UAH Biological Safety Manual

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Preface

With the development and implementation of the Biohazard Safety Manual The University of Alabama in Huntsville has moved forward in promoting the health and safety of the University community and environment. It is imperative that each of our faculty, staff, and students involved in working with biologically hazardous materials be knowledgeable in the proper procedures associated with their handling and disposal.

The primary goal of the Office of Environmental Health and Safety is to assist the University by providing informational resources and consulting that will lead to the safest possible research, work, and learning environment. Input from the University Community is imperative for the

Definitions

Biohazard
Biohazards are biological agents and/or materials that are potentially hazardous to humans, animals and plants, either directly through infection or other deleterious effect or indirectly through damage to the environment. Infectious biohazardous agents have the ability to replicate and give rise to the potential of large populations in nature when small numbers are released from a controlled situation. Biohazardous agents include infectious or etiologic (disease causing) agents, potentially infectious materials, certain toxins and other hazardous biological materials are included in the definition of a biohazard. These agents include but are not limited to: *Certain bacteria, fungi, viruses, rickettsiae, chlamydiae, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents whose cells may contain, viroids, prions and other infectious agents as outlined in laws, regulation, or guidelines.*

Bloodborne Pathogens
Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, Hepatitis B virus (HBV) and Human Immunodeficiency virus (HIV).

Human Subject
An individu8 Tm [(H)20 0 L2(nr2(n,)]TJ -245c)4(H1.155.44 0 Td [(n i)-2(nve)4(s)-1(t)-2(i)-2(g)10(a)4(t)-2

Universa Tm [(H)2Precautions
A method of infection contro [(H)2that treats all human blood and other potentially infectious materials as capable of transmitting HIV, HBV, and other bloodborne pathogens.

Common Acronyms

BL - Biosafety Level
BMBL - Biosafety in Microbiological & Biomedical Laboratories
BSC - Biological Safety Cabinet
EPA - Environmenta Tm [(H)2Protection Agency
HEPA - High Efficiencyurified Air
IBC - Institutiona 6(l B)9(io)2(s)1(a 6(f)-5(e)6(t)-10(y)2H)2Committee
NIH -

TABLE 1
Summary of Biosafety Levels for Infectious Agents

	Biosafety Level 1 (BL-1)
Agents:	Not known to cause disease in healthy adults
Practices:	Standard microbiological practices
Safety Equipment: (Primary Barriers)	None required
Facilities: (Secondary Barriers)	Open bench top sink required
	Biosafety Level 2 (BL-2)
Agents:	Associated with human disease, which is rarely serious for which preventative or therapeutic intervention are often available
Practices:	BL-1 practice plans plus: limited access, biohazard warning signs; "Sharps" precautions; biosafety manual defining any needed waste decontamination or medical surveillance policies
Safety Equipment: (Primary Barriers)	Primary Barriers = Class I or II biosafety cabinets (BSCs) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; Personal protective equipment (PPE): lab coats, gloves, face and eye protection as needed
Facilities: (Secondary Barriers)	BL-1 plus: Autoclave available
	Biosafety Level 3 (BL-3)
Agents:	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences
Practices:	BL-2 practices plus: controlled access; decontamination of all waste; decontamination of lab clothing before laundering, baseline serum
Safety Equipment: (Primary Barriers)	

II. Classification of Infectious Agents (Risk Groups)

According to the particular hazard they may present to an individual and the community there are several systems in place worldwide for classifying human and animal pathogens. Although different, all systems of classification are based on the understanding that certain microorganisms are more hazardous than others. In general, when classifying infectious agents the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures, and/or effective treatment is taken into consideration. In the U.S., the most current classification is found in the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules. Human etiologic agents addressed in these guidelines are classified into four risk groups with Risk Group 1 (RG-1) representing low or no hazard and Risk Group 4 (RG-4) representing highly infectious agents. Table 2 describes the basis for the classification of biohazardous agents by risk group according to NIH guidelines.

Examples of RG-1 agents include microorganisms like Esherichia coli-K12 or Saccharomyces cerevisiae. A comprehensive list of Risk Groups 2, 3, and 4 agents as well as certain animal and plant pathogens can be found in Appendix D. It is important to realize, however, that none of the lists are all inclusive. In addition, those agents not included in Risk Groups 2, 3, and 4 are not automatically or implicitly classified in RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents and their relationship to agents that are listed.

Table 2
Classification of Biohazardous Agents by Risk Group

Risk Group	Risk to the Individual and the Community
Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG-2)	
Risk Group 3 (RG-3)	
Risk Group 4 (RG-4)	

III. Rules, Regulations, and Guidelines

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules.

1) National Institute of Health (NIH): *Guidelines for Research Involving Recombinant DNA Molecules.* These guidelines address the safe conduct of research involving the construction and handling of recombinant DNA (RDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment, as a result of the committee's activity, the initial version of the NIH Guidelines was published. It has been amended and revised numerous times. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or reject proposed RDNA research using NIH Guidelines as a minimum standard. For more information, please refer to the *Recombinant DNA Research* section in this manual and the *NIH Guidelines for Research Involving Recombinant DNA Molecules.*
2) Centers for Disease Control and Prevention (CDC) and the NIH Guidelines on: Biosafety in Microbiological and Biomedical Laboratories (BMBL). In 1984, the CDC/NIH published the first of the BMBL. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-

International Civil Aviation Organization (ICAO): *Technical Instructions for the Safe Transport of Dangerous Goods by Air*

As a minimum safety program all laboratories shall adhere to the recommended safety protocol as set forth in the UAH Laboratory Safety Manual and Biological Safety Manual.

Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or 3 agents are handled or stored or where BL-2 or BL-3 procedures are required. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, on equipment like refrigerators, incubators, transport containers, and/or lab benches. Labels can be obtained from the OEHS at 2352.

Training

Good microbiological and laboratory practices are essential for a safe work environment. Training and education on these practices and procedures must begin at the undergraduate level. In addition, all personnel working with RG-2 or 3 agent must receive adequate laboratory specific training from the Principal Investigator, Professor, or laboratory supervisor.

Training must include at a minimum:

- Good laboratory and animal practices as applicable
- Site specific information on risks, hazards and procedures
- Laboratory or environment specific BL-2 or 3 procedures as applicable

In addition, it is important that all personnel working at BL-2 or 3 or handling RG-2 or 3 agents take the biosafety training offered by the OEHS.

Bloodborne Pathogen Program

In accordance with OSHA requirements, UAH has established a *Bloodborne Pathogen Exposure Control Plan* covering potential exposure to bloodborne pathogens (e.g. HIV, Hepatitis B virus) found in human blood, serum and tissue as well as in other potentially infectious materials. Refer to Section I in this manual for more information.

Recombinant DNA Program

All research at UAH involving recombinant DNA, **independent of the funding source**, must be in compliance with the requirements of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and is subject to the Institutional Biosafety Committee (IBC) approval process. Please refer to Section H in this manual for more information on this subject.

Project Registration Form

For all research at UAH involving RG-2 and 3 or BL-2 and 3 procedures, or certain toxins, a registration form must be filled out with the OEHS prior to initiation of the project. The information provided in the registration document will be used for project review by the IBC as well as for emergency response. More information may be requested to support the Project Registration Form.

CDC Select Agent Requirements

The Centers for Disease Control and Prevention (CDC) mandates specific requirements for facilities possessing, transferring, or receiving certain infectious agents and toxins *(HHS-*

installation, and certification of the biological safety cabinet is critical to its performance in containing infectious aerosols. All BSCs used for RG-2 or 3 and RDNA research must be inspected annually and certified by trained and accredited service personnel according to the National Sanitation Foundation (NSF) Standard 49. Inspection and recertification is mandatory if a cabinet is relocated or after any major repairs, filter changes, etc. The service and repair records must be maintained for annual review by the OEHS. CDC and NIH have published a guide on BSCs: *Primary containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.*

Safe and Effective Use of Biosafety Cabinets

- Make sure that the certification (NSF sticker) is current. Check the magnehelic gauge or electronic controls regularly to be sure they are within the specified parameters.
- Understand how the cabinet works.
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
- Minimize the storage of materials in and around the BSC.
- Always leave the BSC running.
- Before using, wipe work surface with 70% alcohol or any other disinfectant suitable for the agent(s) in use. Wipe off each time you need for your procedures before placing it inside the cabinet.
- Do not place objects over the front air intake grille. Do not block the rear exhaust grille.
- Segregate contaminated and clean items.
- Place a pan with disinfectant and/or sharps container inside the BSC for pipette discard. Do not use vertical pipette discard canisters on the floor outside the cabinet.
- It is not necessary to flame items. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the HEPA filter and release of gas may result in explosion.
- Move arms slowly when removing or introducing new items into the BSC.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a microcentrifuge, blender), place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet. Ensure proper decontamination/sterilization prior to disposal.
- Clean up spills in the cabinet immediately. Wait 10 minutes before resuming work.
- When work is finished, remove all materials and wipe all interior surfaces with 70% alcohol or any other disinfectant suitable for the agent(s) in use.
- Remove lab coat, gloves and other Personal Protective Equipment (PPE) and wash hands thoroughly before leaving the laboratory.

1. Safety Showers. Safety showers provide an immediate water drench of an affected person. Standards for location, design, and maintenance of safety showers are outlined in *Prudent Practices for Handling Hazardous Chemicals in Laboratories.*

2. Eyewash Stations. Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials. Standards for location, design, and maintenance of emergency eyewash facilities are outlined in *Prudent Practices for Handling Hazardous Chemicals in Laboratories.*

3. Ventilation Controls. Ventilation controls are those controls intended to minimize employee exposure to hazardous chemicals and infectious or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:

A. General (Dilution) Exhaust: a room or building-wide system that brings in air from outside and ventilates within. Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for RG-3 agents or BL-3 work.

B. Local Exhaust or Filtration: a ventilated, enclosed workspace intended to capture, contain and exhaust or filter harmful or dangerous fumes, vapors and particulate matter. In the case of hazardous chemicals this includes a fume hood. In the case of infectious agents BSCs should be used. For more information on ventilation requirements involving hazardous chemicals refer to the Lab Safety Manual.

C. Personal Protective Equipment

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may ter04 Tw 3.14 0 Td7Lsa(pr)3+1(ct2)4( )-10 (on)]TJ l onsi142(b)-2(i)-2(o c)4(ont-2(y

Gloves. Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection, contact the OEHS at 2352. To prevent transfer of organisms to personnel and to areas outside of the laboratory, gloves must be removed whenever handling items that are not related to laboratory experiments or when handling items that are removed from laboratories, e.g. calculators, eyeglasses, teled.

Respirators. For cetain protocols and project.
based on the hazard and the protection factor required.
UAH for assistance in selection of
be included in
the UAH

D. Recommended Work Practices

Pipees and Pipeing Aids . Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipeing of biohazardous or toxiluids to a biosafety cabinet if possie. If pipeing is dd on the open bench, use absorbent pads or paper on the bench. Use the following precautions:

- Always use coeon-plugged pipeees when pipeing biohazardous or toxifluids.
- Never prepae any kind of
pipette.

- Work in a biosafety cabinet whenever possible.
- Wear gloves.
- Fill the syringe carefully to minimize air bubbles. Expel air, liquid and bubbles from the syringes vertically into a cotton pad moistened with a disinfectant.

Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device of the one-handed scoop method must be used. Always dispose of needle and syringe unit promptly into an approved sharps container. Do not overfill sharps containers (2/3 filled= full) and contact the OEHS for pick-up (see Biohazardous Waste section).

Cryostats

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
- Consider the trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, M. tuberculosis or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit.

Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and re-suspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

• Add disinfectant to the space between the tube and the bucket to disinfect material in

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in a disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

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

Loop Sterilizers and Bunsen Burners
Sterilization of inoculating loops or needles in an open flame generates small particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable

- Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment and the avoidance of the creation of electrical hazards in wet areas.
- All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

Biohazard Spill Clean-Up Procedures
Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. For most instances the basic kit can be assembled with materials already used in the laboratory. Although it is preferable to have the content of the spill kit in one location, as long as the materials are easily accessible to everyone in the lab, prior assembly might not be necessary. However, ready assembled spill kits are available through laboratory and maintenance supply stores.

Basic Biological Spill Kit:

Disinfectant (e.g., bleach 1: 10 dilution, prepared fresh)
Absorbent Material (e.g., paper towels)
Waste Container (e.g., biohazard bags, sharps containers)
Personal Protective Equipment (e.g., lab coat, gloves, eye and face protection)
Mechanical Tools (e.g., forceps, dustpan and broom)

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, call campus police at 6911 if necessary, and proceed with common sense. Call the OEHS at 2352 if further assistance is required especially if the spill outgrows the resources in the laboratory.

Spill Inside a Centrifuge
Have a complete biological spill kit ready to go before you start the cleanup.

• Clear area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up the spill.
• Wear a lab coat, safety goggles and gloves during clean up.
• Remove rotors and buckets to the nearest biological safety cabinet.
• Thoroughly disinfect inside of centrifuge.
• Remove contaminated debris after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal.

Spill Inside the Laboratory (BL-2, RG-2)
Clear spill area of all personnel. Wait for any aerosols to settle before entering the spill area. Remove any contaminated clothing and place in biohazard bag for further processing by laundry (UAH or department). Wear a disposable gown or lab coat, safety goggles and gloves. Have a complete biological spill kit ready to go before you start the cleanup.
Initiate cleanup with disinfectant as follows:

• Cover spill with paper towels or other absorbent material containing disinfectant.

• Encircle the spill with disinfectant (if feasible and necessary), being careful to minimize aerosolization.
• Decontaminate and remove all items within spill area. Remove broken glassware with forceps or broom and dustpan and dispose in sharps container. Do not pick up any contaminated sharp object with your hands.
• Remove paper towels and any other absorbent material and dispose in biohazard bags.
• Apply disinfectant to the spill area and allow for at least 10 minutes contact time to ensure germicidal action of disinfectant.
• Remove disinfectant with paper towels or other absorbent material and dispose of in biohazard bag.
• Wipe off any residual spilled material and reapply disinfectant before final clean up.
• Wipe equipment with equipment compatible disinfectant (e.g., non-corrosive). Rinse with water if necessary.
• Place disposable contaminated spill materials in biohazard bags for autoclaving.
• Place contaminated reusable items in biohazard bags, or heat resistant pans or

Should a spill of RG-2 material occur in the public, contact the OEHS at 2352. Do not attempt to clean up the spill without the proper personal protective equipment and spill clean up material. Should the spill occur inside a car, leave the vehicle, close all doors and windows, and contact the OEHS at 2352 for assistance.

E. General Guidelines and Policies

Biological Risk Assessment

process. If necessary, written standard operating procedures (SOPs) should be established and distributed.

Guidelines for Working with Tissue Cultured Cell Lines

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG level as that recommended for the agent.

The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin be handled at BL-2. All personnel working with or handling these materials need to be included in UAH's Exposure Control Plan. (See Bloodborne Pathogen Program).

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate virus, which are not contaminated with bacteria, mycoplasma or fungi and which are well established may be considered CG levl -6(t)-6(E6)-6(en)-4(d)-4(-)]TJ -nu(d)-oabisont, the ce7.2e ni bdeLo gT

pr2(r)3(e)-6(c)42(rdv)-1-52.-10(C)-3(ont)i(ont)--2(a)442(rg2.-10(C)-32(a)4-4(r)3(e)4(ve)-1-52(r)3(e)-6(c)4)3(

Use of Animals in Research, Teaching, and Service

The use of animals in research, teaching and outreach activities is subject to state and federal laws and guidelines. UAH's policy specifies that:

- All animals under UAH's care (that is, involved in projects under the aegis or sponsorship of UAH) will be treated humanely;
- Prior to their inception, all animal projects receive approval by the UAH IACUC;
- UAH will comply with state and federal regulations regarding animal use and care.

Principal Investigators planning to use animals for any UAH-related activity must contact the Institutional Animal Care and Use Committee for a review of the anticipated research prior to the start of the project, regardless of the source of funding for the project. Contact Research Administration at 2656 for more information. Information that may be requested include descriptions of experimental protocols, plans for animal care, available facilities, and information on the use of hazardous materials including infectious agents.

All animal protocols involving the use of ton (ar)-1c ingni mleuntgni ter(t)-6(o)-14(g)-4((n)-4(i)-6c(c)-10(h)-4(m
sted(on t)-2(he)4((m)-2(a)4iA)-2((e)4(nt)-2(r)3(a)-6(oc)4e;)]TJ-11212

specific procedures for the correct packaging of these materials, necessary documentation and labeling and permits. More information about specific shipping requirements is available through the OEHS at 2352.

F. Decontamination

Methods of Decontamination
Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means, that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

In order to select the proper method and tools, it is important to consider, for example, the following aspects:

Type of biohazardous agents, concentration and potential for exposure;
Physical and chemical hazards to products, materials, environment and personnel.

Physical and chemical means of decontamination fall into four main categories:
Heat, Liquid Chemicals, Vapors and Gases, and Radiation

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. Vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are commonly used to sterilize materials. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time.
The following paragraphs focus on some of these methods.

Heat
In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in overall shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally or 40 mi 14 Tc rra1

are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general

Table 3
Increasing Resistance to Chemical Disinfectants

LEAST RESISTANT

Lipid or medium-size Viruses

Vegetative Bacteria

Fungi

Nonlipid or Small Viruses

Myobacteria

Bacterial Spores

• **Regulated biological wastes include**:
(a) Liquid or semi-

identify that they have been through the autoclave procedure. They may be disposed of with the trash waste stream after sterilization.

All autoclaves used for the decontamination of biohazardous waste must be tested on an annual basis. After successful autoclaving (decontamination), place all biohazard bags in plastic non-biohazard bags that are leak-proof. These can be put in the waste stream picked up by custodial services. Biohazardous waste that has been successfully sterilized by autoclaving is no longer considered hazardous.

Since autoclaves are an integral part of UAH's biohazardous waste treatment procedure, proper operation and maintenance is very important. All users of autoclaves need to be trained in the proper operating procedures either through the laboratory supervisor or Principal Investigator or whoever was put in charge by the department. Maintenance and repair of autoclaves used for the decontamination of biohazardous waste are the responsibility of the individual departments. If the department chooses to not use autoclaves for their biohazardous waste treatment, alternative procedures (e.g., outside biomedical waste disposal and transport) need to be established.

Waste Specific Procedures for BL-1 and 2 Cultures

Cultures, Stocks and Related Materials

Cultures and stocks of infectious agents and associated biologicals (as defined above), shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags.

Bulk Liquid Waste, Blood and Blood Products

All liquid waste from humans or animals such as blood, blood products and certain body fluids, known to not contain infectious agents, can be disposed of directly by flushing down a sanitary sewer. However, due to coagulation, flushing of large quantities of blood is impractical. Autoclave or treat with a disinfectant all other liquid biohazardous waste.

Sharps

All sharps must be placed in a rigid, puncture resistant, closeable and leak-proof container, which is labeled with the word "Sharps" and the biohazard symbol. Approved sharps containers are available through laboratory supply stores. Food containers (e.g., empty coffee cans) are not permissible as sharps containers. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not recommended. Sharps containers should not be filled more than 2/3 full. After use, the container needs to be closed and the OEHS contacted for pick-up. To comply with the 90-day storage limit, contact the OEHS for pick-up as soon as possible. Never place any type of sharps in the trash.

Contaminated Solid Waste

Contaminated solid waste includes cloth, plastic and paper items that have been exposed to agents infectious or hazardous to humans, animals or plants. These contaminated items shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags. Contaminated Pasteur pipettes are considered sharps and need to be disposed of in a sharps container.

Waste Specific Procedures for Biosafety Level (BL-3)

All biohazardous waste including RG-2 and 3 agents that are handled at BL-3 is to be autoclaved at the point of origin (laboratory, or facility). Transportation of non-autoclaved BL-3 waste outside of the facility is generally not permitted.

Animal Waste
Collect animal carcasses, tissues, or bedding in non-transparent, 4-6 mil plastic bags.

Small animal carcasses may be individually bagged and collected together in a larger leak-proof container. For small animals, do not exceed 35 pounds total weight per bag. Large animals shall be securely packaged in large plastic bags. Bind any limbs or sharp protrusions so they will not puncture the bag. Leaky or punctured bags will not be picked up.

Labels must identify the waste or it will not be removed. Affix labels to the waste container(s) or bag(s) using twist ties or freezer tape. Attach the labels so they will not fall off during transportation and storage. Labels should not be permanently cemented or excessively taped as this prevents the label from being removed for record keeping purposes.

If the waste contains known viable pathogens e.g., the animal had an infectious zoonotic disease or was inoculated with a known pathogen, enter the name of the biohazardous agent on the waste tag and attach a biohazard sticker to the container. If no known viable pathogens are present, mark the waste as noninfectious on the waste tag. Non-infectious animal carcasses can be incinerated locally. Store carcasses in a freezer or cold storage area. Keep freezers/cold storage areas clean and defrost them regularly. Do not mix pathological wastes contaminated with hazardous chemicals or radioisotopes with uncontaminated waste. Pathological wastes containing radioactive materials shall also be labeled with a radioactive waste tag.

Human Waste
Cdloe ti hu stled (s)-1(t)-15 Td [(7HN-15 Td [(z-2(e)t)-15 Td [(a)-4(c)6(tivl(y)1Tdy2(a)4)2(ta)6(in)2(inkg)1

Section III-D, the next category, covers whole animal or plant experiments as well as projects involving DNA from Risk Group 2, 3 or 4 agents. Prior to the initiation of an experiment that falls into Section III-D, the PI must submit a Registration Document for Recombinant DNA Research to the Institutional Biosafety Committee. The IBC reviews and approves all experiments in this category prior to their initiation.

Section III-E experiments require the filing of a Registration Document for Recombinant DNA Research with the IBC at

Petitions
See the appropriate sections of the NIH Guidelines if you wish to petition NIH for exemption.

Compliance Statement
A compliance statement must appear on each Project

3. Petition NIH/ORDA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in Sections III-A-1, Major Actions Under the NIH Guidelines, and HI-B, Experiments that Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation;

I. Bloodborne Pathogens Program and Exposure Control Plan

UAH is committed to protecting its employees from risks associated with exposure to bloodborne pathogens through implementation of its Bloodborne Pathogen Plan (BBP). This plan follows the requirements established by the Department of Public Health Occupational Health Standards Commission as adopted from the rules issued by the U.S. Occupational Safety