

UNIVERSITY OF ARKANSAS
BIOLOGICAL SAFETY MANUAL

University of Arkansas
Fayetteville, Arkansas

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Purpose and Scope

This document applies to all University of Arkansas and Division of Agriculture activities, funded and unfunded, performed on the campus and at the farms, extension stations, and other off-campus facilities, and to activities performed by University personnel at other, non-University facilities. Both teaching and research activities are covered, as well as field operations. Personnel covered by the program include graduate and undergraduate students, part and full time faculty and staff, and visitors.

This manual specifies controls and handling practices required for microbiological agents, (bacterial, viral, and fungal, as well as certain multicellular parasites), biological toxins, recombinant DNA molecules, human or non-human primate blood or tissues, and animal cell cultures.

Radioactive materials are not addressed in this document. Please contact the Radiation Safety Officer (RSO) at 5-5448 for information regarding the use of these materials.

Protocols involving animals must be reviewed and approved by the University's Animal Care and Use Committee. In addition, the reader is referred to the CDC/NIH Publication No. (CDC) 93-8395, *Biosafety in Microbiological and Biomedical Laboratories* for a complete discussion of appropriate precautions for working with infected animals.

Responsibilities of the Institutional Biosafety Committee

Institutional Biosafety Committee (IBC) shall:

1. Advise the Chancellor and the Provost on issues relating to biological safety.
 2. Meet for review of:
 - research protocols involving recombinant DNA (rDNA) research in accordance with National Institutes of Health (NIH) guidelines;
 - protocols involving Risk Group (RG) 3 or Biosafety Level (BSL) 3 agents (Appendix I);
 - protocols involving RG-2 or BSL-2 agents (Appendix I) propagated in production quantities or used in procedures in which the agent is likely to become aerosolized;
 - protocols involving biological toxins, human or non-human primate blood, tissues or cell lines derived thereof.
- (RG-4 or BSL-4 agents (Appendix 1) are excluded from use at the University of Arkansas. For a discussion of the Risk Groups and their corresponding Biosafety Levels, see the section on **Classification of Hazards and Levels of Containment.**)
3. Determine the necessity for health surveillance and prophylaxis for personnel conducting biological research projects.
 4. Review any proposed additions or changes to the University of Arkansas Biological Safety Manual.
 5. Periodically review departmental inventories of rDNA, cell culture lines, and biological agents and toxins.
 6. Respond to reports of significant violations or accidents and report any such occurrence involving rDNA to the NIH Office of Recombinant DNA Activities.
 7. Review and approve any protocol changes that are required for IBC approval of the protocol.

Responsibilities of the Institutional Biological Safety Officer

The Biological Safety Officer (BSO) shall:

1. Provide consultation and technical guidance for the safe handling of biological agents and toxins, assisting in the development of safety and exposure control plans and training programs.
2. Provide advice regarding the disinfection of facilities and equipment, and assist in the disposal of infectious waste.
3. Periodically review and recommend updates of the University of Arkansas Biological Safety Manual to the IBC.
4. Review needs and make recommendations regarding selection, purchase, and certification of biological safety cabinets (BSCs) and other related safety equipment.
5. Maintain a record of agents used, their classification, location and the names of the principal investigators.
6. Audit laboratories for compliance with the approved standards and policies of the University.
7. Enforce the policies of the University to the extent necessary to ensure the safety of the University community and area citizens.
8. Review and forward with recommendation all protocols to the IBC for final consideration.

Responsibilities of the Principal Investigators and Laboratory Supervisors

Principal Investigators and/or Laboratory Supervisors shall:

1. Submit applications (including all required forms and complete protocols) and solicit and receive approval from the IBC prior to initiating any project or curriculum involving the use of agents or materials covered in the scope of this document. Each protocol shall include a safety/exposure control plan and procedures for containment and decontamination of spills.
2. Register all potentially infectious agents, recombinants, and toxic materials with Environmental Health & Safety (EH&S). Use of recombinants and human pathogens requires approval of the IBC.
3. Advise the IBC, in writing, of any significant changes in approved protocol involving use of biological agents and/or toxins. Changes must be approved by the IBC.
4. Maintain and annually review laboratory-specific standard operating procedures.
5. Ensure that laboratory staff and students are trained in these procedures and comply with their requirements.
6. Encourage employees and students to report any changes in health status.
7. Survey laboratories for compliance with standards and policies regarding safe handling and use of biological agents and toxins.
8. Enforce compliance with the approved standards and policies of the University.
9. Comply with the US Department of Transportation (DOT) shipping requirements for biohazardous substances and toxins.
10. Comply with the Centers for Disease Control and Prevention (CDC) Laboratory Registration and Select Agent Transfer and Tracking System (LSAT/TS).
11. Post all signs and procedures, both outside and inside laboratories, as required by the BSO and IBC.
12. Inform the University Health Center, in writing, of
 - A. BSL-2 and above agents or their toxins being used;
 - B. a list of personnel who may be exposed to those agents;
 - C. any available requested information regarding agents or other relevant hazardous materials.
13. Post a succinct, written spill procedure on the laboratory bulletin board.

Responsibilities of all Project Participants

Researchers, technical staff, and students shall:

1. Comply with the established policies, procedures, and guidelines for biological safety as trained.
2. Promptly inform immediate supervisor of any unsafe practice or conditions in the work area.
3. Report any change in health status to the supervisor if there is a possibility it may be work related.
4. Immediately report all biological spills and incidents to the supervisor.
5. Become familiar with written emergency procedures for handling exposure to infectious or potentially infectious biological agents and other hazardous materials.
6. Become trained in the procedures, both archived and wall posted, to safely handle appropriate biological agents.

General Policies and Procedures for Biological Laboratories

Compliance

Good laboratory procedure will be rigorously enforced in both research and teaching laboratories. Eating, drinking, smoking, application of cosmetics, or storage of food are not permitted in any University biological laboratory.

Personnel must wash hands after handling infectious material, after removal of gloves, and before leaving the laboratory.

Work with biological agents and materials will be conducted at the appropriate biological containment level.

Appropriate disinfection and waste disposal procedures will be stringently observed.

Control

It is important to keep the laboratory doors and windows closed at all times.

Ventilation systems in laboratories are very carefully balanced for directional airflow and to ensure that fume hoods and biological safety cabinets function as they were designed. Open doors and/or windows may disrupt airflow and interfere with the function of containment equipment.

Biohazard areas must be posted (by initiative of the PI) with a warning sign with the universal biohazard symbol, identifying the infectious agent present and indicating requirements for entry.

Access to the laboratory or classroom is limited at the discretion of the PI when experiments are in progress. All laboratories are locked after normal University working hours.

The PI's name and telephone number shall be posted along with the telephone number of EH&S (575-5448) and the BSO (5-3597) on the door of biological research laboratories.

Containment

Containment is achieved by a combination of practices, equipment, and facilities. A BSC serves as an effective primary barrier.

The function of the BSC is to complement careful and appropriate work practices, not to replace them. The cabinets are equipped with High Efficiency Particulate Air (HEPA) filters that have 99.97% efficiency against 0.3 micron particles. HEPA filters offer no protection against volatile chemicals.

There are several classes/types of BSCs. Selection of the correct BSC is based on the classification of the agent, the associated biosafety level for the particular agent, and chemicals that will be used in the research. For assistance with choosing the appropriate class/type of BSC, call EH&S at 5-5448.

If possible, leave the BSC on at all times. Otherwise, turn the blower on and purge air for at least five minutes prior to use.

Never work with the UV light illuminated. Skin and eye damage can occur from the direct and reflected light.

Wipe down the work surface with an appropriate disinfectant. Do not depend on the UV germicidal lamp to provide a sterile surface.

Needed items should be placed inside the BSC prior to beginning work, arranged in a manner to segregate clean and contaminated materials.

Keep the glass sash lowered and conduct work at least four inches inside the sash. To minimize the escape of aerosols, keep necessary arm movements slow and smooth and avoid moving arms in and out of cabinet.

Avoid using an open flame inside the cabinet.

An open flame in a BSC creates turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface. Excessive heat that is generated may damage and compromise the HEPA filters. In addition, combustion gases accumulate and constitute an explosion hazard. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used

Upon completion of work in the BSC, disinfect all surfaces and leave the blower on for five minutes to purge the air from the cabinet.

A BSC is not to be used with infectious materials until it has been certified as meeting minimal safety specifications (e.g., NIH-03- 112 or National Sanitation Foundation Standard 49) on site. Cabinets are to be certified in situ by a trained technician when installed and annually thereafter, and, also, whenever moved.

For additional information regarding the use of the BSC, the reader is referred to the CDC/NIH publication on the use of the BSC: *Primacy Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets. 2nd Edition*
(<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>)

General Guidelines Concerning Aerosols

The incidental production of aerosols while handling infectious agents is thought to account for the vast majority of all laboratory-acquired infections. Aerosolized particles, too small to be seen, remain suspended for long periods of time, and may become entrained in the air stream and spread by ventilation to areas outside the laboratory.

Aerosols may be generated by the use of centrifuges, blenders, shakers, magnetic stirrers, sonicators, pipettes, vortex mixers, syringes and needles, separatory funnels, grinders, inoculating loops, by pouring of a liquid onto the free surface of another liquid, by sparging a gas through a liquid, by boiling a liquid or by the opening of pressure or vacuum containers. They may be released from lyophilized samples or from vacuum-sealed samples.

Clearly, if generation of aerosols is anticipated, then those activities manipulating biological agents should be performed in a BSC and *additional* precautions or containment may be necessary. (See discussion of BSL requirements.) If working with a toxin, a chemical fume hood may be required. However, NEVER use a chemical fume hood in place of a BSC. The discretion of the IBC should be used in certain circumstances where the use of a BSC is not possible.

The following general guidelines are offered for helping to control the production of infectious or toxic aerosols in routine laboratory' activities whether such agents are being handled in the hood or at the bench.

1. Keep tubes stoppered when vortex mixing or centrifuging and allow aerosolized material to settle in the container before opening the centrifuge, blender, or mixed tubes.
2. Place towels soaked with disinfectant over work surfaces to help contain and disinfect possible spills or droplets of biohazardous agents. Soaked gauze can be wrapped around ampules when breaking, needles being removed from a vial, or stoppers being removed from tubes.
3. Slowly reconstitute or dilute contents of an ampule. Mix solutions by discharging the secondary fluid down the side of the container or as close as possible to the surface of the primary solution.
4. Allow inoculating needle to cool before touching biological specimens.

Pipetting

Mouth pipetting is strictly forbidden. No infectious mixture is to be prepared by bubbling air through the liquid with the pipette. No infectious materials are to be forcibly discharged from pipettes.

Syringes and needles

Avoid the use of syringes and needles wherever possible.

Use the needle-locking type or a disposable syringe needle unit.

Needles should not be re-sheathed, bent, broken, or removed from disposable syringes.

Needles and syringes should be discarded into biohazard-labeled, approved sharps containers for later disposal.

Do not discard needles into disinfectant pans containing disposable pipettes or other glassware. These items must be disposed of separately.

Disinfection and Sterilization

Frequently disinfect floors, cabinet tops, and equipment where biohazard material is stored.

Sterilize all infectious materials and contaminated equipment prior to being washed, stored, or discarded.

All infectious waste is to be autoclaved.

Use disposable materials wherever possible, keeping reusable items and disposable materials separated.

Clearly mark all holding containers as "NON-INFECTIOUS - TO BE CLEANED" or "BIOHAZARDOUS - TO BE AUTOCLAVED".

A list of disinfectants and sterilization procedures and their appropriate uses can be found in Appendix III.

Waste disposal

Infectious waste must be decontaminated on site, preferably by autoclaving. Transport of waste for off-site decontamination and disposal must have the approval of the BSO (5-3597).

After autoclaving, disposable, non-glass materials may be placed in ordinary trash bags for disposal. **Do not** dispose of red biohazard bags without first placing them in an unmarked bag.

Disposable glass should be autoclaved or otherwise disinfected and placed in an approved glass disposal container.

Sharps should be placed in an approved container and disposed of by EH&S as medical waste.

Do not put disposable glass in sharps containers.

Liquid waste can be disposed of down the sink, provided it contains no infectious agents, hazardous chemicals, or radioactive materials.

Do not pour melted agar down the drain. It will solidify and clog the pipe.

Animal carcasses must be incinerated. Call EH&S for assistance.

Personnel Exposure Control Plans/Procedures

Each area with potentially exposed employees or students must have a written Exposure Control Plan. The plan describes specific practices and procedures designed to minimize or eliminate exposure to these hazards. It must be reviewed annually by the PI and updated as necessary with written records kept.

Appropriate immunization may be required for some personnel. Hepatitis B vaccine must be provided for all employees who have the potential for an occupational exposure to human or non-human primate blood or other potentially infectious human or non-human primate materials within 10 days of assignment.

Other prophylaxis or surveillance may be necessary for personnel working with feral or non-domesticated animals. Consult the University Health Center for assistance.

Accidents

Accidents that result in injury or overt exposure to infectious materials are immediately reported to the PI. Medical evaluation, surveillance and treatment are provided as appropriate and written records are maintained.

Spills

Laboratories are required to develop procedures for dealing with spills and should have available appropriate equipment and materials. A basic spill kit should include a concentrated disinfectant (chlorine bleach or Wescodyne) a package of paper towels, sponges, household "rubber" gloves, forceps for broken glass, and an autoclavable container.

Procedures for handling spills must be posted on the laboratory bulletin board.

A site-specific spill plan must be developed by the PI that is appropriate to the biosafety level of the project. Consult EH&S for assistance.

Training

Training of technical personnel, teaching assistants and students must be accomplished prior to beginning the project and repeated at least annually. At a minimum it will consist of methods to minimize exposure, proper shipping procedures, and if working with human or non-human primate blood or blood containing products, access to a copy of the OSHA Blood borne Pathogen Standard, explanation of its contents, and a general explanation of the Exposure Control Plan.

Training records must be kept on file by the PI. Training assistance is available from EH&S.

Personal Protective Equipment (PPE)

Protective clothing designed to keep street clothes and forearms free of contamination shall be worn when working with microorganisms in the laboratory. Long sleeve lab coats are recommended at minimum.

Protective clothing is never to be worn outside the laboratory.

Protective gloves must be worn when hands may contact infectious material. Gloves should be changed if damaged and removed before contact with clean surfaces such as the telephone or doorknob. Hands must be washed as soon as gloves are removed.

Face protection (goggles, mask, face shield or other spatter guard) must be used for anticipated splashes or sprays of infectious or hazardous materials when microorganisms are manipulated outside the BSC.

Where personnel cannot be adequately protected via procedural or ventilation controls, it is important that *appropriate* respiratory protection be used with respect to the hazards associated with the agent or procedure used. For example, surgical masks may be worn for product protection, but offer no personal protection against infectious materials. All questions concerning the selection or use of respiratory protection or other PPE should be referred to EH&S.

Use of disposable respirators for personnel protection must follow procedures outlined in the EH&S respiratory protection program. EH&S offers fit testing and training for respiratory protection. Call 5-5448 for assistance.

Classification of Hazards and Levels of Containment

Agents listed by the National Institutes of Health (NIH) are those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded. The NIH lists can be found as Appendix 1 of this document. These lists are of the more commonly encountered agents and are not meant to be all-inclusive. The agents are divided into risk groups, which correspond to the equivalent biosafety level. For a complete discussion of the levels of containment, the reader is referred to the CDC/NIH manual *Biosafety in Microbiological and Biomedical Laboratories* available from the U.S. Government Printing Office. (HHS Publication No (CDC) 93-8395)

(Note: There are certain non-indigenous animal pathogens the importation, possession or use of which is restricted by law. Please see Appendix II.)

Biosafety Level I

RG1 agents are not associated with disease in healthy adult humans.

BSL-1 represents a basic level of containment, standard microbiological practices, and no special equipment or facilities, except a sink for hand washing.

BSL-1 is suitable for work involving agents of known or of minimal potential hazard to laboratory personnel and the environment.

The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container, which is closed before removed from the laboratory.

The following Standard Microbiological Practices apply to agents assigned to BSL-1:

- I. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator/Supervisor when experiments are in progress.
2. Work surfaces are decontaminated once a day and after any spill of viable material.
3. All contaminated liquid or solid wastes are decontaminated before disposal.
4. Technical pipetting devices are used; mouth pipetting is prohibited.
5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food stored in cabinets or refrigerators should be located outside of the work area.
6. Persons must wash their hands after handling viable materials and animals and before leaving the laboratory.
7. All procedures are performed carefully to minimize the creation of aerosols.

8. Laboratory coats or gowns are worn to prevent contamination or soiling of street clothes.

Biosafety Level 2

RG-2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

BSL-2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

BSL-2 agents can be safely used in activities conducted on the open bench, using good microbiological techniques, provided the potential for producing splashes and aerosols is low.

BSL-2 requires the same Standard Microbiological Practices as BSL-1. *In addition,*

1. Laboratory personnel must have specific training in handling pathogenic agents and must be directed by competent scientists.
2. Access to the laboratory is limited or restricted at the discretion of the PI/Supervisor when experiments are in progress.
3. Procedures that may create infectious aerosols must be conducted in a BSC or other suitable containment or with the use of personal protective equipment.

Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms.

The PI/Supervisor shall establish policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are to be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

Laboratory coats, gowns, or smocks are to be worn while in the laboratory. Before leaving the laboratory for non-laboratory area (e.g., library, administrative offices), this protective clothing is to be removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Special care is taken to avoid skin contamination with infectious materials; gloves are to be worn when handling infected animals and when the skin contact with infectious materials is unavoidable.

A BSC (Class 1 or 2) or other appropriate personal protective or physical containment devices is to be used whenever:

1. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, by sparging a gas through a liquid, by boiling a liquid or by the opening of pressure or vacuum containers, inoculating animals intra-nasally, and harvesting infected tissues from animals or eggs.
2. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Biosafety Level 3

RG-3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

The BSL-3 laboratory must have special engineering and design features to be applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by the inhalation route.

For a detailed description of BSL-3 requirements, please see the above referenced CDC/NIH manual. *Currently there are no facilities on the University of Arkansas campus that are approved for BSL-3 work involving human pathogens.* Any proposals involving RG-3 agents must come to the attention of the IBC.

Biosafety Level 4

RG-4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

The University of Arkansas does not have containment facilities that support BSL-4 research and RG-4 agents are not to be used.

Working with Environmental Isolates

It is important to remember that those agents not listed in RG-2, 3 or 4 are NOT automatically or implicitly classified in RG-1.

When dealing with newly isolated (clinical or environmental) or unknown agents, a hazard evaluation must be made based on the known and potential properties of the agents and their relationship to agents that are listed. The PI will perform the hazard evaluation in consultation with the BSO, if warranted, and may submit an amended protocol for approval of the IBC. If required or recommended by regulatory authority, a formal risk assessment may be performed in cooperation with and is subject to approval by the IBC.

Uncharacterized environmental isolates may be handled at BSL-2 as long as they are not aerosolized or grown in production quantities. Upon identification, isolates must be handled at the appropriate BSL. The agent will be classified as being BSL-2 or an appropriate other level and thereafter handled with the appropriate level of control specified in the BMBL 4th Edition. If the agent identified is a human pathogen, an amendment must be submitted to the IBC. Any isolate identified as a human pathogen must be registered as such using the Human Pathogen Registration form.

Working with Human Tissues or Cell Lines

BSL-2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues in a manner consistent with the concept known as "Universal Precautions".

The OSHA Bloodborne Pathogen Standard requires limiting exposure to blood and other potentially infectious materials. Any exposure could result in transmission of bloodborne pathogens and lead to disease or death.

A site specific (laboratory, departmental, etc.) Exposure Control Plan must be developed and made readily available to all at-risk employees, the primary goal of which is to prevent transmission of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), and other bloodborne pathogens.

HBV vaccination is available at the University Health Center. All occupationally at-risk employees and students shall be immunized.

Under no circumstances shall anyone work with cells derived from themselves or from first-degree relatives since the host immune systems may not provide adequate protection. Training in "Universal Precautions" is mandatory for all employees or students working with cells or products derived from human tissues.

Employees with no prior experience in handling human pathogens must be trained in the laboratory prior to handling infectious materials.

Participation in work involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the PI/Supervisor.

Cell Culture

The following must be handled at BSL-2 or higher containment level:

1. Cell lines of human/primate origin
2. Cell lines derived from human or non-human primate lymphoid or tumor tissue
3. Cell lines exposed to or transformed by any oncogenic virus and cell lines exposed to or transformed by amphotropic packaging systems
4. Any clinical material (e.g., human blood or other fluids; samples of human tissues obtained from surgical resection or autopsy)
5. All cell lines new to the laboratory (until proven to be free of all adventitious agents)
6. All mycoplasma-containing cell lines

A cell line must be classified at the same level as that recommended for the agent when cell cultures are known to contain an etiologic agent, an oncogenic virus or amphotropic packaging system.

Working with Recombinant DNA

Federal Guidelines and Registering Experimental Protocols

All research conducted at the University of Arkansas involving recombinant DNA molecules must meet current NIH guidelines.

Experimental protocols must be approved by the IBC and, in special instances, by a committee at the NIH or USDA as well.

The principal investigator is responsible for determining the status of his/her experiments and filing the proper documents if review is required.

The NIH Guidelines instruct the PI to prepare a set of emergency plans covering accidental spills and resulting personnel contamination for work involving rDNA.

Research that is carried out at physical containment level BSL-2 or higher requires the principal investigator prepare or adopt a biosafety manual. The University Biosafety Manual may serve as the basis for preparing a more specific document.

Investigators who create transgenic animals must complete a rDNA registration document and submit it to the IBC for approval. In addition, the protocol must receive approval from the IACUC.

Experiments to genetically engineer plants by rDNA methods require approval from the IBC.

The NIH guidelines provide specific plant biosafety containment recommendations to prevent release of transgenic plant materials to the environment. Protocols must be registered with the IBC.

Working with Toxins of Biological Origin

The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed.

If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written.

If animals are used, animal safety practices must also be considered.

When vacuum lines are used with systems containing toxins, they shall be protected with appropriate filters to prevent entry of toxins into the lines.

Sink drains shall be similarly protected when water aspirators are used. Do not dispose of toxic or hazardous materials down the sink.

Practices

Special practices listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

Additional requirements for working with biological toxins are as follows:

1. Each laboratory shall develop a chemical hygiene plan specific to the toxin(s) used in that laboratory. The chemical hygiene plan must: (a) identify the hazards that will be encountered in normal use of the toxin, and those that could be encountered in case of a spill or other accident, and (b) specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance. (See the University of Arkansas Chemical Hygiene Plan available from EH&S.)
2. Training specific to the toxin(s) used is required and shall be documented for all laboratory personnel working with toxins, before starting work with the toxin and at intervals thereafter.
3. An inventory control system shall be in place. Toxins shall be stored in locked storage rooms, cabinets, or freezers when not in use.
4. Access to areas containing toxins shall be restricted to those whose work assignments require access.
5. The user shall verify inward airflow of the hood or BSC before initiating work. All work should be done within the operationally effective zone of the hood or biological safety cabinet.
6. The laboratories shall be posted with signs indicating the toxin and listing the phone numbers of the PI and other emergency contacts.
7. Any special entry requirements shall be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.
8. All high-risk operations shall be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.

9. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container must be decontaminated and placed in a clean secondary container. Toxins shall be transported only in leak/spill-proof secondary containers.
10. Contaminated and potentially contaminated protective clothing and equipment shall be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning or repair.
11. If decontamination is not possible/practical materials (e.g., used gloves) shall be disposed of as toxic waste. (Call EH&S for assistance.)
12. Materials contaminated with infectious agents as well as toxins shall also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
13. The interior of the hood, glove box, or cabinet shall be decontaminated periodically (e.g., at the end of a series of related experiments).
14. Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.
15. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins shall be conducted in a chemical fume hood (**NOTE: A chemical fume hood is not acceptable for handling biological agents**), a glove box, or a BSC or equivalent containment system approved by EH&S. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.

Personal Protective Equipment

When using an open-fronted fume hood or BSC, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.

Eye protection shall be worn if an open-fronted containment system is used.

Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, it may be necessary to use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.

Gloves shall be of a type that does not generate static electricity. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin. For assistance in choosing a glove or for additional information, call EH&S at 55448.