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* (5^{th} edition) 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 I) 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 approval 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 U. S. 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 or above agents and 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.

Biological safety cabinets must be certified yearly.

Autoclaves must be tested once per month using the biological indicator *Bacillus stearothermophilus*.

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, disinfectant 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: *Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets.?*" (5th edition). (This document is currently printed as Appendix A of the *Biosafety in Microbiological and Medical Laboratories* (*BMBL).*)
(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 ampoules 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.

- As described below, to ensure biological materials are autoclaved appropriately, autoclaves must be tested once per month. Use the biological indicator *Bacillus stearothermophilus* for the monthly testing by placing it at the center of a load processed under standard operating conditions to confirm the adequate sterilization conditions were reached. Maintain records of *B. stearothermophilus* testing and maximum autoclave temperature recordings.

<u>Monthly Spore Testing Procedure</u>

1. Place ampoule of *B. stearothermophilus* spores and holding thermometer in the center of an autoclave load.
2. Process the load under normal operating procedures.
3. The highest temperature indicated on the holding thermometer is entered on the Autoclave QC Log. If this temperature is less than 121ºC, then autoclave is not to be used to treat infectious materials until it is checked by authorized personnel (someone from warranty granting company, if applicable or someone from a company certified to maintain autoclaves).

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 or disposed of by a contractor such as Stericycle.

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 autoclaveable 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 Bloodborne 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 list can be found as Appendix I of this document. These lists are 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 that the importation, possession or use of is restricted by law. Please see Appendix II.)

Biosafety Level 1

RGI 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 removal from the laboratory.

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

1. 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 or 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 5th 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 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 5-5448.

Appendix I

Select Agents and Toxins List

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. The list of excluded agents and toxins can be found at: http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html.

HHS SELECT AGENTS AND TOXINS

Abrin
Botulinum neurotoxins
Botulinum neurotoxin producing species of *Clostridium*
Cercopithecine herpesvirus 1 (Herpes B virus)
Clostridium perfringens epsilon toxin
Coccidioides posadasii/Coccidioides immitis
Conotoxins
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola virus
Francisella tularensis
Lassa fever virus
Marburg virus
Monkeypox virus
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
Shigatoxin
South American Haemorrhagic Fever viruses
Flexal
Guanarito
Junin
Machupo
Sabia
Staphylococcal enterotoxins
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
Central European Tick-borne encephalitis
Far Eastern Tick-borne encephalitis
Kyasanur Forest disease
Omsk Hemorrhagic Fever
Russian Spring and Summer encephalitis
Variola major virus (Smallpox virus)
Variola minor virus (Alastrim)
Yersinia pestis

OVERLAP SELECT AGENTS AND TOXINS
Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei (formerly Pseudomonas mallei)
Burkholderia pseudomallei (formerly Pseudomonas pseudomallei)
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

USDA VETERINARY SERVICES (VS) SELECT AGENTS
African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Bluetongue virus (exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Ehrlichia ruminantium (Heartwater)
Foot-and-mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
(Alcelaphine herpesvirus type 1)
Menangle virus
Mycoplasma capricolum subspecies *capripneumoniae*
(contagious caprine pleuropneumonia)
Mycoplasma mycoides subspecies *mycoides* small
colony (*Mmm* SC) (contagious bovine pleuropneumonia)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (exotic): Indiana subtypes
VSV-IN2, VSV-IN3
Virulent Newcastle disease virus [1]

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS
Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly *Pyrenochaeta glycines*)
Ralstonia solanacearum race 3, biovar 2
Rathayibacter toxicus
Sclerophthora rayssiae var zeae
Synchytrium endobioticum
Xanthomonas oryzae
Xylella fastidiosa (citrus variegated chlorosis strain)

9/19/11 (http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html)

Select Agents Exclusions

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) to establish regulations regarding the possession, use, and transfer of select biological agents and toxins. In accordance with the Act, HHS and USDA published new regulations in the Federal Register on December 13, 2002 (67 FR 76886-76905 and 67 FR 76908-76938, respectively). The HHS regulations are set out in 42 CFR Part 73 and the USDA regulations are set out in 7 CFR Part 331 and 9 CFR Part 121.

The regulations in 42 CFR Part 73 and 9 CFR Part 121 establish a procedure by which an attenuated strain of a select biological agent or toxin that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the list of select biological agents and toxins.

HHS has received requests for exclusions for *Yersinia pestis* strains, *Bacillus anthracis* strains, *Francisella tularensis* subspecies *novicida* and *Francisella tularensis* subspecies *holartica* LVS.

USDA has received requests for exclusions for *Bacillus anthracis* Sterne strain and *Francisella tularensis* subspecies *holartica* LVS.

Based upon consultations with subject matter experts and a review of relevant published studies and information provided by the entities requesting the exclusions, HHS and USDA have determined that the following attenuated strains are not subject to the requirements of 42 CFR Part 73 and 9 CFR Part 121 if used in basic or applied research, as positive controls, for diagnostic assay development, or for the development of vaccines and therapeutics.

However, an individual or entity that possesses, uses, or transfers an excluded attenuated strain will be subject to the regulations if there is any reintroduction of factor(s) associated with virulence or other manipulations that modify the attenuation such that virulence is restored or enhanced.

Attenuated strains of HHS Select Agents and toxins excluded:

- Coccidioides posadasii **Δchs5 strain**. (effective 10-14-2003)

  The data demonstrates that deletion of the single copy of the class V chitin synthase using a gene replacement strategy results in a stable avirulent phenotype that lacks the ability to form infectious arthroconidia. This mutant is unable to form spherules in vivo as exemplified by its inability to survive or kill mice following intraperitoneal inoculation. Based upon consultations with subject matter experts and information provided by the requestor, HHS has determined that *Coccidioides posadasii* Δchs5 mutant strain does not pose a severe threat to public health and safety.

- Coccidioides posadasii **Δcts2/Δard1/Δcts3 strain**. (effective 03-03-2006)

  A triple gene knock-out mutant of *Coccidioides posadasii* Δcts2/Δard1/Δcts3 is attenuated through the deletion of two coccidioidal chitinase genes and a D-arabinitol 2-dehydrogenase gene. Unpublished data demonstrate that this mutant strain, unlike the wild-type Coccidioides posadasii, is unable to produce endospores which give rise to the next generation of spherules. These data further demonstrate that the mutant strain is stable, and that there is no possibility of spontaneous reversion to the wild-type gene. Based upon consultations with subject matter experts and information provided by the requestor, HHS has determined that *Coccidioides posadasii* (Δcts2/ Δard1/ Δcts3) mutant strain does not pose a severe threat to public health and safety.

- **Conotoxins** specifically excluded are: the class of sodium channel antagonist μ-conotoxins, including GIIIA; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVII, MVIIA, MVIIC, and

their analogs or synthetic derivatives; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives. (effective 4-29-2003)

The term "conotoxin" is used broadly to comprise a very large number of polypeptides isolated from the venom of fish-hunting marine snails of the Conus genus of gastropod mollusks; many of these molecules are neurologically active in mammals [1-3]. Based upon available experimental evidence, however, the following conotoxins (i.e. conopeptides) do not possess sufficient acute toxicity to pose a significant public health threat, and many are employed as useful research tools or potential human therapeutics. Select Agents conotoxins excluded are: the class of sodium channel antagonist μ-conotoxins, including GIIIA [3]; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVII, MVIIA, MVIIC, and their analogs or synthetic derivatives [3,4]; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives [5]; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives [6].

References:

1. Olivera, B.M., Gray, W.R., Zeikus, R., McIntosh, J.M., Varga, J., Rivier, J., de Santos, V. and Cruz, L.J. (1985). Peptide neurotoxins from fish-hunting cone snails. Science 230:1338-1343.
2. Olivera, B.M., Rivier, J., Scott, J.K., Hillyard, D.R. and Cruz, L.J. (1991). Conotoxins. J. Biol. Chem. 266:22067-22070.
3. Olivera, B.M. and Cruz, L.J. (2001). Conotoxins, in retrospect. Toxicon 39:7-14.
4. Yoshikami, D., Bagabaldo, Z. and Olivera, B.M. (1989). The inhibitory effects of omega-conotoxins on Ca channels and synapses. Ann. N.Y. Acad. Sci. 560:230-248.
5. Prorok, M. and Castellino, F.J. (2001). Structure-function relationships of the NMDA receptor antagonist conantokin peptides. Curr. Drug Targets 2:313-322.
6. Craig, A.G., Norberg, T., Griffin, D., Hoeger, C., Akhtar, M., Schmidt, K., Low, W., Dykert, J., Richelson, E., Navarro, V., Mazella, J., Watkins, M., Hillyard, D., Imperial, J., Cruz, L.J., and Olivera, B.M. (1999). Contulakin-G, an O-glycosylated invertebrate neurotensin. J. Biol. Chem. 274:13752-13759.

- **Junin virus** vaccine strain Candid 1. (effective 2-7-2003)
- Yersinia pestis **strains** which are Pgm- due to a deletion of a 102-kb region of the chromosome termed the pgm locus (i.e., Δpgm). Examples are *Y. pestis* strain E.V. or various substrains such as EV 76. (effective 3-14-2003)

Pgm- mutants of *Yersinia pestis* occur at a high frequency (ca 10-5) (1) and result in avirulence and Pgm- strains such as the EV 76 strain have been used for years as live human vaccines with no significant plague-associated problems. The mutation in question is due to the excision of about 102-kb of chromosomal DNA via reciprocal recombination between adjacent IS 100 elements (2). The lost DNA sequence encodes the ability to synthesize and utilize the siderophore yersiniabactin, which is necessary for growth in mammalian peripheral tissue, as well as the Hms+ locus, which is necessary for biofilm production in the flea vector (3). However, PCR and/or Southern blot analysis will be required to ensure that "Pgm-" derivatives have undergone this deletion rather than a mutation in the hemin storage genes (hms), which also causes loss of Congo red (CR) binding, which is the most common characteristic used to evaluate the pigmentation phenotype (4).

References:

1. Brubaker, R. R. 1970. Mutation rate to nonpigmentation in Pasteurella pestis. J. Bacteriol. 98:1404-1406.
2. Fetherston, J.D., P. Scheutze, and R.D. Perry. 1992. Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element. Mol. Microbiol. 6:2693-2704.

3. Bearden, S.W., and R.D. Perry. 1999. The Yfe system of *Yersinia pestis* transports iron and manganese and is required for full virulence of plague. Mol. Microbiol. 32:403-414.
4. Une, T. and R.R. Brubaker. 1984. In vivo comparison of avirulent Vwa- and Pgm- or Pstr phenotypes of Yersiniae. Infect. Immun. 43:895-900.

- Yersinia pestis strains (e.g., Tjiwidej S and CDC A1122) devoid of the 75 kb low-calcium response (Lcr) virulence plasmid. (effective 2-27-2003)

  Strains of *Yersinia pestis* that lack the 75 kb low-calcium response (Lcr) virulence plasmid are excluded. Strains lacking the Lcr plasmid (Lcr-) are irreversibly attenuated due to the loss of a virulence plasmid. An Lcr- strain of *Yersinia pestis* (Tjiwidej S) has been extensively used as a live vaccine in humans in Java. Thus, these strains pose no significant threat to public health.

  References:

  1. Plague immunization. I. Past and present trends, K.F. Meyer et al., J. Infect. Dis. 1974; 129 (suppl.): S13-S18.

Attenuated strains of Overlap Select Agents and toxins excluded:

- Bacillus anthracis strains devoid of both plasmids pX01 and pX02. (effective 2-27-2003)
  1. *Bacillus anthracis* strains that are devoid of both virulence plasmids, pX01 and pX02 are excluded based on published studies evaluating the attenuation of strains containing different combinations of the two plasmids.
  2. *Bacillus anthracis* strains lacking the virulence plasmid pX02 (e.g., Sterne pX01+ and pX02) are excluded based on information indicating that these strains were 105- to 107-fold less virulent than isogenic strains with both plasmids. These strains have been used to vaccinate both humans and animals and do not pose a severe threat to the public health and safety.

  References:

  3. Human live anthrax vaccine in the former USSR, E. N. Shlyakhov and E. Rubenstein, Vaccine, Vol 12, No. 8, 1994, pages 727-730.
  4. Avirulent Anthrax Vaccine, Max Sterne, Onderstepoort Journal, Vol. 21, No. 1, 1946, pages 41-43.
  5. Anthrax: The Disease in Relation to Vaccines, P. Hambleton et al., Vaccine, Vol. 2, June 1984, pages 125-132.
- Bacillus anthracis strains devoid of the plasmid pX02 (e.g., *Bacillus anthracis* Sterne, pX01+pX02). (effective 2-27-2003)
  1. *Bacillus anthracis* strains that are devoid of both virulence plasmids, pX01 and pX02 are excluded based on published studies evaluating the attenuation of strains containing different combinations of the two plasmids.
  2. *Bacillus anthracis* strains lacking the virulence plasmid pX02 (e.g., Sterne pX01+ and pX02) are excluded based on information indicating that these strains were 105- to 107-fold less virulent than isogenic strains with both plasmids. These strains have been used to vaccinate both humans and animals and do not pose a severe threat to the public health and safety.

  References:

  3. Human live anthrax vaccine in the former USSR, E. N. Shlyakhov and E. Rubenstein, Vaccine, Vol 12, No. 8, 1994, pages 727-730.
  4. Avirulent Anthrax Vaccine, Max Sterne, Onderstepoort Journal, Vol. 21, No. 1, 1946, pages 41-43.

5. Anthrax: The Disease in Relation to Vaccines, P. Hambleton et al., Vaccine, Vol. 2, June 1984, pages 125-132.

- Brucella abortus Strain 19. (effective 6-12-2003)

  The *Brucella abortus* Strain 19 live vaccine, used in the U.S. Department of Agriculture Brucellosis Eradication Program from 1941 to 1996, is effective in the control of clinical brucellosis in cattle.1 For over a decade, *B. abortus* Strain 19 was also used to immunize more than 8 million people in the USSR.2 While there have been occasional reports of human brucellosis caused by B. abortus Strain 19 as a result of accidental aerosolization or needle sticks, 3, 4 this strain does not pose a severe threat to human or animal health.

  References:

  1. Proceedings of the United States Animal Health Association 93:640-655.
  2. Joint FAO/WHO Expert Committee on Brucellosis, 1986. No. 740, p. 34-40.
  3. Young, E., 1983. Human Brucellosis in Reviews of Infectious Diseases, Vol. 5, No 5.
  4. Pivnick, H, et al. 1966. Infection of Veterinarians in Ontario by *Brucella abortus* St 19.

- Brucella abortus strain RB51 (vaccine strain). (effective 5-7-2003)

  Brucella abortus strain RB51 was conditionally licensed as a vaccine by USDA in 1996 and granted a full license in March 2003. It is used as part of the cooperative State-Federal Brucellosis Eradication Program.1 *Brucella abortus* strain RB51 is a genetically stable, rough morphology mutant of field strain Brucella. It lacks the polysaccharide O-side chains on the surface of the bacteria. Strain RB51 is less virulent than the *Brucella abortus* Strain 19 vaccine and field strain2 *Brucella abortus*. The RB51 strain does not pose a significant threat to human or animal health.

  References:

  1. Brucellosis http://www.aphis.usda.gov/vs/nahps/brucellosis/.
  2. Schurig GG, Roop RM II, Bagchi T, Boyle S, Buhrman D, Sriranganathan N. Biological properties of RB51: a stable rough strain of *Brucella abortus*. Vet Microbiol 1991, 28:171-88.
  3. Stauffer B, Reppert J, Van Metre D, Fingland R, Kennedy G, Hansen G, Pezzino G, Olsen S, Ewalt D. Human Exposure to *Brucella abortus* Strain RB51 – Kansas, 1997. MMWR 1998 47(09):172-175.

- Coxiella burnetii Phase II, Nine Mile Strain, plaque purified clone 4 (effective 10-15-2003)

  LPS is the only confirmed virulence factor of *C. burnetii*. Organisms isolated from natural infections or laboratory are in phase I and have a smooth-type LPS. Repeated passage of phase I organisms through embryonated eggs or cultured cells resulted in the conversion to phase II and a change in the LPS to a rough-type. Injection of such laboratory-derived phase II variants into guinea pigs resulted in infection and reversion to phase I. However, plaque-purified (cloned) isolates of the Nine Mile Strain phase II organisms do not undergo phase reversion and are avirulent since inoculation of susceptible animals with phase II cells does not result in infection nor can viable phase II or phase I organisms be recovered from the spleens of these animals. The Nine Mile Strain plaque purified phase II is stable and does not revert to phase I; restriction fragment-length polymorphisms detected after HaeIII digestion of chromosomal DNA and DNA-DNA hybridization, suggests that the Nine Mile Strain plaque purified phase II variant has undergone a deletion. Based upon consultations with subject matter experts and a review of relevant published studies, HHS and USDA have determined that *Coxiella burnetii*, Phase II, Nine Mile Strain, plaque purified clone 4, does not pose a significant threat to human or animal health.

References:

1. O'Rourke, A.T., M.. Peacock, J.E. Samuel, M.E. Frazier, D.O. Natvig, L.P. Mallavia, and O. Baca. 1985. Genomic analysis of phase I and II *Coxiella burnetii* with restriction endonucleases. J. Gen. Microbiol. 131:1543-1546.
2. Vodkin, M.H., J.C. Williams, and E.H. Stephenson. 1986. Genetic heterogeneity among isolates of *Coxiella burnetii*. J. Gen. Microbiol. 132:455-463.
3. Moos, A. and T. Hackstadt. 1987. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. Infect. Immun. 55:1144-1150.

- Francisella tularensis subspecies *novicida* (also referred to as Francisella *novicida*) strain, Utah 112 (ATCC 15482). (effective 2-27-2003)
    1. The type strain Utah 112 of *Francisella tularensis* subspecies *novicida* (also referred to as Francisella *novicida*) is excluded. The exclusion is only for the type strain, Utah 112. This strain was originally isolated from a water sample taken from Ogden Bay, Utah in 1951. It is experimentally pathogenic for mice, guinea pigs and hamsters, producing lesions similar to those of tularemia; rabbits, white rats and pigeons are resistant. The Utah 112 strain is not known to infect man and thus, is not of public health concern.
    2. *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain) is excluded. This and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

        References:

        1. Tularemia, by J. Ellis et al, Clinical Microbiological Reviews, Vol 15, No. 4, Oct 2002, p 631-646.

    3. *Francisella tularensis* biovar tularensis strain ATCC 6223. This strain has fastidious growth requirements and grows poorly in the laboratory. Mice are used as a model to study the pathogenesis of tularemia (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). However, mice infected intraperitoneally with 105 CFU or intradermally with 107 CFU of strain ATCC 6223 were not killed. Thus, strain ATCC 6223 does not pose a threat to human or animal health.

    Reference:

    4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.

- Francisella tularensis subspecies *holartica* LVS (live vaccine strain; includes NDBR 101 lots, TSI-GSD lots, and ATCC 29684). (effective 2-27-2003)
    1. The type strain Utah 112 of *Francisella tularensis* subspecies novicida (also referred to as *Francisella novicida*) is excluded. The exclusion is only for the type strain, Utah 112. This strain was originally isolated from a water sample taken from Ogden Bay, Utah in 1951. It is experimentally pathogenic for mice, guinea pigs and hamsters, producing lesions similar to those of tularemia; rabbits, white rats and pigeons are resistant. The Utah 112 strain is not known to infect man and thus, is not of public health concern.
    2. *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain) is excluded. This and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

        References:

        1. Tularemia, by J. Ellis et al, Clinical Microbiological Reviews, Vol 15, No. 4, Oct 2002, p 631-646.

3. *Francisella tularensis* biovar tularensis strain ATCC 6223. This strain has fastidious growth requirements and grows poorly in the laboratory. Mice are used as a model to study the pathogenesis of tularemia (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). However, mice infected intraperitoneally with 105 CFU or intradermally with 107 CFU of strain ATCC 6223 were not killed. Thus, strain ATCC 6223 does not pose a threat to human or animal health.

References:

4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.

- Francisella tularensis ATCC 6223 (also known as strain B38). (effective 4-14-2003)
  1. The type strain Utah 112 of *Francisella tularensis* subspecies novicida (also referred to as *Francisella novicida*) is excluded. The exclusion is only for the type strain, Utah 112. This strain was originally isolated from a water sample taken from Ogden Bay, Utah in 1951. It is experimentally pathogenic for mice, guinea pigs and hamsters, producing lesions similar to those of tularemia; rabbits, white rats and pigeons are resistant. The Utah 112 strain is not known to infect man and thus, is not of public health concern.
  2. *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain) is excluded. This and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

     References:

     1. Tularemia, by J. Ellis et al, Clinical Microbiological Reviews, Vol 15, No. 4, Oct 2002, p 631-646.
  3. *Francisella tularensis* biovar tularensis strain ATCC 6223. This strain has fastidious growth requirements and grows poorly in the laboratory. Mice are used as a model to study the pathogenesis of tularemia (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). However, mice infected intraperitoneally with 105 CFU or intradermally with 107 CFU of strain ATCC 6223 were not killed. Thus, strain ATCC 6223 does not pose a threat to human or animal health.

  References:

  4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.
- **Rift Valley Fever (RVF) virus** vaccine strain MP-12. (effective 2-7-2003)
- **Venezuelan Equine Encephalitis (VEE) virus** vaccine candidate strain V3526. (effective 5-5-2003)

Venezuelan Equine Encephalitis (VEE) strain V3526 is an attenuated strain of VEE, which was constructed by site-directed mutagenesis. V3526 contains two mutations relative to the virulent parental clone (1). One of these mutations is a deletion, which renders the virus non-viable; the other mutation restores viability without restoring the pathogenic properties of the parental virus. The stability of the deletion mutation in V3526 fundamentally and significantly decreases the hazard associated with this strain, and makes it unlikely that it can revert to wild type. This strain is considerably less virulent than the excluded vaccine strain TC83. This strain does not pose a significant threat to human or animal health.

References:

1. Davis, N.L., et al. 1995. Attenuated mutants of Venezuelan equine encephalitis virus containing lethal mutations in the PE2 cleavage signal combined with a second site suppressor mutation in E1. Virology 212:102-110.

- **Venezuelan Equine Encephalitis (VEE) virus** vaccine strain TC-83. (effective 2-7-2003)

Attenuated strains of USDA-only select biological agents and toxins excluded:

- **Highly pathogenic avian influenza (HPAI) virus**, recombinant vaccine reference strains of the H5N1 and H5N3 subtypes (effective 5-7-2004)

  Several recombinant reference vaccine strains of highly pathogenic subtypes have been excluded based on results from in-vitro and in-vivo studies indicating that these strains were not pathogenic in avian species. The data requirements necessary for exclusion consideration under 9 CFR 121.3 (g) can be downloaded in pdf format (83KB PDF). Specific reference vaccine strains have not been listed here for proprietary reasons.

- **Japanese encephalitis virus**, SA14-14-2 strain. (effective 3-12-2003)

  (1) Japanese encephalitis virus, SA14-14-2 strain is excluded. This strain is the vaccine strain of choice in the People's Republic of China to protect against Japanese encephalitis. It is non-pathogenic in weanling mice and rhesus monkeys.

  References:

  1. Japanese encephalitis: a Chinese solution?, The Lancet, Vol. 347, June 1996, p. 1570.
  2. Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA14-14-2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use, Vaccine, Vol. 6, Dec. 1988, pp. 513-518.

Information taken from: http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html

Permissible Toxin Amounts

HHS Toxins [§73.3(d)(3)]	Amount
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Clostridium perfringens epsilon toxin	100 mg
Conotoxin	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Shigatoxin	100 mg
Staphylococcal enterotoxins	5 mg
T-2 toxin	1000 mg
Tetrodotoxin	100 mg

Information taken from: http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html

Appendix II

Pathogens of Veterinary Significance (Appendix D: Agriculture Pathogen Biosafety, BMBL, 5th Edition)

Some pathogens of livestock, poultry and fish may require special laboratory design, operation, and containment features. This may be BSL-3, BSL-3 plus enhancements or BSL-4 and for animals ABSL-2, ABSL-3 or BSL-3-Ag. The importation, possession, or use of the following agents is prohibited or restricted by law or by USDA regulations or administrative policies.

This Appendix does not cover manipulation of diagnostic samples; however, if a foreign animal disease agent is suspected, samples should be immediately forwarded to a USDA diagnostic laboratory (The National Veterinary Services Laboratories, Ames, IA or the Foreign Animal Disease Diagnostic Laboratory, Plum Island, NY). A list of agents and their requirements follows.

African horse sickness virus [a, b]

African swine fever virus [a, b, c]

Akabane virus [b]

Avian influenza virus (highly pathogenic) [a, b, c]

Bacillus anthracis [a, b]

Besnoitia besnoiti

Bluetongue virus (exotic) [a, b]

Borna disease virus

Bovine infectious petechial fever agent

Bovine spongiform encephalopathy prion [b]

Brucella abortus [a, b]

Brucella melitensis [a, b]

Brucella suis [a, b]

Burkholderia mallei/Pseudomonas mallei (Glanders) [a, b]

Burkholderia pseudomallei [a, b]

Camelpox virus [b]

Classical swine fever virus [a, b, c]

Coccidioides immitis [b]

Cochliomyia hominivorax (Screwworm)

Coxiella burnetti (Q fever) [b]

Ephemeral fever virus

Ehrlichia (Cowdria) ruminantium (heartwater) [b]

Eastern equine encephalitis virus [a, b]

Louping ill virus [a]

Lumpy skin disease virus [a, b, c]

Malignant catarrhal fever virus (exotic strains or alcelaphine herpesvirus type 1) [b]

Menangle virus [b]

Mycobacterium bovis

Mycoplasma agalactiae

Mycoplasma mycoides subsp. mycoides (small colony type) [a, b, c]

Mycoplasma capricolum [a, b, c]

Nairobi sheep disease virus (Ganjam virus)

Newcastle disease virus (velogenic strains) [a, b, c]

Nipah virus [a, b, d]

Peste des petits ruminants virus (plague of small ruminants) [a, b, c]

Rift Valley fever virus [a, b, c]

Rinderpest virus [a, b, c]

Sheep pox virus [a, b]

Spring Viremia of Carp virus

Swine vesicular disease virus [b]

Teschen disease virus [a]

Theileria annulata

Theileria lawrencei

Theileria bovis

Theileria hirci

Trypanosoma brucei

Foot and mouth disease virus [a, b, c]	*Trypanosoma congolense*
Francisella tularensis [b]	*Trypanosoma equiperdum (dourine)*
Goat pox [a, b]	*Trypanosoma evansi*
Hemorrhagic disease of rabbits virus	*Trypanosoma vivax*
Hendra virus [a, b, d]	*Venezuelan equine encephalomyelitis virus* [a, b]
Histoplasma (Zymonema) farciminosum	*Vesicular exanthema virus*
Infectious salmon anemia virus	*Vesicular stomatitis virus (exotic)* [a, b]
Japanese encephalitis virus [a, b]	*Wesselsbron disease virus*

Notes:

[a] Export license required by Department of Commerce (See: *http://www.bis.doc.gov/index.htm*).

[b] Agents regulated as Select Agents under the Bioterrorism Act of 2002. Possession of these agents requires registration with either the CDC or APHIS and a permit issued for interstate movement or importation by APHIS-VS. Most require BSL-3/ABSL-3 or higher containment (enhancements as described in this Appendix or on a case-by-case basis as determined by APHIS-VS).

[c] Requires BSL-3-Ag containment for all work with the agent in loose-housed animals.

[d] Requires BSL-4 containment for all work with the agent.

A USDA/APHIS import or interstate movement permit is required to obtain any infectious agent of animals or plants that is regulated by USDA/APHIS. An import permit is also required to import any livestock or poultry product such as blood, serum, or other tissues.

For Summaries of Selected Agriculture Agents, see Appendix D: Agriculture Pathogen Biosafety, BMBL, 5th Edition

Appendix III

BIOLOGICAL WASTE MANAGEMENT AND DISPOSAL AT UNIVERSITY OF ARKANSAS

A. INTRODUCTION

The purpose of this document is to provide information, requirements, guidelines and procedures for the handling and disposal of hazardous and non-hazardous biological waste for all departments and units on the University of Arkansas campus. The conclusion of this section will briefly focus on the term biomedical waste and its definition.

"BIOLOGICAL WASTE" means discarded biological material from teaching, clinical, and research laboratories and operations; examples include cell culturing media, culture flasks, Petri dishes, gloves, and absorbent pads. This does not include household or office trash, waste from Food Services, Facilities Management, bedding and manure from normal agricultural operations or bedding and litter from noninfectious animals. **"BIOHAZARDOUS WASTE"** means any solid or liquid biological laboratory waste that may be infectious due to its physical and/or biological nature. All waste that contains infectious material or which, because of its biological nature, may be harmful to humans, animals, plants or the environment is biohazardous waste. This includes: waste from infectious animals; bulk human blood or blood products; microbiological waste; pathological waste; viruses; sharps; and hazardous products of recombinant DNA biotechnology and genetic manipulation.

Treatment of all laboratory biological waste prior to disposal is good laboratory practice, and is highly recommended, but biohazardous waste must be treated prior to disposal. Acceptable treatment methods include thermal or chemical disinfection, encapsulation (solidification), or incineration. However, if those treatment methods are not available, then the Office of Environmental Health and Safety must be contacted for biohazardous waste disposal.

The key requirements for disposal of biohazardous waste are that it must be (1) segregated from other waste; (2) securely packaged; (3) specifically labeled to indicate the method of treatment; (4) transported to the point of treatment or disposal by appropriately trained personnel; (5) treated to eliminate the biological hazard; and (6) documented by maintenance of appropriate records.

Biohazardous waste that is mixed with hazardous chemical waste, radioactive waste, or both must be treated to eliminate the biohazard prior to disposal. After treatment, the waste must be managed as hazardous chemical waste through the Office of Environmental Health and Safety (EH&S) or as radioactive waste through the Office of Environmental Health and Safety (EH&S).

"BIOMEDICAL WASTE" means biological waste that is contaminated by human blood or fluids. Such examples of this type of waste include sharps and gauze. Biomedical waste is always picked up by the Office of Environmental Health and Safety (EH&S).

B. RESPONSIBILITY

The Principal Investigator, faculty member or other person with operational responsibility shall assure compliance with these requirements within his/her laboratory or area of responsibility.

C. SEGREGATION

1. Any waste that could produce laceration or puncture injuries must be disposed of as "sharps". Sharps must be segregated from other waste. Metal sharps and broken glass may be commingled with each other, but not with non-sharp waste. Metal sharps such as carpet blades must be put into a metal sharps container and put in a dumpster. For information about an appropriate metal sharps container, contact EH&S at 575-5448. Broken glass must be put into a broken glass container or a cardboard box lined with at least one garbage bag. Once the box is filled, then tape it shut and label it broken glass. The box can then be put in a dumpster. For information about a broken glass container, contact EH&S at 575-5448.
2. Waste that is to be incinerated should not be commingled with glass or plastics.
3. Biological waste must not be commingled with chemical waste or other laboratory trash.
4. Biohazardous waste should be segregated from nonhazardous biological waste.

D. CONTAINERS

Containers must be appropriate for the contents; not leak; be properly labeled; and maintain their integrity if chemical or thermal treatment is used. Containers of biohazardous material should be kept closed.

1. LIQUIDS – Use leak-proof containers able to withstand thermal or chemical treatment.
2. METAL SHARPS – Use a rigid, puncture-resistant container (heavy-walled plastic is recommended) suitable for encapsulation and disposal. Container and encapsulated contents must withstand an applied pressure of 40 psi without rupture.
3. NON-HAZARDOUS SOLID BIOLOGICAL WASTE – Use heavy-duty plastic bags or other appropriate containers without a Biohazard Symbol. Red or orange biohazard bags or containers should not be used for non-hazardous material.
4. PASTEUR PIPETS and BROKEN GLASSWARE – Use the appropriate rigid, puncture-resistant container (e.g., plastic, heavy cardboard or metal) that can be sealed.
5. SOLID BIOHAZARDOUS WASTE – Use heavy-duty plastic "BIOHAZARD BAGS" (autoclave bags) or containers for solid biohazardous waste.

E. STORAGE

Biological waste may be held temporarily under refrigeration, prior to disposal, in a safe manner that does not create aesthetic (visual or odor) problems. Biohazardous waste should be treated and disposed of promptly and not allowed to accumulate. Containers holding biohazardous material must be clearly labeled, including the Biohazard Symbol. Temporary holding areas for biohazardous waste must be clean and orderly with no access to unauthorized persons (warning signs should be posted).

F. LABELING BIOHAZARDOUS WASTE CONTAINERS

1. Each container of untreated biohazardous waste must be clearly identified as such and must be labeled with the Biohazard Symbol.
2. Each container of treated biohazardous waste to be placed in a UA trash dumpster must be labeled to indicate the method of treatment and to cover biohazard markings. The contents must be placed inside a garbage bag before placing in a UA trash dumpster.
3. Label autoclave bags with commercially available autoclave tape that produces the word **"AUTOCLAVED"** upon adequate thermal treatment. Apply this tape across the Biohazard Symbol on the bag before autoclaving.
4. All containers of encapsulated sharps must be labeled as **"ENCAPSULATED SHARPS"**.

Note: It is not a requirement to label containers of non-hazardous biological waste, but it is recommended to label such containers as "NON-HAZARDOUS BIOLOGICAL WASTE".

G. HANDLING AND TRANSPORT

1. Only properly trained technical personnel can handle or transport untreated biohazardous waste.
2. Treated waste must also be transported by properly trained technical personnel (not custodial).
3. Avoid transporting untreated biohazardous materials or foul or visually offensive material through non-lab or populated areas.

H. TREATMENT AND DISPOSAL METHODS

NOTE: Waste should be treated as near the point of origination as possible.

1. ANIMAL CARCASSES AND BODY PARTS may be incinerated, biodigested, landfilled, or rendered. EH&S uses the services of Stericycle for this type of disposal.
2. ANIMAL WASTE, SOLID (bedding, manure, etc):
   BIOHAZARDOUS ANIMAL WASTE:
   1) Incinerate; or
   2) Disinfect by thermal or chemical treatment; place in a UA trash dumpster; or
   3) Alternative method, with approval of the Campus Biosafety Officer.
3. CHEMICAL WASTE: Biohazardous waste that also contains hazardous chemicals must be managed as hazardous chemical waste trough the Office of Environmental Health and Safety.
4. GENETIC MATERIAL: Disposal of materials containing recombinant DNA or genetically altered organisms must be consistent with applicable NIH Guidelines, in addition to complying with the requirements contained in this document.
5. HUMAN PATHOLOGICAL WASTE:
   a. Human cadavers, recognizable body parts: dispose by cremation or interment.
6. METAL SHARPS: Discarded metal sharps **MUST** be contained, encapsulated and disposed of in a manner that prevents injury to laboratory, custodial and landfill workers. Needles, blades, etc., are considered HAZARDOUS even if they are sterile, capped and in the original container.
   Never place sharps in a trash container or plastic bag that might be handled by custodial staff.
   a. Place sealed containers of encapsulated sharps and blades in a UA trash dumpster.
   b. Gas chromatography needles should be thoroughly rinsed to remove hazardous chemicals, then disposed with non-contaminated broken glassware.
   c. Do not attempt to recap, bend, break or cut discarded needles.
7. MICROBIOLOGICAL WASTE:
   a. Solid – Disinfect by thermal or chemical treatment; place in a UA trash dumpster.
   b. Liquid – Disinfect by thermal or chemical treatment; discharge into the sewer system.
8. NON-HAZARDOUS BIOLOGICAL WASTE:
   a. It is good laboratory practice to autoclave or chemically treat all microbial products prior to disposal, even if the material is not hazardous.
   b. Solid – Place in a UA trash dumpster.
   c. Liquid – Discharge into the sewer system.
      Note: Never pour hot/warm agar down the sink because it will solidify after cooling and clog the drain.

9. PASTEUR PIPETS and BROKEN GLASSWARE:
    a. CONTAMINATED WITH BIOHAZARDOUS MATERIAL:
        1) Disinfect by thermal or chemical treatment; place in a UA trash dumpster; or
        2) Encapsulate and place in a UA trash dumpster. **NOTE: Encapsulation is required if metal sharps are commingled with glass sharps.**
    b. NOT CONTAMINATED: Place in a UA trash dumpster.
    c. **DO NOT INCINERATE GLASSWARE.**
10. PLASTIC WASTE:
    a. CONTAMINATED WITH BIOHAZARDOUS MATERIAL: Disinfect by thermal or chemical treatment; place in a UA trash dumpster.
    b. NOT CONTAMINATED: Place in a UA trash dumpster.
    c. **DO NOT INCINERATE PLASTICS.**
11. RADIOACTIVE WASTE: Biological waste that contains radioactive material must be disposed according to the procedures of the Radiation Safety Officer in EH&S.

I. TRAINING AND HAZARD COMMUNICATION

The Principal Investigator or individual with primary supervisory responsibility must assure that all personnel who work with, or who may contact potentially biohazardous material are informed of the hazards and are trained in the proper procedures and equipment needed to avoid exposure, proper treatment and disposal of biohazardous wastes, and recognition of symptoms of infection or exposure.

J. REFERENCES:

1. *Management and Disposal of Biological Waste at Texas A&M University* http://ehsd.tamu.edu/documents/AgriculturalSafety/biowaste03.pdf
2. Centers for Disease Control/ National Institutes of Health, *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, 2009.
3. *University of Arkansas Biological Safety Manual*, 2011.
4. *Arkansas Department of Health, Rules and Regulations Pertaining to the Management of Medical Waste from Generators and Health Care Related Facilities*, 2000.

Note: Definitions of terms used in this document can be found in APPENDIX A.

APPENDIX A. DEFINITION OF TERMS

1. **ANIMAL WASTE** includes carcasses; body parts; bulk whole blood and blood products, serum, plasma and other blood components; and bedding of animals.
2. **BIODIGESTION** is a heated alkaline hydrolysis tissue digestion system
3. **BIOHAZARDOUS WASTE** is any kind of laboratory waste that may be infectious or, because of its physical and/or biological nature, may be harmful
    a. Animal waste known or suspected of being contaminated with a pathogen
    b. Bulk human blood or blood products
    c. Microbiological waste
    d. Pathological waste
    e. Infectious waste
    f. Waste products of recombinant DNA biotechnology and genetic manipulation
    g. Sharps
4. **BIOMEDICAL WASTE** is waste contaminated by human blood or fluids, such as sharps or gauze.
5. **BIOLOGICAL INDICATOR** – Commercially available microorganisms (e.g. spore strips or vials of *Bacillus* species) which can be used to verify the performance of waste treatment equipment and/or processes.
6. **BULK BLOOD AND BLOOD PRODUCTS** – Discarded bulk (>100 ml.) blood and blood products in a free draining, liquid state; body fluids contaminated with visible blood; and materials saturated or dripping with blood.
7. **CHEMICAL DISINFECTION** means the use of a chemical agent such as 10% bleach or EPA-approved chemical disinfectant/sterilant (used according to manufacturer's direction) to significantly reduce biological activity of biohazardous material.
8. **DISCHARGE INTO THE SEWER SYSTEM** means flushing treated liquid biological waste into the UA sanitary sewer system followed by copious quantities of water.
9. **ENCAPSULATION** is the treatment of sharps waste using a material such as Plaster of Paris (or a commercial product such as Isolyser) which when fully reacted, will encase the waste in a solid protective matrix. The encapsulating agent must completely fill the container. The container and solidified contents must withstand an applied pressure of 40 psi without disintegration.
10. **INCINERATION** means burning biological waste in an incinerator
11. **INFECTIOUS WASTE** is waste containing pathogens or biologically active material which because of its type, concentration, and quantity is capable of transmitting disease.
12. **MICROBIOLOGICAL WASTE:**
    a. Discarded cultures and stocks of infectious agents and associated biological material
    b. Discarded cultures of specimens from medical, pathological, pharmaceutical, research, and clinical laboratories
    c. Discarded live and attenuated vaccines
    d. Discarded used disposable culture dishes
    e. Discarded used disposable devices used to transfer, inoculate, and mix cultures

    NOTE: in vitro tissue cultures that have not been intentionally exposed to pathogens are exempt from the definition of microbiological waste.
13. **PATHOGENS** include any diseases that are transmissible to humans.
14. **PATHOLOGICAL WASTE** pertains to human materials and includes, but is not limited to:
    a. Human materials removed during surgery, labor, delivery, spontaneous abortion, autopsy or biopsy including: body parts; tissues and fetuses; organs; bulk blood and body fluids
    b. Laboratory specimens of blood, tissue or body fluids after completion of laboratory examination
    c. Anatomical remains.
15. **SHARPS** – Any device having acute rigid corners or edges, or projections capable of cutting or piercing, including:
    a. Hypodermic needles, syringes, and blades
    b. Glass pipets, microscope slides, and broken glass items.
16. **THERMAL TREATMENT** means:
    a. Autoclaving at a temperature of not less than 121°C and a minimum pressure of 15psi for at least 30 minutes (longer times may be required depending on the amount of waste, water content and the type of container used); or
    b. Subjecting biological material to dry heat of not less than 160°C under atmospheric pressure for at least two hours. (Exposure begins after the material reaches the specific temperature and does not include lag time).
17. **TREATMENT** refers to chemical, thermal or mechanical processes that significantly reduce or eliminate the hazardous characteristics, or that reduce the amount of a waste.