

# BIOSAFETY REFERENCE GUIDE

Updated 5/14/2015

*This document contains the rules and regulations for the safe use of recombinant and biohazardous material at the University of Arizona.*



THE UNIVERSITY OF ARIZONA  
**Research**  
Research Laboratory & Safety Services

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## Certification and Approvals

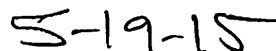
This Biosafety Plan has been approved by:

Daniel Silvain,  
Responsible Official  
Senior Director, Research Laboratory and Safety Services

This Biosafety Plan for the University of Arizona has been prepared in compliance with the *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* and 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73. This plan was organized based on information provided in the APHIS/CDC Select Agent and Toxins Security Information Document from March 8, 2007. This plan is required to be reviewed annually and updated whenever changes occur. The signature below verifies the annual review for this plan was completed.



Signature of Authorized Responsible Official



Date

Daniel Silvain

### Research Laboratory and Safety Services (RLSS)

1717 E. Speedway Blvd. (Bldg. 151)

Suite 1201

Tucson, AZ 85724

P.O. Box 245101

[rlss.arizona.edu](http://rlss.arizona.edu)

Phone (520) 626-6850 Fax (520) 626-2583

For emergency assistance after hours,  
call University of Arizona Police Department at 621-UAPD (621-8273)

## **Mission Statement**

Research Laboratory and Safety Services (RLSS) serves The University of Arizona, University of Arizona Health Network, and various regulatory, research, clinical and educational units around the state. We make every effort to provide quality and timely service.

RLSS assists, monitors, and provides oversight to ensure that federal, state, local, and University regulations and policies are implemented in a safe and secure manner. We are a service-oriented department committed to professionalism through friendly and helpful interactions.

This is the written biosafety plan for the University of Arizona. This plan addresses and meets the requirements of the Select Agent Final Regulations. The Biosafety Plan is reviewed annually and revised as necessary. Drills and exercises that satisfy the requirements of the Biosafety, Security and Incident Response Plans are conducted annually by RLSS. Documentation for all drills is maintained by RLSS. All written plans are updated when drills and exercises warrant change.

## **Definitions of Recombinant and Biohazardous Material**

Biohazardous Material:

- An organism or samples from that organism that have the potential to cause disease in animals, humans, or plants
- Animal (vertebrate and invertebrate) and/or human blood, tissue, bone or excreta; or animal and/or human and non-human primate cell lines
- Bacteria, chlamydia, fungi, parasites, prions, rickettsia and viruses which cause disease in humans, animals (vertebrate and invertebrate), and/or plants

Recombinant Material:

- Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids
- Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids
- Molecules that result from the replication of those described above

## **Recombinant Nucleic Acid Requirements**

Prior to acquiring or conducting any work involving recombinant material or any genetically modified plants or animals at or sponsored by the University of Arizona, Approval Holders (individual Principal Investigator approved for such work) must adhere to the following requirements:

- Acquire appropriate permits and/or authorizations from outside agencies to include but not limited to the USDA/APHIS and the NIH Recombinant Advisory Committee.
- Obtain approval from the University of Arizona's Institutional Biosafety Committee (IBC).
- Complete the appropriate training offered by the Research Laboratory and Safety Services (RLSS). RLSS is available for onsite training.
- Ensure all their staff completes appropriate training from RLSS and are provided research specific training.

Once research has begun, the policies and procedures of the University of Arizona and all other applicable agencies must be followed. Inspections will be performed annually by RLSS.

## **Human and Non-Human Primate Tissues and Cell Culture**

All human and non-human primate blood, tissue cell lines and other potentially infectious material is handled at a minimum of biosafety level 2 (BSL-2). This includes the following:

- All cell lines (primary and established) of human/primate origin
- All cell lines derived from lymphoid or tumor tissue
- All cell lines exposed to or transformed by any oncogenic virus
- All cell lines exposed to or transformed by amphotropic packaging systems
- All human clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy)
- All mycoplasma-containing cell lines

It is not acceptable to handle any of the above cell lines in a clean bench or horizontal laminar flow hood.

## **Bloodborne Pathogens**

Bloodborne Pathogens are any microorganism that is carried in the blood and can cause disease in humans. Anyone whose job requires possible exposures to Bloodborne Pathogens is required to complete the Bloodborne Pathogen training offered by Risk Management and Safety via the D2L online system. Risk Management and Safety maintains the University of Arizona

Bloodborne Pathogen Policy. The Exposure Control Plan is available on their website at:

<http://risk.arizona.edu/healthandsafety/bloodbornepathogens.shtml>

### **Safety Precautions for Handling Human Brain Tissue**

All human brain tissue, to include spinal cord material, must be treated as a potential contamination risk for certain biohazardous agents and must be handled with care. Human brain tissue can transmit prion diseases such as variant Creutzfeld-Jakob Disease and Kuru. Although prion diseases linked to handling brain tissue are rare, they are able to remain infectious for long periods of time in fixed tissues and require special treatment to ensure their destruction before discarding as waste. Laboratorians working with human brain tissue must:

- Obtain demographics of the sample to include where collected, reason, part of study, autopsy, etc., or obtain a pathology report.
  - If no pathology report is available or demographics indicate the possible presence of prions, additional procedures are required to ensure complete destruction of any agent.

The recommended procedure to ensure prion destruction is incineration. If this is not possible, then autoclaving at 134 C for a minimum of 30 minutes is required. Chemical disinfection requires 1N NaOH immersion for 1 hour, rinsing, followed by autoclaving at 121 C for 1 hour. Other methods of sterilization can be found in the reference. If you wish to incinerate your material, you must contact Risk Management Services for assistance.

### **Use of Recombinant and Biohazardous Material with Animals**

- All laboratory personnel and animal care handlers must be fully informed of the biosafety practices necessary to prevent an accidental exposure from an infected animal. It is the Approval Holder's responsibility to inform animal care staff associated with the research of the potential risks and appropriate biosafety practices.
- All animals inoculated with human cells and most recombinant material are handled at ABSL-2 for 72 hours post inoculation. Review the following guidelines to assist in determining containment level for viral vectors. Consult RLSS if you have additional questions.
- At a minimum, personnel handling animals containing recombinant and/or biohazardous material must wear a laboratory coat, and gloves and any additional PPE as prescribed by your animal protocols.
- All personnel working at ABSL-3 must be respirator fit tested in case of an accidental aerosolization or spill.



## **Guidelines for Research Involving Viral Vectors in Animal Use at the University of Arizona**

All work involving recombinant nucleic acids must be approved by the UA Institutional Biosafety Committee (IBC) as required by the NIH Guidelines for Research involving Recombinant or Synthetic Nucleic Acid Molecules. The

IBC requires that all viral vectors used for transgene expression must:

- Be free of detectable replication competent virus
- Minimize probability of homologous and end joining recombination which might reestablish wild type virus
- Be produced in the absence of helper virus
- Utilize a homologous packaging system
- Utilize self-inactivating derivatives

The biosafety level of a viral vector defaults to the Risk Group (BSL-1, ABSL-1 or BSL-2 or ABSL-2) of the wild type viral strain from which the vector is derived. This biosafety level is applied during preparation, during use in cell culture systems, and for the first 72 hours after inoculation into animals while the vector is considered infectious (though non-replicating). In addition, the transgene being inserted and the source of the viral vector should be considered.

The following checklist will assist a researcher in determining the proper biosafety level:

- 1) Is the viral vector derived from a wild-type virus pathogenic to humans or primates (HIV, SIV, Human Adenovirus, etc.)?
- 2) Does the transgene encode a product that is potentially hazardous (oncogene, toxin, etc.)?
- 3) Does the vector or transgene encode more than 2/3 of the viral genome?
- 4) Is the viral vector obtained from a non-commercial source?

If all answers are “NO”, then the viral vector can be handled at BSL-1 or ABSL-1 pending IBC approval. Examples of these types of vectors include AAV, murine retrovirus, FIV and VSV.

Exceptions must be requested via IBC protocol review process. For example an AAV vector made “in-house” must have safety data that shows it can be handled at ABSL-1.

### **Select Agents**

Select Agents and Toxins are biological agents that the U.S Department of Health and Human Services and the US Department of Agriculture have declared to pose a severe threat to public health and safety. As part of the 1996 Anti-terrorism and Effective Death Penalty Act (PL 104-132), the CDC and the US Department of Health and Human Services issued a final rule in October 1996 regarding the transfer of Select Agents and Toxins that could be used in terrorist

activities. The law requires additional requirements for facilities that utilize select agents and toxins in their research.

All researchers that want to conduct research with Select Agents or Toxins must first contact RLSS for guidance on the registration process. Please plan for a minimum of six months to complete the registration process; this will be longer for tier one agents.

## **Responsibilities**

### **Approval Holder**

The Approval Holder is a university employee who has been approved by the IBC to conduct research with recombinant and/or biohazardous material.

The Approval Holder is responsible for full compliance with the policies, practices and procedures set forth in this reference guide. This responsibility extends to all aspects of biosafety involving all individuals who enter or work in the Approval Holder's laboratory. The Approval Holder is responsible for assuring the appropriate safety training of employees, for correcting errors and unsafe working conditions, and for documentation of these elements.

As part of the general responsibilities, the Approval Holder shall:

- Be trained in standard microbiological techniques
- Submit all protocols for review by the Institutional Biosafety Committee (IBC) prior to beginning experiments
- Ensure no project is significantly modified prior to IBC approval
- Develop and implement laboratory-specific biosafety procedures that are consistent with the nature of current and planned research activities
- Ensure personnel have been trained in biosafety and laboratory-specific procedures and are enrolled in the Medical Surveillance Program as appropriate
- Ensure that all laboratory personnel, maintenance personnel and visitors who may be exposed to any recombinant or biohazardous material are informed in advance of their potential risk and of the behavior required to minimize that risk. It is essential that everyone who may have any potential exposure to recombinant or biohazardous materials enter and/or work in the laboratory under the Principle of Informed Consent
- Ensure that all maintenance work in, on or around contaminated equipment is conducted only after that equipment is thoroughly decontaminated by the laboratory staff or Approval Holder
- Ensure that research materials are properly decontaminated before disposal and that all employees are familiar with the different methods of waste disposal

- Comply with shipping requirements for recombinant and biohazardous material
- Report accidents, exposures or violations to RLSS

During the conduct of research the Approval Holder shall:

- Supervise the safety performance of the laboratory staff to ensure that the required safety practices are employed
- Investigate and report in writing to the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures
- Immediately notify RLSS of any laboratory spills, accidents, containment failure or violations of biosafety practice which result in the release of recombinant or biohazardous material and/or the exposure of laboratory personnel (or the public) to infectious agents
- Correct work errors and conditions that may result in the release of recombinant and biohazardous materials
- Ensure the integrity of all containment systems used in the project
- Restrict access as required by the laboratory-specific biosafety practices procedures and by the biosafety containment level approved by the IBC

### **Approval Safety Coordinator**

The Approval Holder may choose to delegate aspects of the biosafety program in his/her laboratory to another laboratory worker. This individual will be designated as the Approval Safety Coordinator. This individual will be a secondary contact for laboratory workers and RLSS.

### **Laboratory Workers**

Anyone in the laboratory that will work with recombinant and/or biohazardous material is defined as a laboratory worker, whether the person is a faculty member, a student, an intern, a visiting scholar or a volunteer.

It is the laboratory staff's responsibility to:

- Conscientiously follow biosafety practices and lab-specific procedures
- Inform the Approval Holder or Approval Safety Coordinator of any condition that may require additional safety precautions
- Report to the Approval Holder or Approval Safety Coordinator and RLSS all problems, violations in procedure or spills as soon as they occur
- Refuse to take any adverse action against any person for reporting real or perceived problems or violations of procedures to supervisors, the Approval Holder, or RLSS

## **Risk Management and Safety**

- Provide University's Bloodborne Pathogen Training (D2L)
- Maintain the University's Exposure Control Plan
- Provide shipping training
- Maintain Bloodborne Pathogen training and vaccination records
- Pick up and decontaminate recombinant and biohazardous waste

## **Research Laboratory and Safety Services**

- Provide training and assistance
- Submit protocols to the IBC
- Inspect laboratories
- Assist laboratories in maintaining safety and regulatory compliance
- Available for emergency response

## **Institutional Biosafety Committee (IBC)**

The Institutional Biosafety Committee (IBC) reviews all research involving recombinant and biohazardous material that is performed at or sponsored by the University of Arizona. It is the committee's responsibility to review and enforce policies and procedures which meet or exceed applicable standards or regulations for recombinant and biohazardous material. They must ensure experiments are evaluated and designed to minimize aerosolization of recombinant and biohazardous material. The committee makes recommendations or requirements when necessary to modify experimental procedures to reduce the possibility of inadvertent generation aerosols. Non-committee faculty or staff with special expertise will be asked to advise the Committee when the need arises.

The IBC is comprised of University faculty and staff and two representatives from the community. The members are selected for their expertise which ensures that the committee has collective experience to evaluate the risks associated with the use of recombinant and biohazardous material in a wide variety of research proposals. Members of the IBC must recuse themselves from voting on projects in which they are or expect to be engaged in, or have a direct financial interest. IBC meetings are held monthly and are open to the public.

The Approval Holder must complete a New Protocol Application Form for all research involving the use of recombinant and/or biohazardous material. The Approval Holders must obtain IBC approval before changing any variable in their research. After Committee review and approval the Approval Holder is sent a letter of approval.

RLSS signs the Sponsored Projects Proposal Routing Sheets for any protocol in which recombinant and/or biohazardous materials are used.

## **Standard Microbiological Practices**

All individuals working with recombinant and/or biological material at all containment levels must follow the standard microbiological practices:

- Wash hands after work with recombinant and biological material and before leaving the laboratory. When washing hands use soap and water for a minimum of 15 seconds.
- No eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food in laboratory
- Wear appropriate personal protective equipment – lab coats and gloves at a minimum.
- Follow precautions when working with sharps
- No mouth pipetting
- No open toed shoes
- Minimize aerosols; aerosols/splatters of infectious materials and toxins must be avoided and procedures that might create them must be carefully performed
- Decontaminate work surfaces at least daily and after spills
- Decontaminate all recombinant and biological materials before disposal using an effective method
- Use of glassware should be avoided; plastic ware should be substituted

## **University Standards for Recombinant and Biohazardous Material**

### **Training**

Appropriate training is required for all individuals that work with and/or supervise the use of recombinant and biohazardous material. The Biosafety Protection Courses are an annual requirement. The refresher courses can be taken online. You can see the status of your training by logging into the User Dashboard on RLSS website:

**Basic Biosafety Protection Course** – required for all personnel working with BSL-1 or BSL-2 agents

**Plant Hazard Protection Course** – required for research involving transgenic plants, plant pathogens and organisms associated with plants

**BSL-3 Protections Course** – required for anyone working within a BSL-3 laboratory

**Select Agent and Toxin Protection Course** – required for all individual that have undergone a security risk assessment for access to select agent and toxins at the University of Arizona

The University of Arizona’s Bloodborne Pathogen Course offered through D2L by Risk Management and Safety is a prerequisite for the Basic Biosafety Protection Course and required for anyone else that may come in contact with bloodborne pathogens.

### **Signs and Labels**

- The entrance to all laboratories working with recombinant and/or biohazardous material must be posted by RLSS.
- All tools and equipment that are used with recombinant and/or biohazardous material must be labeled as biohazardous. This includes but is not limited to: centrifuges, refrigerators, freezers, incubators, growth chambers, storage cabinets, liquid nitrogen dears, and transport containers.

### **Sharps Precautions Requirements**

The UA sharps requirements for the safe handling of sharps, including needles, scalpels, broken glassware, and sharp-like objects including pipette tips must be adhered to, in order to reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

- Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Used disposable needles and syringes must be carefully placed in conveniently located puncture proof containers used for sharps disposal.
- Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

Sharp-like objects, including plastic pipette tips of 1 mL volume or less, contaminated with biohazardous and/or recombinant nucleic acid material, must be placed in a container that is puncture resistant prior to placing in a red bag for disposal. For example, acceptable puncture resistant containers include: used plastic bottles with a loose seal (1/4 turn from being fully tightened) and bag-lined cardboard boxes.

## Protecting Vacuum Systems

- The aspiration of tissue culture media from cultures and supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure. Protection against pulling biological aerosols or overflow fluid into the vacuum system is necessary. An overflow flask and a cartridge type filter are required to provide protection for the vacuum line.
- For assembling the apparatus, flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flask of capacities from 250 to 4,000 ml may be used for the overflow flask depending on the amount of fluid that could be aspirated out of the collection flask.
- The overflow flask contains a disinfectant solution appropriate for the recombinant and/or biological material in use. Bubbling of air through the disinfectant can cause foam which can shut off the vacuum if it reaches the filter.
- Change the filter if it becomes contaminated.

## Pipetting

Pipetting is the act of transferring, measuring or dispensing a liquid through a small piece of apparatus typically consisting of a narrow tube. Pipets can be constructed of a variety of glass or plastic materials. Liquids can be drawn into the pipet through the use of hand-held bulbs, manual pipet aids, motorized pipet aids, or various other vacuum sources. Pipetting is a routine function in most laboratories; therefore, the safety concerns must not be overlooked. The following safety rules must be followed when using pipets:

- Never pipet by mouth
- Visually inspect the pipet prior to inserting it into any pipet aid. Make sure the pipet does not have any cracks.
- Always dispose of pipets in hard walled containers
- Routinely clean and inspect pipet aids and bulbs. Damaged pipet aids and weakened bulbs must be discarded.
- Motorized pipet aids should have some type of filter (typically 0.45  $\mu\text{m}$  or 0.22  $\mu\text{m}$ ) to prevent liquid from accidentally being drawn into the housing.

## Storage of Biological Materials

- A biological hazard sign must be clearly posted on storage areas such as refrigerators, freezers, cabinets, etc. containing recombinant and/or biohazardous materials.
- All containers and/or racks are to be clearly labeled to identify the contents.
- Storage containers must be intact (no tears or cracks), leak-proof and covered or closed to avoid spills or contamination. Secondary containment must be used when possible.

- To prevent unnecessary handling of specimens, all materials should be inventoried and organized.
- Any substance being stored in a freezer must be placed in a labeled container designed for low temperature storage.
- If flammable materials are used, they must be stored in equipment that is designed for this purpose.
- No personal items must be stored in lab refrigerators, freezers or incubators (e.g. food, beverages).
- When storage equipment needs repair, calibration or transport, it must be completely decontaminated prior to starting work or being removed.

Each approval is responsible for performing a physical inventory of their long term storage (i.e. freezers and liquid nitrogen tanks) at least once a year. This annual physical inventory must be documented and the records kept available for inspection.

### **Transporting Recombinant and Biohazardous Material**

Any time recombinant and/or biohazardous material or a toxin solution including biohazardous waste is moved outside the lab, into public space or into a high traffic area (such as within an open bay) it must be transported in a closed, rigid secondary container:

- Rigid secondary containers are containers such as pails, cartons, drums, dumpsters or bins for storage
- Secondary containers must be leak-proof and have tight-fitting covers
- Secondary containers must be labeled as biohazardous or recombinant
- The outside of the secondary container must be decontaminated before leaving the lab.
- Reusable secondary containers must be easy to clean and must be washed and decontaminated each time they are emptied, unless they have been completely protected from contamination
- Toxins must be in leak proof secondary container if not inside a biosafety cabinet.

Recombinant and biohazardous material must not be transported in a personal vehicle for any reason. However, use of a university or university sponsored vehicle is permitted.



## **Shipping Regulations**

All individuals that ship recombinant and/or biohazardous materials must receive training. Training for shipping hazardous material is offered by Risk Management and Safety.

## **Pest Control Management**

Approvals must have a method in place for reporting and controlling pest species. If the agent in use is transmitted or spread by a pest, special precautions and containment must be implemented and documented in the laboratory's Standard Operating Procedures.

## **Record Retention**

Records relating to recombinant and/or biohazardous materials must be retained for 3 years and include the following: inventory, training, shipping, protocol, approval documentation, and all records relating to Select Agents and Toxins.

## **Personal Protective Equipment**

Personal Protective Equipment (PPE) is used to protect personnel from contact with recombinant and biohazardous materials. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. Remove all PPE prior to exiting the laboratory. The following PPE is recommended for regular use:

### **Laboratory Clothing**

Laboratory clothing includes: laboratory coats, smocks, scrub suits and gowns. Long sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect skin from contamination. If the garment is not disposable, it must be capable of withstanding sterilization in the event it becomes contaminated. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables must be available for visitors and maintenance and service workers entering the lab if they are required. All protective clothing must be either discarded in the laboratory or laundered by the facility. Personnel must not launder laboratory clothing at home.

### **Gloves**

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with recombinant and biohazardous material. Delicate work requiring a high degree of precision dictates the use of thin walled gloves.

When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment must be overlapped by the glove. A long sleeved glove or disposable arm shield may be worn for further protection of the garment.

Double gloving may be appropriate or required. However, if a medical condition dictates that only a single pair is worn, then that is acceptable. If a spill occurs, hands will be protected after the contaminated outer gloves are removed. Gloves must be disposed of when contaminated or removed when work with recombinant and/or biohazardous materials is completed. Gloves must not be worn outside the laboratory. Disposable gloves must not be washed or reused. Always wash hands after removing gloves.

### **Face Protection**

Goggles or safety glasses with solid side shields in combination with masks or chin length face shields, or other splatter guards, are required for anticipated splashes, sprays or splatters of recombinant and/or biohazardous materials. Application or removal of contact lenses is not permitted in the laboratory setting. Persons who wear contacts must wear eye protection when in areas with potentially aerosolizable agents.

### **Footwear**

Open-toed shoes are not permitted in the laboratory. Protective footwear such as shoe covers may be necessary to minimize contamination of the laboratory and prevent the accidental release of recombinant and biohazardous materials from a laboratory. If disposable shoe covers are used in the laboratory, waste containers must be available to dispose of used shoe covers. Shoe covers must not be reused.

### **Respirators**

Additional respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Respirators must be carefully fitted to the individual and fit tested before use. Personnel who require respiratory protection must contact Risk Management and Safety for assistance in selection of equipment, training in proper usage and enrollment in the Risk Management Respiratory Protection Program.

## **Laboratory Equipment**

### **Biosafety Cabinets**

Some laboratory workers refer to biosafety cabinets as "hoods" or "laminar flow hoods". It is important to know the difference between a biosafety cabinet, a chemical fume hood, and a clean bench. Biosafety cabinets (BSC) are designed to protect the individual and the environment from biological agents, and to protect the research materials from contamination.

Chemical fume hoods; however, are designed solely to protect the individual from exposure to chemicals and noxious gases. Chemical fume hoods are not equipped with HEPA filters; therefore, chemical fume hoods must not be used for work with biohazardous materials. Horizontal laminar flow hoods or "clean benches" are not acceptable for work with biohazardous materials. The air is HEPA filtered and directed across the bench top toward the user. Thus, it offers no protection to the user, only the product.

Various laboratory procedures generate aerosols that may spread recombinant and biohazardous material in the work area and pose a risk of infection to the worker. Biological safety cabinets are used to prevent the escape of aerosols or droplets.

### **Types of Biosafety Cabinets**

- Class I biological safety cabinets are enclosures similar to chemical fume hoods, with an inward airflow through the front opening. The exhaust air from the biological safety cabinet is passed through a HEPA filter so that the equipment provides protection for the worker and the public. The product (research material) in the cabinet; however, is subject to contamination.
- Class II biological safety cabinets are designed to protect the worker, the environment, and the product. Class II cabinets are vertical laminar-flow cabinets with a partially open front. Airborne contaminants in the cabinet are prevented from escaping across this opening by a curtain of air formed by unfiltered air flowing from the room into the cabinet and HEPA filtered air supplied from an overhead grill down into the cabinet. A portion of the filtered air is used to maintain the air curtain, and the remainder passes down onto the work surface, and is drawn out through the grills at the back and front edges of the work surface. The HEPA filtered air from the overhead grill flows in a uniform downward movement to minimize the air turbulence. It is this air that provides and maintains a clear air work environment. A percentage of air drawn through the front and back grills of the work surface is also HEPA filtered and exhausted from the cabinet.
- Class III cabinets or glove boxes are gas tight cabinets and all operations within the cabinet are conducted through arm-length rubber gloves. Air entering class III cabinets is HEPA filtered and exhaust air is filtered through two HEPA filters in a series and exhausted directly to the outside.

Class I and Class II cabinets are partial containment devices which, if used in conjunction with good laboratory practices, can dramatically reduce the risk of operator exposure to recombinant or biohazardous aerosols and droplets. Class III cabinets are generally used for extremely hazardous work (e.g. BSL- 4 labs at the CDC) or experiments with a high potential for aerosolization of an agent that is transmitted by aerosolization.

### **Before Using the Biosafety Cabinet**

- Turn off ultraviolet light (if so equipped) as soon as you enter the room.
- Turn on all blowers and cabinet illumination lights.
- Allow five minutes of operation to purge the system. Check flow alarm system audio and visual alarm function if so equipped.
- Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.

### **During Use of the Biosafety Cabinet**

- Minimize disruption of airflow.
- Open continuous flames are not permitted to be used inside the BSC.
- Keep front and back grills free of materials that might block airflow.
- Minimize items within the cabinet.

### **After Use of the Biosafety Cabinet**

- All items removed from the Biosafety Cabinet must be decontaminated first.
- Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents or suspected agents present.
- Allow five minutes of operation to purge the system.
- Turn off cabinet blower.

### **Moving and Installation**

Disinfect biosafety cabinet work surfaces prior to moving them to new facilities. Biosafety Cabinets used for work with pathogenic organisms may require paraformaldehyde decontamination before being moved. Contact Facilities Management for instructions.

Each biological safety cabinet must be recertified for correct air flow and filter integrity after it has been moved and placed in its final location. Call Facilities Management for biosafety cabinet certification.

## **Decontamination and Maintenance**

The Approval Holder is responsible for cleaning and decontaminating his or her biological safety cabinets. Facilities Management personnel will disconnect the cabinet and label when the cabinet was disconnected and decontaminated. If the safety cabinet is equipped with a UV light, do not use this as your primary disinfectant.

## **Certification**

All biological safety cabinets must be recertified annually. They must also be recertified if they are moved or have had repairs. Contact Facilities Management (520) 626-6880 if you have a biosafety cabinet that needs recertification.

## **Centrifuges**

Hazards associated with centrifuging include mechanical failure (e.g. rotor failure, tube or bucket failure) and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users must be properly trained, and operating instructions that include safety precautions must be prominently posted on the unit. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging BSL-2 or BSL-3 agents the following procedures are required:

- Use sealed tubes, safety cups, or sealed rotors that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Open sealed tubes, safety cups, or sealed rotors inside a biosafety cabinet.
- Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Do not decant or pour off supernatant of tubes containing biohazardous materials. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a biosafety cabinet when resuspending sedimented material from a biohazardous source. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a biosafety cabinet during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions must be taken to filter the exhaust air from vacuum lines. Manufacturers, recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.

- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.

### **Aerosol-generating Equipment**

The use of blenders, ultrasonic disrupters, grinders and lyophilizers can result in considerable aerosol production. This equipment and any other device that may generate an aerosol must be used in a biosafety cabinet when working at BSL-2 or BSL-3.

#### **Blenders**

Safety blenders are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. Test blender rotors with sterile saline or dye solution to determine if they are leak-proof prior to use with recombinant and/or biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars must be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant must be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle and then open in a BSC. The device must be decontaminated promptly after use.

#### **Sonicators and French Presses**

Sonication of living microorganisms is potentially a source of aerosols. Whether using a sonicating bath or probe sonicator, precautions must be taken to protect personnel. Ordinarily, this will be done by performing the sonication in a biosafety cabinet or glove box. It is prudent to consider all surfaces in the vicinity of the sonicator to be contaminated following its use, and they must be thoroughly disinfected. Modern sonicators have containment mechanisms. Ensure these mechanisms are utilized if available.

The use of French pressure cells requires similar caution. The greatest potential for aerosols is at or near the end of a pressing cycle, when air bubbles at the top of the column of suspension can escape with little or no warning. This can result in microaerosols, which will contaminate the work area, but also in macroaerosols which can effectively inoculate the mucus membranes and conjunctivae of the operator. Due to the size of the press, it is usually impractical to perform this operation inside a biosafety cabinet. Thus, one must avoid pressing live organisms which are human pathogens. Operators must use face shields or other eye protection.

Arcing, which sometimes occurs during electroporation of bacteria, can also cause aerosols. These range from minimal spattering of the bacteria/DNA solution to major broadcast of potentially infectious material when a cuvette shatters. The shields supplied with most electroporators are usually sufficient to protect the operator from flying plastic and gross contamination, but will not contain microaerosols. Thus, if one must electroporate bacteria at BSL-2 or 3, it must be done in a biosafety cabinet.

### **Cell Concentrators**

Cell concentrators are also employed in laboratories as a means of handling viable organisms. There are two principal types of cell concentrators. The first involves the removal (through evaporation) of liquid from solid material thereby increasing the concentration versus volume. The second involves the retention of the solid material on the surface of a filter and the subsequent harvesting of the material from the filter surface. The following safety rules must be applied when using such an apparatus:

- Before starting, check all of the equipment to be used for signs of stress or fatigue. Pay close attention to tubing and glassware
- When possible conduct the procedure in a biosafety cabinet.
- Upon the completion of the run, thoroughly sanitize the apparatus before the next experiment.
- For rotary type concentrators, make sure the load is balanced.
- If a vacuum is to be used, make sure the appropriate exhaust filter is present on the vacuum line to prevent contamination (normally a 0.22 $\mu$ m hydrophobic filter).
- Do not exceed recommended pressures or speed for operation of equipment.

### **Lyophilizers and Ampoules**

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If working at BSL-2 or BSL-3, sample material must be loaded in a biosafety cabinet. The vacuum pump exhaust must be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a biosafety cabinet. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent must be disinfected. If the lyophilizer is equipped with a removable chamber, it must be closed off and moved to a biosafety cabinet for unloading and decontamination. Handling of cultures must be minimized and vapor traps must be used.

Opening ampoules containing liquid or lyophilized culture material at BSL-2 and BSL-3 must be performed in a biosafety cabinet to control the aerosol produced. To open, nick the neck of the ampoule with a file. Wrap it in a disinfectant soaked towel. Hold the ampoule upright and snap

it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as biohazardous material waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free and presterilized, and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

## **Decontamination**

Decontamination is a term used to describe a process or treatment that renders a medical device, instrument or environmental surface safe to handle. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection and antisepsis are all forms of decontamination.

- All recombinant and biohazardous material and all contaminated equipment or apparatus must be decontaminated before being washed, stored or discarded.
- All equipment must be decontaminated before repair, maintenance or removal from areas where infectious agents/animals are in use.
- Recombinant and biohazardous material must not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- Autoclaves must not be operated by untrained personnel.
- Special precautions must be taken to prevent accidental removal of material from an autoclave before it has been sterilized, or simultaneous opening of both doors on a double door autoclave.
- Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth or oil:
- Oxidizer + Organic Material + Heat = possible explosion
- Liquid, gas or vapor disinfectants, dry heat, ultraviolet or ionizing radiation appropriate for some applications are not universal and may not substitute for autoclaving or incineration before disposal in all situations.

The preferred chemical for decontamination is 10% sodium hypochlorite. If your approval chooses to use a different chemical for decontamination the process and efficacy must be documented in your Standard operating procedures. Although some other chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not



guarantee adequacy for the other. Ensure the chemical disinfectant and procedure is appropriate for the organisms in use.

## **Recombinant and Biohazardous Waste**

All recombinant and biohazardous material must be rendered inactive prior to disposal. Contaminated clothing and equipment must be decontaminated using suitable method or disposed of as biohazardous waste. All sharps must be disposed of in a biological sharps container that does not allow for easy access to the contents.

The following are acceptable methods of discarding waste:

### **Utilizing Risk Management and Safety (RM&S) waste containers**

All biohazardous and recombinant nucleic acid material must be packaged properly for disposal through Risk Management Services (RMS). Most buildings with biological laboratories have designated collection points, some of which are in cold rooms. The following are required for proper waste packaging:

- Un-autoclaved waste may be disposed of in these containers.
  - Waste must be double bagged in red or orange bags
  - Bags must be closed with ties or tape
  - No sharps or sharp-like objects, including contaminated broken glass and pipette tips, unless contained in puncture resistant devices
  - Transport bags to the waste containers in a leak proof container.
  - Check bags for punctures
  - Sharps containers specifically designed for needles/syringes must be sealed but do not require bagging
  - All sealed boxes must be placed in the bags
  - Bags must be placed within the collection containers; do not overfill
  - Containers cannot contain loose waste; everything must be in a bag
  - Containers must be kept closed and cannot exceed 75 lbs. Risk Management and Safety will not pick up waste if it is overflowing or not inside the container. Contact RM&S to empty the container before it is overflowing.
  - If waste may become odiferous, then put in a red bin in a cold room
  - Animal carcasses have designated cold room collection points in Animal Care facilities
  - Do not use these containers for liquid waste, carcasses, or recognizable human anatomical remains.
- 
- Contact RMS for additional red bins or for more frequent pick-up

### **Autoclave Your Own Waste**

Individuals that autoclave their own recombinant and/or biohazardous waste must follow the Arizona Administrative Code R18-13-1401 and the University of Arizona's biological waste disposal standards. In accordance with these standards the following is required:

- Maintain a logbook for each load of waste that is run that has date, time, temperature, pressure, and the initials of who ran the load.
- Perform and document a monthly biological indicator test
- Keep records of autoclave maintenance
- Ensure all recombinant and biohazardous waste is properly bagged in a red or orange bag prior to autoclaving
- Transport biohazard bag in a leak proof container to autoclave
- Autoclave the waste according to your Standard Operating Procedures (SOPs). The autoclave must be run at a minimum of 121°C (250°F) for at least ½ hour.
- Attach the Regulated Biological Waste Label to bag. Regulated Biological Waste labels are available on RLSS website.
- Check autoclave tape and document in logbook
- Place the autoclaved recombinant and biohazardous waste bag into a black or beige bag after autoclaving and then place in appropriate bin
- Maintenance of these records must be kept for 3 years

### **Other waste options**

- Liquid waste can be brought to 10% bleach for 15 minutes and disposed of down the drain. Halogenated solvents, flammable solvents, phenolic compounds, and corrosive materials (6<pH>9) may not be poured down the sink.
- Recognizable human anatomical remains must be cremated or interred. Consult the Human Subjects Committee for further information.
- Research animal carcasses must be incinerated. Drop off sites for carcasses are located in the Animal Care Facilities.

### **Closing out a Recombinant and/or Biohazardous Laboratory**

Approval Holders at the University of Arizona are responsible for leaving all assigned laboratory areas in a safe condition when vacated.

- Several months before the planned lab close-out, a Laboratory Close-Out Form available from RLSS website must be completed.
- If recombinant or biohazardous material will be shipped to a new location, then the Approval Holder must ensure that proper procedures are followed and that the receiving location be notified of shipping dates.
- RLSS must be notified for a final lab audit.

## Biosafety Containment Levels

### BSL-1

When working at BSL-1, workers must follow the standard microbiological practices and wear the minimum PPE of a lab coat and gloves. Examples of agents worked with at BSL-1 include: *E. coli*, murine cell lines and samples from lab mammals.

Laboratories must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids must be stored in locked containers.

### BSL-2

At BSL-2, workers are required to follow the standard microbiological practices and wear the minimum PPE of a lab coat and gloves. Additional requirements at BSL-2 are:

- Access to the lab is limited when work is being conducted
- All aerosol-generating procedures must be performed in the biosafety cabinet or otherwise appropriately contained
- All approved laboratories where work is performed must be under negative pressure
- Medical surveillance and vaccines required when appropriate
- Standard Operating Procedures are required to be available to all laboratory workers and any visitor or contractor upon request. A template for the SOPs is available on the RLSS website. The SOPs must contain:
  - Agent specific information
  - Lab specific bio-containment procedures
  - Decontamination and waste procedures
  - Applicable Medical Surveillance Program information
  - Acknowledgement Pages signed by all laboratory workers.

Examples of agents worked with at BSL-2 include: Human samples and cell lines, *Aspergillus fumigatus*, *Toxoplasma gondii*, *Salmonella typhimurium* and Influenza A.

Laboratories must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids and BSL-2 agents must be stored in locked containers. All material in the open bay or common use areas must be secured when not in use.

### **BSL-3**

Biosafety Level 3 work involves agents that may cause serious and potentially lethal infection. The primary routes of exposure to personnel working with these agents are: inhalation, auto inoculation, and ingestion. BSL-3 is the highest containment level laboratory at the University of Arizona. Workers must have experience working in BSL-2 and be very familiar with standard microbiological practices prior to working at BSL-3.

Examples of agents worked with at BSL-3 include: *Coccidioides immitis and posadasii*, *Mycobacterium tuberculosis*, Chikungunya Virus and West Nile Virus.

BSL-3 requires a specialized facility with an anteroom. The anteroom must be posted with the current agents in use. The anteroom is where PPE is donned and supplies are stored. Each anteroom is required to have a posting that lists the PPE and the proper way to put it on and remove it. The minimum PPE requirements at BSL-3 are:

- a water-resistant closed-front lab coat
- double gloves

Certain agents and any work with live animals may require additional PPE. BSL-3 workers must be fit tested for an appropriate respirator and individuals wearing contact lenses must wear eye protection.

Other additional requirements for working in the BSL-3:

- Hand washing sinks are required to be hands free.
- Pass through autoclaves are available in most BSL-3 laboratories at the University of Arizona. If the autoclave is outside of the laboratory the outer bag must be decontaminated prior to leaving the laboratory. The waste must be carried in a leak proof container to the nearest autoclave and be immediately loaded into autoclave.
- All work at BSL-3 must be performed in a biosafety cabinet. Work on the bench top with recombinant and biohazardous materials is not permitted.
- Physical containment devices, such as centrifuge safety cups and sealed centrifuge caging for animals, are used for all activities with biohazardous materials that pose a threat of aerosol exposure.
- Vacuum lines must have a HEPA filter.
- The BSL-3 labs must be under negative pressure at all times. Workers must check the gauges to ensure negative pressure prior to entering the laboratory each time.
- There is no recirculation of air to other areas of the building and all exhaust is HEPA filtered.

- Windows must be completely sealed and cannot open.

The Approval Holder must monitor and authorize access of all individuals entering the BSL-3 laboratory. Access is limited to those who understand the nature of the biohazard, have adequate laboratory-specific biosafety training and agree to comply with all precautions. Visitors and maintenance personnel who enter the BSL-3 laboratory must be fully informed of the potential risks, required practices and procedures that they must follow. They must be instructed about the signs and symptoms of any and all biohazardous materials manipulated or stored in the laboratory and sign a statement that they understand the risks.

Laboratories must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids and BSL-3 agents must be stored in locked containers.

### **Laboratory Maintenance and Repair**

If the ventilation system or other physical containment component of the laboratory fails, work in the BSL-3 must be halted. Notify Facilities Management and RLSS to help determine appropriate action.

Any time the BSL-3 facility must be closed for maintenance or repair, the laboratory must be decontaminated prior to the entry of facilities personnel. No further work with recombinant and biohazardous materials may be conducted until all maintenance and repair work is completed. A thorough inspection of the laboratory must be conducted by the Approval Holder or Approval Safety Coordinator and RLSS to ensure that the laboratory is functioning properly before work with recombinant and biohazardous materials may recommence.

- Routine maintenance that affects ventilation, affects containment provided by the facility or requires entry into the lab by non-laboratory staff must be scheduled with RLSS and the laboratory workers at least two weeks in advance.
- Special containment systems such as an exhaust HEPA filtration system must be tested and certified annually to meet National Sanitation Foundation standard 49.
- The Approval Holder must keep a log of all maintenance conducted by non-laboratory staff when the BSL-3 facility was not closed for maintenance. The log must record:
  - type of work/maintenance completed (with enough detail so those unfamiliar with the work can reconstruct the sequence of work/maintenance events)
  - date of entry
  - names of workers
  - start time
  - completion time

- disinfection technique for contaminated tools
- special personal protective equipment required to protect the workers (e.g., boots, heavy gloves, face shield etc.)
- work order number
- disinfection or other steps taken to protect maintenance workers

## **Plant Biosafety Levels**

Plant biosafety levels are designated with a “P” after the containment level. These agents do not usually pose a threat to human health; however, they may pose a threat to plants and the environment. Plants pathogens can be spread by, direct contact between plants, arthropods, soil borne nematodes, plant damage, and pollinators.

Plants can be grown in the greenhouse, laboratory, growth chamber, and or field. The NIH Guidelines define a greenhouse as a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment.

### **BSL-1P**

Recommended for all experiments with transgenic plants and associated agents that have no or limited threat potential. For example: transgenic plants that are not noxious weeds or agents that have no recognized potential for rapid dissemination. Examples of agents worked with at BSL-1P include: *Agrobacterium tumefaciens*, and *Rhizobium* spp..

Requirements at BSL-1P:

- Access to the laboratory and greenhouse, shall be limited or restricted when experiments are in progress.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.
- A record shall be kept of experiments currently in progress in the greenhouse facility.
- Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal.
- A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and federal laws.

- Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
- The greenhouse floor may be composed of gravel or other porous material. Impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the laboratory and greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds). Screens are recommended.
- Laboratories and greenhouses must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids must be stored in locked containers.

### **BSL-2P**

Recommended for transgenic plants that are noxious weeds, plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent, plants associated with transgenic non-exotic microbe that has a recognized potential for serious detrimental impact on managed or natural ecosystems, or plant pathogens that have a recognized potential for serious detrimental impact on managed or natural ecosystems. Examples of agents worked with at BSL-2P include: *Melioidogyne incognita* (root-knot nematode), Pepino Mosaic Virus, and *Pseudomonas syringae*.

The following are required when working at BSL-2P:

- A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws
- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagates of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagates of experimental organisms are readily disseminated through soil.
- Materials containing experimental microorganisms must be transferred in a closed non-breakable container.
- An autoclave must be available for the treatment of contaminated plant material including soil.
- If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.



- BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses
- Laboratories and greenhouses must be locked when unoccupied. All agents must be secured against accidental exposure, unauthorized use, and theft. All recombinant nucleic acids and BSL-2P agents must be stored in locked containers. All material in the open bay or common use areas must be secured when not in use.

### **Standard Operating Procedures**

Standard operating procedures are required for all field work involving transgenic plants and all work at BSL-2P. The SOP's must be easily accessible in the laboratory or field and contain:

- Location of field site/facility/greenhouse
- Physical containment standards
- Devitalization procedures
- How persistence in the environment is controlled
- Volunteer management procedures
- Acknowledgement pages

Templates are available on the RLSS website.

### **Field Work Involving Transgenic Plants**

The U.S. Department of Agriculture-Animal and Plant Health Inspection Service (USDA/APHIS) regulates plants, plant pests, and plant products. APHIS also regulates the movement, importation and field release of genetically engineered plants and arthropods. Field work with genetically engineered plants requires permits from APHIS before work can begin. If you are not sure about the required permits, ask before you start. Records required to be maintained:

- Log for cleaning equipment after use in the field
- Isolation distance
- Log of volunteer management
- Planting reports and final reports

## **Select Agent and Toxin Laboratory Safety Requirements**

### **Responsibilities**

The Responsible Official and Alternate Responsible Officials (RO/AROs) are the individuals at the University of Arizona that ensure compliance with the Select Agent and Toxin Rule. They are the point of contact for the CDC for all communications regarding Select Agent and Toxins (SA/T).

### **Isolation of Select Agent and Toxins from diagnostic sample**

Laboratorians that suspect that an unauthorized select agent or toxin has been isolated must immediately report the finding to RLSS and either:

- Destroy it promptly and properly document the destruction
- Properly transfer it to an approved laboratory

There is a complete list of all select agents and toxins linked to our website.

The Responsible Official or Alternate Responsible Official at RLSS must report the identification and final disposition of any select agent or toxin contained in a specimen presented for diagnosis or verification. Select agents and toxins must be reported within 24 hours to the CDC. Tier 1 select agents or toxins must be immediately reported by phone, fax or e-mail. Final disposition must be reported by submitting APHIS/CDC Form 4 within 7 calendar days. In the case of proficiency testing for select agents or toxins, APHIS/CDC Form 4 must be submitted within 90 calendar days of receipt of the select agent or toxin. The UA does not possess any Tier 1 Select Agents.

### **Entry Requirements**

Access is defined if the University of Arizona employee is required as part of their job to enter an SA/T laboratory, or possess a select agent or toxin (e.g., ability to carry, use, or manipulate).

No individual is provided access to select agents or toxins at the University of Arizona unless that individual is approved by the HHS Secretary or Administrator, following a security risk assessment (SRA) by the Attorney General. This includes filling out an FD 961 form and being fingerprinted using the card sets and applications provided by the Department of Justice CJIS Bioterrorism Act Fingerprint Checks Program.

The Responsible Official or Alternate Responsible Official provides the FD 961 form and the fingerprint card sets to University of Arizona employees who are required to have access to select agents and toxins as part of their jobs. The Responsible Official will coordinate with both

the applicant, University of Arizona Police Department and the Laboratory Principal Investigator on completing the documentation and submitting it to Department of Justice CJIS.

Authorized visitors must be escorted at all times by an SRA-approved individual and the visitor must be in line of sight at all times.

Each laboratory will be specifically registered under the provisions of 42 CFR Part 73 for select agents and toxins. The possession, transfer, use, storage or disposal by Principal Investigators and laboratory staff, of select agents or toxins other than those registered for a particular lab, is in violation of federal law and University of Arizona policy.

### **Administrative Controls**

No work will be conducted with select agents or toxins at the University of Arizona unless it has been specifically approved in compliance with the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and the Select Agent and Toxin Rule.

No work will be conducted with select agents or toxins at the University of Arizona unless it has been specifically approved by the Institutional Biosafety Committee.

No restricted experiment will be conducted unless approved in accordance with any conditions prescribed by the HHS Secretary.

Only SRA-approved individuals may work with select agents and toxins.

All SRA-approved individuals will receive annual training regarding both operational and emergency response.

### **Visitor Training**

All visitors that enter a Select Agent Laboratory must be provided with a safety orientation regarding the agent specific pathogens in use. This includes route of exposure, signs and symptoms, and emergency procedures and contacts. Visitor's must be escorted and under line of sight of an SRA approved individual at all times.

### **Transporting Select Agent and Toxins**

In addition to the University's standard transport requirements to transport Select Agents and Toxins individuals must:

- Only transfer material to individuals or entities registered to possess, use, or transfer that agent or toxin.
- [APHIS/CDC Form 2](#) must be used to document transfers from or to other institutions with a certificate of registration from CDC or APHIS

- All intra-entity transfers will be handled by the Responsible Official. The RO will maintain all documents.

The recipient must immediately notify The RO or ARO who will inform the CDC or APHIS if the select agent or toxin has not been received within 48 hours after expected delivery time, or if the package containing select agents or toxins has been damaged to the extent that a release of the select agent or toxin may have occurred.

## **Biosecurity**

There are additional requirements for laboratories that use Select Agents and Toxins (SA/T); these security requirements are outlined in the University of Arizona Biosecurity Plan for Select Agents and Toxins.

## **Injuries and Worker Health**

### **Laboratory Acquired Infections**

A laboratory-acquired infection (LAI) is an infection that resulted from laboratory work, whether it occurred in a laboratory worker or in another person who happened to be exposed, as a result of research or clinical work with infectious agents.

Microorganisms can enter the body through the mouth, the respiratory tract, broken or intact skin and the conjunctivae. In laboratory-acquired infections, the route may not be the same as when the disease is acquired naturally.

Modes of infection can be classified into two categories:

- Infections preceded by overt personal accidents, which include:
  - Inoculation (i.e. resulting from pricking, jabbing or cutting the skin with contaminated instruments such as hypodermic needles, scalpels and glassware; and from animal bites or scratches).
  - Ingestion (i.e. resulting from mouth-pipetting, eating, drinking and smoking, which is why these practices are not permitted in the lab).
  - Splashing into the face and eyes.
  - Spillage and direct contact.
- Infections not preceded by personal accidents:
  - Aerosols, droplets and fomites. Aerosols are defined as a cloud of very small liquid droplets produced whenever energy is applied to a liquid, and such liquid is allowed to escape into the environment. The larger droplets (greater than 0.1 mm in diameter) will settle quickly and contaminate the surfaces upon which

they come to rest. The smaller droplets do not settle, but rather evaporate very rapidly.

It has been shown that many laboratory techniques using both simple and complex mechanical equipment, as well as laboratory accidents, produce aerosols.

### **Vaccines**

The Hepatitis B vaccine or titer check is offered to all employees that may come in contact with human blood and non-human primate blood, tissue, body fluids, and cell cultures. The vaccine is available through Occupational Health Clinic. Other vaccines may be available upon request.

### **Medical Surveillance**

Medical Surveillance must be provided for all persons who perform work with certain agents, which are primarily at BSL-3, including select agents and toxins. An annual questionnaire must be completed as well as additional tests required based on the agent(s) in use and the answers provided in the questionnaire.

### **Pregnancy and Individuals with Compromised Immune System**

Lab personnel should self-identify any condition regarding immune competence and medical conditions that may predispose to infection. It is recommended that individuals who are pregnant, planning on becoming pregnant, or have an impaired immune system consult with a professional regarding their unique situation. RLSS offers confidential counseling or you may speak to your personal physician or a physician at campus health. While agents at BSL-2 are not a serious threat to a healthy adult some of these agents can be detrimental to a fetus or an individual with a compromised immune system. For example *Listeria monocytogenes*, *Toxoplasma gondii*, Lymphocytic Choriomeningitis virus are detrimental to a fetus and *Pseudomonas aeruginosa* can cause complications for individuals with Cystic Fibrosis.

Additional PPE and other enhanced biosafety practicing can be recommended.

### **Injuries**

Each laboratory containing recombinant and/or biohazardous material must have a first aid kit. The kit must contain a disinfectant and bandages. It is the responsibility of the Approval Holder to ensure that these items are readily available, stocked at all times and are within their expiration dates.

If an injury occurs:

- Remove gloves if worn
- Wash injured skin with soap and water for 3-5 minutes

- If splashed in eyes or mucous membranes, rinse with water for 15 minutes
- Apply first aid
- Contact your supervisor and RLSS
- You may go to Campus Health. If closed, you may go to any hospital or urgent care.

### **Injury Reporting**

- Report all injuries to RLSS
- Your supervisor must fill out “The University of Arizona Supervisor’s Report of Employee Injury/Illness” with the department within 7 calendar days and return it to Risk Management and Safety. This form is available on-line from Risk Management and Safety.
- A “Worker’s Physician’s Report of Injury” must be filled out at the location where you receive treatment for medical care (102 form, pink, four page carbon copies). You fill out the top of this form. Your physician fills out the bottom.

### **Exposure to potentially infectious bodily fluid**

- If there are no infiltrations of mucous membranes or open skin surfaces, it is not considered an occupational exposure
- Report all accidents involving blood or bodily fluids
- Confidential post-exposure medical evaluations are offered by Campus Health at no cost to the employee

### **Emergency Procedures**

#### **Recombinant and Biohazardous Material Spill Cleanup Procedures**

Each laboratory containing recombinant and/or biohazardous material must have a spill kit. The kit must contain appropriate disinfectants, absorbent material, personnel protective equipment, biohazard bags, and a dust pan and broom. It is the responsibility of the Approval Holder to ensure that these items are readily available and stocked at all times. The Approval Holder must also develop appropriate containment, inactivation and spill cleanup procedures for the specific biological materials to be used in the laboratory.

If a spill occurs outside of containment, the spill is considered too large or too dangerous for laboratory personnel to safely clean up, involves select agents, or occurs in a public space, secure the spill and the area and call ORCB,S or UAPD if after hours, immediately for assistance.

Check yourself and your clothing for potential contamination. Remove all contaminated clothing and place it in a bag for decontamination. Contaminated body areas must be washed immediately with soap and water, use an emergency shower if available.

Biological spills outside biological safety cabinets will generate aerosols that can be dispersed in the air throughout the laboratory. Serious exposure can result from an aerosol outside of containment at BSL-2 and BSL-3. To reduce the risk of exposure in such an incident, occupants must immediately evacuate the laboratory or don an appropriate respirator. Allow the aerosol to settle for at least 30 minutes prior to reentering the laboratory to decontaminate and clean up the spill. Ensure the appropriate PPE is worn when decontaminating any spill. The following procedures are provided as a guideline to recombinant and biohazardous spill cleanup.

### **Spills with non-infectious and/or recombinant agents or inside the Biosafety Cabinet**

- Alert people in immediate area of spill
- Put on proper personnel protective equipment
- Cover spill with paper towels or absorbent pads
- Carefully pour a freshly prepared 10% (vol./vol. w/water) dilution of household bleach around the edges of the spill and then into the spill. Avoid splashing
- Allow a 15 minute contact period
- Use paper towels or absorbent pads to wipe up the spill, working from the edges into the center
- Clean spill area with fresh towels soaked in disinfectant
- Place towels or absorbent pads in red plastic bag for disposal in the Biohazardous waste container

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

If a spill occurs outside of the biosafety cabinet, but remains within the laboratory, the laboratorian must secure the spill and the area and call RLSS or UAPD if after hours immediately for assistance and perform the following:

- Clear area of all personnel
- Follow instructions provided by RLSS.
- Wait at least 30 minutes for aerosol to settle before entering the spill area. RLSS may need to notify the building manager to shut down air the handling system.
- Remove any contaminated clothing and place it in a biohazard bag to be autoclaved.
- Prior to starting the decontamination process, put on agent specific PPE and a respirator if appropriate.
- Initiate cleanup with 10% sodium hypochlorite solution as follows:

- Place absorbent material on spill; then layer a second set of disinfectant soaked paper towels over the spill
- Encircle the spill with additional disinfectant being careful to minimize aerosolization while assuring adequate contact
- Decontaminate all items within spill the area
- Allow a minimum of 20 minutes contact time. Note: a longer contact time may be necessary for some agents
- Wipe equipment with appropriate disinfectant
- Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures (e.g., autoclave)

### **Spills with an infectious agent outside of containment**

If a spill occurs outside containment of the laboratory, the laboratorian must secure the spill and the area and call RLSS, or UAPD if after hours, immediately for assistance and perform the following:

- Clear area of all personnel and do not leave until assistance arrives to secure the spill.
- Follow instructions provided by RLSS.
- If indoors, wait at least 30 minutes for aerosol to settle before entering the spill area. RLSS may need to notify the building manager to shut down the air handling system.
- Remove any contaminated clothing and place it in a biohazard bag to be autoclaved.
- Prior to starting the decontamination process, put on agent specific PPE and a respirator if appropriate.
- Initiate cleanup with 10% sodium hypochlorite solution as follows:
  - Place absorbent material on spill; then layer a second set of disinfectant soaked paper towels over the spill
  - Encircle the spill with additional disinfectant being careful to minimize aerosolization while assuring adequate contact
  - Decontaminate all items within spill the area
  - Allow a minimum of 20 minutes contact time. Note: a longer contact time may be necessary for some agents
  - Wipe equipment with appropriate disinfectant
  - Discard contaminated disposable materials using appropriate biohazardous waste disposal procedures (e.g., autoclave)



## **Inventory Control**

The suspected loss, theft or release of any recombinant or biohazardous material, or any suspicion that inventory has been altered or compromised to RLSS immediately.

## **Medical and Fire Emergencies involving Recombinant and/or Biohazardous Material**

Call 911 and report the emergency. Give as much information about the situation as possible.

Include:

- Location
- Condition of victim or cause of fire
- Your name
- The type of recombinant and/or biohazardous material and how it is involved