

# BIOSAFETY PLAN

Updated 12/12/2022

*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

# TABLE OF CONTENTS

Mission Statement.....	1
Definitions of Recombinant and Biohazardous Material.....	1
Recombinant Nucleic Acid Requirements .....	1
Use of Recombinant and Biohazardous Material with Animals .....	2
Human and Non-Human Primate Tissues and Cell Culture.....	2
Bloodborne Pathogens.....	3
Human Brain Tissue .....	3
Viral Vector Guidelines.....	3
Select Agents and Toxins .....	4
Responsibilities .....	5
Approval Holder.....	5
Approval Safety Coordinator.....	6
Laboratory Workers .....	6
Risk Management Services .....	6
Research Laboratory and Safety Services.....	7
Institutional Biosafety Committee (IBC).....	7
Biosafety Containment Levels.....	8
Biological Safety Level-1 (BSL-1).....	8
Biological Safety Level-2 (BSL-2).....	8
Biological Safety Level – 3 (BSL-3) .....	9
Plant Biosafety Levels .....	11
Plant Biological Safety Level – 1 (BSL-1P).....	12
Plant Biological Safety Level – 2 (BSL-2P).....	12
Standard Operating Procedures .....	13
Field Work Involving Transgenic Plants .....	14

---

Trainings .....	14
Standard Microbiological Practices .....	15
Work Practices for Recombinant and Biohazardous Material.....	15
Signs and Labels.....	15
Sharps Precautions Requirements.....	15
Vacuum Systems .....	16
Pipetting .....	16
Storage of Biological Materials .....	17
Transporting Recombinant and Biohazardous Material.....	17
Shipping Regulations Recombinant and Biohazardous Materials.....	18
Pest Management .....	18
Record Retention.....	18
Personal Protective Equipment.....	19
Laboratory Clothing .....	19
Gloves .....	19
Face Protection.....	19
Footwear.....	20
Respirators.....	20
Laboratory Equipment .....	20
Biosafety Cabinets.....	20
Centrifuges .....	23
Aerosol-generating Equipment.....	23
Decontamination.....	25
Recombinant and Biohazardous Waste .....	26
Waste Disposal Using Risk Management Services.....	26
Autoclave Your Own Waste.....	27
Other waste options.....	27
Closing out a Recombinant and/or Biohazardous Laboratory.....	27
Injuries and Worker Health.....	28
Laboratory Acquired Infections.....	28

---

Vaccines .....	28
Medical Surveillance .....	29
Pregnancy and Individuals with Compromised Immune System .....	29
Injuries .....	29
Injury Reporting .....	29
Emergency Procedures.....	30
Recombinant and Biohazardous Material Spill Cleanup Procedures .....	30
Spills inside the Biosafety Cabinet .....	30
Spills Outside of the Biosafety Cabinet and Primary Containment.....	31
Spills Outside of the Laboratory .....	31
Inventory Discrepancy.....	32
Medical and Fire Emergencies involving Recombinant and/or Biohazardous Material.....	32

---

## Certification and Approvals

This Biosafety Plan has been approved by:

James Spencer, MS, MPH, CSP, RBP  
Biological Safety Officer

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 is required to be reviewed annually and updated whenever changes occur. The signature below signifies completion of the annual review for this plan.

12Dec2022

---

**Signature of Biological Safety Officer**

**Date**

**James Spencer, MS, MPH, CSP, RBP**

**Research Laboratory & Safety Services (RLSS)**

1717 E. Speedway Blvd. (Bldg. 151)

Suite 1201

Tucson, AZ 85724

P.O. Box 245101

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

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

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

---

## **Mission Statement**

Research Laboratory & Safety Services (RLSS) serves the University of Arizona and various regulatory, research, clinical, and educational units around the State of Arizona.

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 plan is reviewed annually and revised as necessary.

## **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 RAC.
- 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 of the University of Arizona, and all other applicable agencies, must be followed.

### **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 Animal Biosafety Level – 2 (ABSL-2) for 72 hours post inoculation. Review the 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. Additional personal protective equipment (PPE) may be required.

All personnel working at ABSL-3 must be respirator fit tested in case there is a need to perform a procedure with a higher risk of aerosolization, as notated in the specific protocol. Individuals using respirators are required to enroll in the [Respiratory Protection Program](#) (RPP).

### **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:

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

It is not acceptable to handle any of the above cell lines on a clean bench or in a horizontal laminar flow hood unless approved by RLSS and the Institutional Biosafety Committee (laboratory equipment defined in later section).

## **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 Services (RMS). RMS maintains the University of Arizona Bloodborne Pathogen Policy and the [Exposure Control Plan](#).

## **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 at a minimum of BSL-2. Human brain tissue can transmit prion diseases such as variant Creutzfeldt-Jakob Disease and Kuru. Although prion diseases linked to handling brain tissue are rare, they are able to remain infectious for long periods in fixed tissues and require special treatment to ensure their destruction before discarding as waste. If you are 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, autoclaving at 134°C for a minimum of thirty (30) minutes is required.
- Chemical destruction requires 1N NaOH immersion for one (1) hour, rinsing, followed by autoclaving at 121°C for one (1) hour.
- Other methods of sterilization can be found in the below reference. If you wish to incinerate your material, you must contact RMS for assistance.

[World Health Organization \(WHO\) Infection Control Guidelines](#)

## **Viral Vector Guidelines**

All work involving recombinant nucleic acids must be approved by the University of Arizona 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 two-thirds 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 Adeno-Associated Virus (AAV), Murine Retrovirus, Feline Immunodeficiency Virus (FIV) and Vesicular Stomatitis Virus (VSV).

Exceptions must be requested using the 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 and Toxins**

Select Agents and Toxins (SA/T) are biological agents that the U.S Department of Health and Human Services (HHS) and the US Department of Agriculture (USDA) 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 Center for Disease Control and Prevention (CDC) and HHS issued a final rule in October 1996 regarding the transfer of SA/T that could be used in terrorist activities. The law requires additional requirements for facilities that utilize SA/T in their research.

All researchers that want to conduct research with Select Agents or Toxins must first contact Research Laboratory & Safety Services for guidance on the registration process. Please plan for a minimum of six (6) months to complete the registration process. **The University of Arizona currently does not have an active Select Agent & Toxin Registration with the CDC.**

### **Physical Inventory Requirements:**

In 2014, there were several reported lapses involving biosafety practices in Federal laboratories. These lapses have prompted the Federal government to announce a new biosafety stewardship initiative designed to remind all laboratorians of the importance of constant vigilance over our implementation of biosafety standards. As part of this initiative, the University of Arizona requires every laboratory to annually document all biohazardous material in their possession. This requires visual inspection of all freezers, including liquid nitrogen (LN2) Dewars. Any material that is unknown, unlabeled, or unnecessary must be disposed properly. You may use any method that ensures proper tracking of agents in your laboratory.

## **Responsibilities**

### **Approval Holder**

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

The AH 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 AH's laboratory. The AH is responsible for assuring the appropriate safety training of employees, for correcting errors and unsafe working conditions, and for documentation of these elements.

The following are general responsibilities of the AH:

- Be trained in standard microbiological techniques.
- Submit all protocols for review by the 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 and/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 and/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 AH.
- 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.
- Keep biosafety approval information up to date on the RLSS online Dashboard.

During the conduct of research, AH 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 and/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/or 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 AH may choose to delegate aspects of the biosafety program in their laboratory to another laboratory worker. This individual will be designated as the Approval Safety Coordinator (ASC), which is similar to a laboratory manager. 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 workers responsibility to:

- Follow biosafety practices and lab-specific procedures designated in the University Biosafety Plan and laboratory specific Biosafety SOP (when applicable).
- Complete required online training in the RLSS Dashboard and/or in-person training from RLSS staff.
- Inform the AH or ASC of any condition(s) that may require additional safety precautions.
- Report to the AH or ASC and RLSS all problems, concerns, violations in procedure, and/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, AH, ASC, or RLSS.

### **Risk Management Services**

Risk Management Services (RMS) provides the following services:

- Provide Bloodborne Pathogen Training (UAccess Learning).
- Maintain the University's Exposure Control Plan.
- Provide DOT/IATA shipping training for biohazardous materials and dry ice.
- Maintain Bloodborne Pathogen training records.
- Collect and properly dispose of recombinant and biohazardous waste.

- Partner with other campus entities to assist in preparing for, and responding to, emergency situations on campus.

### **Research Laboratory and Safety Services**

RLSS provides the following services:

- Provide training and assistance to laboratory staff.
- Assist researchers with gaining IBC approval for submitted protocols and amendments.
- Audit laboratories.
- Assist laboratories in maintaining safety and regulatory compliance.
- Assist with emergency response.
- Maintain laboratory biosafety program.
- Provide administrative and technical support to the IBC.
- Submit reports to regulatory authorities.
- Plans and conducts biosafety drills.

### **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, or for entities that have a contract with the University of Arizona. They must ensure experiments are evaluated and designed to minimize laboratory acquired infections of recombinant and biohazardous material. The committee makes recommendations or requirements when necessary to modify experimental procedures to reduce the possibility of inadvertent generation of 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. Members are selected for their expertise, ensuring that the committee has the 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 expected, to be engaged in, or have a direct financial interest. IBC meetings are held the third Wednesday of each month (unless otherwise specified), and are open to the public.

The AH or ASC must complete a New Protocol Application Form for all research involving the use of recombinant and/or biohazardous material. The AH must obtain IBC approval before changing any variable in their research. After committee review and approval, the AH is sent a letter of approval from the committee chair.

## **Biosafety Containment Levels**

### **Biological Safety Level-1 (BSL-1)**

Suitable for work involving well-characterized agents not known to cause disease in healthy humans and are of minimal potential hazard to laboratory personnel and the environment.

Examples include: *Bacillus subtilis*, *Bacillus cereulans*, *Escherichia coli* (non-pathogenic strains of *E. coli* such as K-12), Murine cell lines, samples from laboratory mammals.

When working at BSL-1, workers must follow all standard microbiological practices, receive all specific training on procedures and equipment and wear the minimum personal protective equipment (PPE) of a lab coat and gloves.

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.

### **Biological Safety Level-2 (BSL-2)**

BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
- Access to laboratory is restricted when work is being conducted.
- All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

At BSL-2, workers are required to follow the standard microbiological practices and wear minimum personal protective equipment (PPE) of a lab coat and gloves. Additional PPE may be required depending on procedures. The AH must ensure that laboratory personnel demonstrate proficiency in standard microbiological practices before working with BSL-2 agents.

Additional requirements at BSL-2 include:

- Access to the lab is limited when work is being conducted and must be locked when unoccupied.
- All aerosol-generating procedures must be performed in the BSC or otherwise appropriately contained.
- Infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Lab equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
- Medical surveillance and vaccines may be required for specific work.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the lab supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.
- Standard Operating Procedures (SOPs) are required to be available to all laboratory workers and any visitor or contractor upon request. SOPs must contain:
  - Agent specific information.
  - Lab specific bio-containment procedures.
  - Decontamination and waste procedures.
  - Applicable Medical Surveillance Program information.
  - Acknowledgement pages signed by laboratory workers and visitors.

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

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 open bay or common use areas must be secured when not in use.

### **Biological Safety Level – 3 (BSL-3)**

BSL-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 SARS-CoV-2.

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 required PPE and the proper way to put it on and remove it. Additional PPE requirements at BSL-3 are:

- Water-resistant closed-front lab coat
- Double gloves
- Eye protection when there is a splash hazard
- Respiratory protection as required

Certain agents, and any work with live animals may require additional PPE. BSL-3 workers must be medical cleared and fit tested for an appropriate respirator. 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 BSC. Work on the bench top with recombinant and biohazardous materials is not permitted.
- Physical containment devices, such as centrifuge safety cups, sealed rotors, and HEPA-filtered isolation 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 ensure the laboratory is under negative pressure prior to each time they enter the laboratory.
- 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.
- Agents must be secured in storage locations at all times when not in use.

The AH 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. Undergraduates are not allowed to work alone in the BSL-3 and must be always escorted by a member of the laboratory. 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 that will be kept long term must be stored in a secure, locked location.

### **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, agents must be secured in storage locations and 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 AH or ASC and RLSS to ensure that the laboratory is functioning properly before work with recombinant and biohazardous materials may resume.

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 (2) weeks in advance.

Special containment systems, such as an exhaust HEPA filtration system, must be tested and certified annually to meet NSF International/ANSI standard 49.

The AH or ASC 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 and completion time.
- Disinfection technique for contaminated tools.
- Special PPE, if applicable, required to protect the workers (e.g., boots, heavy gloves, face shield etc.).
- Work order number.

### **Plant Biosafety Levels**

Plant research generally, but not always, does not pose a human health hazard; therefore biosafety principles are designed to protect the natural and agricultural environment. Recombinant DNA practices, under the National Institutes of Health (NIH) Guidelines, drive most of the plant biosafety issues. Field work with genetically engineered plants requires permits from USDA/APHIS before work can begin.

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. 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.



The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants.

### **Plant Biological Safety Level – 1 (BSL-1P)**

BSL-1P is 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 is limited or restricted when experiments are in progress.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL-1P greenhouse practices and procedures.
- All procedures must be performed in accordance with accepted greenhouse practices appropriate to the experimental organism.
- Records will be kept of experiments currently in progress in the greenhouse facility.
- Render experimental organisms 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.
- House arthropods and other motile macroorganisms 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.

### **Plant Biological Safety Level – 2 (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: *Meliodogyne incognita* (root-knot nematode), *Pepino mosaic virus* (PepMV), *Pectinophora gossypiella* (Pink bollworm), and *Pseudomonas syringae*.

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

- A program 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, leak proof 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.
- 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.
- All incidents regarding BSL-2P agents that have a USDA/APHIS permit must be reported to the supporting regulatory agency and RLSS.

### **Standard Operating Procedures**

Standard operating procedures (SOPs) are required for all field work involving transgenic plants and all work at BSL-2P. The SOPs 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, contact Research Laboratory & Safety Services before beginning your project. 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.
- Harvesting & destruction reports.

### **Trainings**

Appropriate training is required for all individuals that work with and/or supervise the use of recombinant and biohazardous material. The Biosafety Level-3 Protection Course is the only training with an annual refresher requirement which can be taken online. You can see the status of your training by logging into the RLSS [User Dashboard](#).

**Basic Biosafety Protection Course** – required for all personnel working in BSL-1 or BSL-2 registered laboratories.

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

**BSL-3 Protection Course** – required for anyone working within a registered BSL-3 laboratory.

**Bloodborne Pathogen Course** – prerequisite for the Basic Biosafety Protection Course and required for any individual who may come in contact with bloodborne pathogens. The course is provided by [Risk Management Services](#).

## **Standard Microbiological Practices**

All individuals working with recombinant and/or biohazardous material must follow the standard microbiological practices:

- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in the laboratory. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Wear appropriate personal protective equipment; including no open-toed shoes.
- Follow policies for safe handling of sharps, such as needles, scalpels, pipette tips, and broken glass.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Long hair should be pulled back to prevent exposure to agents, chemicals, and open flames.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and any other potentially infectious materials before disposing using an effective method.
- A sign incorporating the universal biohazard symbol, and the room's biosafety level, must be posted at the entrance to the laboratory when infectious agents are present.
- Use of glassware should be avoided; plastic ware should be substituted.
- AH must ensure that the laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

## **Work Practices for Recombinant and Biohazardous Material**

### **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 dewars, and transport containers.

### **Sharps Precautions Requirements**

The University of Arizona 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.

- 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. Acceptable puncture resistant containers include used plastic bottles with a loose seal (1/4 turn from being fully tightened) and bag-lined cardboard boxes.

### **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. A HEPA type filter is required for work in a BSL-3 laboratory.
- 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 an apparatus typically consisting of a narrow tube. Pipets can be constructed of a variety of glass or plastic materials. Liquids are drawn into the pipet using 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 pipet tips in hard walled, leak proof 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 biohazard.
- 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.
- 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 may be stored in refrigerators, freezers or incubators (e.g. food, medication, beverages) designated for lab use.
- When storage equipment needs repair, calibration or transport, it must be completely decontaminated prior to starting work or being removed. Biohazard stickers must be removed from the equipment once it has been decontaminated.

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

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

Whenever 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, leak-proof secondary container.

- 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 Recombinant and Biohazardous Materials**

Individuals that ship infectious, potentially infectious, or regulated plant material with commercial carriers (i.e. FedEx, UPS, etc.) are required to have the proper training. This is mandated by the U.S. Department of Transportation (DOT) and /or the international Air Transport Association (IATA).

Accepted forms of training are:

- Documented training by Risk Management Services or,
- Any accredited Shipping Infectious Materials class from the DOT.

Examples of material that falls under this requirement are:

- Human cell lines
- Pathogenic organisms
- Viral vectors
- Recombinant plants
- Recombinant DNA

Extracted DNA does not fall under these rules.

### **Pest Management**

Approvals must have a method in place for reporting and controlling pests. 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 a minimum of three (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.

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.

Double gloving may be appropriate or required. However, if a medical condition dictates that only a single pair is worn, that can be 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. Gloves must not be worn when handling non-infectious material, equipment, or instrumentation. Remove gloves in a manner that does not contaminate your hands. 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. If disposable shoe covers are used in the laboratory, they must not be reused and waste containers must be available to dispose of used shoe covers.

### **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 complete the medical surveillance form with Occupational Health, complete the university RPP online training, and be fit tested by RLSS on an annual basis.

## **Laboratory Equipment**

### **Biosafety Cabinets**

Biosafety cabinets are often referred to 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. Some laboratory procedures generate aerosols that may spread recombinant and biohazardous material in the work area and pose a risk of infection to the worker. BSCs are used to prevent the escape of those aerosols or droplets.
- Chemical fume hoods are designed solely to protect the individual from exposure to chemicals and noxious gases. They are not equipped with HEPA filters and therefore 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.

Below are the distinctions between the various types of BSCs:

- **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 Biological Safety 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 or experiments with a high potential for aerosolization of an agent that is transmitted by aerosolization.

Below are steps that should be taken before using a BSC:

- 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 worked with in the laboratory.

Below are procedures that should be observed while using a BSC:

- Minimize disruption of airflow.
- Open continuous flames are not permitted to be used inside the BSC unless the procedure has been review and approved by the UA IBC.
- Keep front and back grills free of materials that might block airflow.

- Minimize items within the cabinet.
- Disinfect items that are removed during the course of work.

Below are steps that should be taken when working in a BSC has concluded:

- All items removed from the BSC must be appropriately decontaminated first.
- Decontaminate readily accessible interior surfaces with a disinfectant appropriate for the agents used in the BSC.
- Allow five minutes of operation to purge the system.
- Turn off cabinet blower.

### **Movement and Instillation of a BSC**

Disinfect BSC work surfaces prior to moving them to new facilities and remove biohazard labeling. BSCs used for work with pathogenic organisms may require paraformaldehyde decontamination before being moved. Contact a Facilities Management at (520) 621-3000 to have a BSC decontaminated and moved.

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. Contact Facilities Management at (520) 621-3000, or an accredited outside company, for BSC certification.

### **Decontamination and Maintenance of a BSC**

The AH is responsible for cleaning and decontamination their BSC with an appropriate disinfectant. When paraformaldehyde decontamination is performed by Facilities Management, personnel will disconnect the cabinet and label when the cabinet was decontaminated. If the safety cabinet is equipped with a UV light, do not use this as your primary disinfectant.

### **Certification of a BSC**

Biosafety cabinets should be registered with RLSS and be certified annually by Facilities Management or an accredited third party. Certification ensures that the cabinet is working properly and that the HEPA filters are in good condition. The certification sticker on the BSC must be visible and the date legible.

In addition to annual certification, BSCs must also be certified:

- After a cabinet has been moved, even if it shifted a short distance.
- After maintenance procedures are performed on internal parts.
- After HEPA filters have been changed.

## **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, and users must be properly trained. 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 BSC.
- Avoid overfilling centrifuge tubes. 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 inside of a BSC.
- Work in a BSC 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. Manufacturer 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 BSC when working at BSL-2 or BSL-3, or may require assessment for the use of respiratory protection.

### **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 entire device and jar 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 BSC. 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 that must be utilized if available.

The use of French presses 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 BSC. The pressing of human pathogens outside of a BSC must be approved by RLSS, and operators must use face shields or other means of 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 on 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 BSL-3, it must be done in a BSC.

### **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 BSC.

- 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.
- 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 BSC. The vacuum pump exhaust must be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. 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 BSC 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 BSC 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, 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 pre-sterilized, 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, and ultraviolet or ionizing radiation are not universal and may not substitute for autoclaving or incineration before disposal in all situations.

The preferred chemical for decontamination is a 10% concentration of sodium hypochlorite. If your approval chooses to use a different chemical for decontamination the process and efficacy must be documented in your SOPs and approved by the IBC. 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.

## **Waste Disposal Using Risk Management Services**

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 is required for proper waste packaging:

- Waste must be double bagged in red or orange bags.
- Bags must be closed properly with tape.
- No sharps or sharp-like objects, including contaminated broken glass and pipette tips, unless contained in puncture resistant devices.
- Check bags for punctures prior to disposal.
- Sharps containers specifically designed for needles/syringes must be sealed but do not require over-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.
- RMS will not pick up waste if it is over flowing or not inside the container.

- If waste becomes odiferous, the RMS containers can be relocated to a cold room.
- Animal carcasses have designated cold room collection points in Animal Care facilities.
- Do not use RMS containers for liquid waste, carcasses, or recognizable human anatomical remains. These types of waste can be collected separately with a request to RMS.

Contact RMS for additional red bins, for more frequent waste pick-ups, or with questions concerning biohazardous waste collection.

### **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 that is run in the autoclave including: duration of cycle, temperature and pressure, date, and initials of individual running the cycle.
- 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 SOPs.
- Attach the Regulated Biological Waste Label to bag.
- Check autoclave tape and document in logbook.
- Place the autoclaved recombinant and biohazardous waste bag into an opaque bag after autoclaving and then place in the RMS container.
- Log book and indicator test records must be kept for at least three (3) years.

### **Other waste options**

- Liquid waste can be brought up to a 10% bleach concentration for 15 minutes and disposed of down the drain. Halogenated solvents, flammable solvents, phenolic compounds, and corrosive materials ( $5 < \text{pH} < 11$ ) may not be poured down the sink.
- Recognizable human anatomical remains must be cremated or interred. Contact RMS for disposal of these remains.
- 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**

AH at the University of Arizona are responsible for leaving all assigned laboratory areas in a safe condition when vacated. Things to consider before closing a laboratory:



- Several months before the planned lab close-out, inform RLSS of the planned closure date.
- If recombinant or biohazardous material will be shipped to a new location, then the AH 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.

## **Injuries and Worker Health**

### **Laboratory Acquired Infections**

A laboratory-acquired infection (LAI) is an infection that results 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 LAI, 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 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 with direct contact aerosolization.
- 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 the [Occupational Health Clinic](#). Other vaccines may be available when required or 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 SA/T. 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 medical professional. RLSS offers confidential counseling, or you may speak to your personal physician or someone in Occupational 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* and *Toxoplasma gondii* are detrimental to a fetus, and *Pseudomonas aeruginosa* can cause complications for individuals with Cystic Fibrosis.

Additional PPE and other enhanced biosafety practicing are commonly recommended for individuals that fall into these categories. Please see the RLSS webpage on [Pregnant Laboratory Workers](#) for more information.

### **Injuries**

Each laboratory containing recombinant and/or biohazardous material must have a first aid kit. The kit must contain a disinfectant and bandages ([OSHA recommended kit](#)). It is the responsibility of the AH 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 Occupational Health Services. If closed, you may go to any hospital or urgent care.

### **Injury Reporting**

- Report all injuries to RLSS and your supervisor.

- Your supervisor must fill out the injury form located on the Risk Management Services [website](#).
- Risk Management Services can assist with worker compensation filing.

## **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 AH to ensure that these items are readily available and stocked at all times. The AH 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 a Biosafety Cabinet, is considered too large or too dangerous for laboratory personnel to safely clean up, involves select agents, or occurs in a public space, you must secure the spill and the area and call Research Laboratory & Safety Services at (520) 626-6850 (or the University of Arizona Police Department if after hours) immediately for assistance.

Remove all potentially contaminated clothing and place it in a bag for decontamination. Exposed or contaminated skin must be washed immediately with soap and water. Use an emergency shower if available.

Biological spills can generate aerosols that can be dispersed 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 hold their breath, notify others in the surrounding area, and immediately evacuate the area. Allow the aerosol to settle for at least thirty (30) minutes prior to reentering the laboratory to decontaminate and clean up the spill. Ensure the appropriate personal protective equipment (PPE) is worn when decontaminating any spill. RLSS may require individual cleaning up the spill to wear respiratory protection.

### **Spills inside the Biosafety Cabinet**

- Alert people in immediate area of spill.
- Remove any contaminated personnel protective equipment (PPE) and don fresh PPE.
- 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 and Primary Containment**

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

- Hold breath and clear the area of all personnel.
- Follow instructions provided by RLSS.
- Wait at least thirty (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 a 10% solution of 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 area.
  - Allow a contact time of a minimum of twenty (20) minutes (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 Outside of the Laboratory**

If a spill occurs outside of the laboratory, the worker must should follow these steps:

- Hold your breath, remove any contaminated clothing, clear the area of all personnel, and remain near the area until assistance arrives.
- Ensure that no one enters the area where the spill has occurred, and contact RLSS to inform them of the spill (contact UAPD afterhours). The RO/ARO will meet you at the location of the spill, and bring the proper PPE, disinfectant, clean-up items, and respiratory protection needed.
- Wait at least 30 minutes for potential aerosols to settle before entering the spill area. If the spill is indoors, RLSS may need to notify the building manager to shut down the air handling system.
- Ensure that proper PPE is donned before entering the spill area. Discuss with RLSS if respiratory protection will be required for spill clean-up.

- Initiate cleanup with freshly made 10% bleach solution as follows:
  - Place absorbent material on the spill.
  - Apply disinfectant in a circular pattern around the spill starting from the outside moving inward, until the entire spill area has been covered by disinfectant.
  - Allow a minimum of 15 minutes contact time to ensure complete disinfection. Note that RLSS may require a longer contact time for certain agents.
  - Items located near the spill area that may have been splashed should be wiped down with 10% bleach.
  - Carefully discard of the absorbent material into double bagged biohazardous waster bags, and dispose of in autoclave or biohazardous waste accumulation site.
  - Wipe up any leftover disinfectant from the floor.

### **Inventory Discrepancy**

Report 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. After speaking with emergency services, call RLSS at (520) 626-6850. Provide as much information about the situation as possible, including:

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