must be stored at +4°C before use.
7. 55°C incubator or heating block

Procedure:

1. Operate the autoclave following the manufacturer’s instructions.
2. Place the (BI) in an autoclave bag (clear bag only for BSL-1).
3. Loosely seal the autoclave bag with autoclave tape
4. Place the biohazard bag containing the BI into the autoclaving bin or tray
5. Close the autoclave door
6. Run the cycle that is used for autoclaving wastes.
7. When the cycle is completed, remove the ampoule from the autoclave bag.
8. Save the printout of the ran cycle and place it in the autoclave verification binder.
9. Place the autoclaved ampoule into a 55°C incubator or heat block (Heating plate can be used too). Make sure there is a thermometer present to ensure temperature is correct.
10. Place a NON autoclaved ampoule (positive control) into a 55°C incubator or heat block (Heating plate can be used too). Make sure there is a thermometer present to ensure temperature is correct.
11. Incubate both ampoules (autoclaved and NON autoclaved for a minimum of 48H)
12. After 48H, do a visual reading of both ampoules and record the findings in the autoclave log (included below).
Reading the results:

For the autoclaved ampoule:

- **No change of color (purple)**: the autoclave cycle passed the testing procedure. (Note: the color of the autoclaved sample may turn brown due to the presence of carbohydrate in the ampule)

- **Change of color (yellow)**: the autoclave failed the testing procedure
For the NON autoclaved ampoule:

- **Change of color (yellow)**: confirms quality of the product used for biological test.
What should I do if a spore test result is positive?

If the mechanical parameters (e.g., time, temperature, pressure) suggest that the sterilizer is functioning properly, a single positive spore test result probably does not indicate sterilizer malfunction. However, sterilizer operators should repeat the spore test immediately using the same cycle that produced the positive biological indicator.

If the result of the repeat spore test is negative and operating procedures were correct, then the sterilizer can be returned to service.

If the repeat spore test result is positive, inform the person responsible for autoclave (PI, facility manager, lab manager) and do not use the sterilizer until it has been inspected or repaired and re-challenged with a biological indicator tests. Results of biological monitoring and (cycle printout/reports) should be recorded.
**Biological Indicator Test Results Log**

Building/Room#: Autoclave Make: Serial #: Model:__________

Biological Indicator Type and Brand: ____________________________

Contact Person/Department:______________________________

<table>
<thead>
<tr>
<th>Operator Name</th>
<th>Date of Autoclaving and Incubation</th>
<th>Cycle Selected and Temperature</th>
<th>Cycle Time</th>
<th>Incubation Time (Hours)</th>
<th>Biological Indicator Lot Number and Expiration Date</th>
<th>Result Pass Fail</th>
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Please keep completed log in a binder near the autoclave or in the laboratory.