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## **Definitions and Acronyms**

### *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 individual about whom an investigator conducting research obtains: 1) data or materials (blood, tissue, etc.) through intervention or interaction with the person, or, 2) “identifiable information.” (Public officials elected or in non-elected, decision-making positions, are not considered human subjects under these regulations).

### *Research*

Final investigation designed to develop or contribute to generalized knowledge.

### *Universal Precautions*

A method of infection control that 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 – Environmental Protection Agency  
HEPA – High Efficiency Purified Air  
IBC – Institutional Biosafety Committee  
NIH – National Institutes of Health  
NSF – National Sanitation Foundation  
OEHS – Office of Environmental Health & Safety  
ORDA – The Office of Recombinant DNA Activities  
OSHA – Occupational Safety and Health Act  
RAC – Recombinant DNA Advisory Committee

RDNA – Recombinant DNA

RG - Risk Group

**I. Biological Safety (Biosafety) Levels**

Biological safety or biosafety, is the application of knowledge, techniques, and equipment, to prevent personal, laboratory, and environmental exposure to potentially infectious agents or biohazards. Biosafety level defines the containment conditions under which infectious agents can be safely handled and manipulated. The objective of containment is to confine the biohazards and to reduce the potential exposure of laboratory personnel, persons outside the laboratory, and the environment to potentially infectious agents. These goals can be accomplished through the following means:

**Primary Containment**

Protection of personnel and the immediate laboratory environment through adherence to good laboratory practices and biological techniques is the most important element in maintaining a safe work environment.

**Secondary Containment**

Protection of the environment external to the laboratory from exposure to infectious materials through facility design and operational practices. Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four biosafety levels (1 – 4) define the level of containment necessary to protect personnel and the environment. A Biosafety Level 1 (BL-1) is the least restrictive, while Biosafety Level 4 (BL-4) requires a special containment laboratory facility not currently available at UAH. Since no BL-4 research is conducted at UAH, this manual will focus on Biosafety Levels 1-3. For more information on Biosafety Level 4 requirements refer to the appropriate literature or contact the OEHS. Table 1 summarizes the requirements of BL’s 1-3.

Adherence to good laboratory practices and biological techniques is the most important element in maintaining a safe work environment. All UAH employees and students working with infectious agents or potentially infected materials must be made aware of the potential risks. In addition, they are required to be trained and proficient in the practices and techniques required for the handling of such materials. It is the responsibility of the Principal Investigator, Professor, or laboratory supervisor to provide or arrange for appropriate training of all personnel.

**Table 1  
Summary of Biosafety Levels for Infectious Agents**

**Biosafety Level 1 (BL-1)**

<b>Agents</b>	Not known to cause disease in healthy adults
<b>Practices</b>	Standard Microbiological Practices
<b>Safety Equipment (Primary Barriers)</b>	None Required
<b>Facilities (Secondary Barriers)</b>	Open bench top and sink required

## Biosafety Level 2 (BL-2)

<b>Agents</b>	Associated with human disease, which is rarely serious for which preventative or therapeutic intervention are often available
<b>Practices</b>	BL-1 practice plans plus: limited access; biohazard warning signs; “Sharps” precautions; biosafety manual defining any needed waste decontamination or medical surveillance policies
<b>Safety Equipment (Primary Barriers)</b>	Primary Barriers = Class I or II Biological Safety 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): laboratory coats, gloves, face and eye protection as needed
<b>Facilities (Secondary Barriers)</b>	BL-1 plus: Autoclave available Biosafety Level 3 (BL-3)

## Biosafety Level 3 (BS-3)

<b>Agents</b>	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences
<b>Practices</b>	BL-2 practices plus: controlled access; decontamination of all waste; decontamination of lab clothing before laundering; baseline serum
<b>Safety Equipment (Primary Barriers)</b>	Primary Barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents; PPE; protective lab clothing, gloves, face and eye protection, and respiratory protection as needed
<b>Facilities (Secondary Barriers)</b>	BL-2 plus: Physical separation from access corridors; self-closing, double door access; exhausted air not recirculated, negative airflow into laboratory

## 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**

<b>Risk Group</b>	<b>Risk to the individual and the community</b>
<b>Risk Group 1</b> (RG-1)	Agents that are not associated with disease in healthy adult humans
<b>Risk Group 2</b> (RG-2)	Agents that are associated with human disease but are rarely serious and for which preventative or therapeutic interventions are often available
<b>Risk Group 3</b> (RG-3)	Agents that are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available (high individual risk and high community risk)
<b>Risk Group 4</b> (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available (high individual risk and high community risk)

**Risk Groups and Biosafety Levels**

Determining the risk group (RG) of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BL-2, and RG-3 agents at BL-3. However, the use of certain RG-2 agents in large quantities might require BL-3 containment conditions, while some RG-3 agents may be safely manipulated at BL-2 under certain conditions. For more information refer to the section on risk assessment or contact the OEHS at 2171.

**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. Copies of these documents are available from the OEHS.

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 in 1976. 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. A link to this document via the internet is provided on the UAH OEHS website.

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 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. The BMBL has been revised several times and is commonly seen as the standard for biosafety. UAH is using this manual as the basis for its biosafety manual.

3. Occupational Safety and Health Administration (OSHA): Bloodborne Pathogens Standard. In 1992, OSHA promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions.

4. Department of Health and Human Services (HHS): Additional Requirements for Facilities Transferring or Receiving Select Agents. In 1996, HHS published a set of rules that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. HHS therefore requires that UAH comply with the BMBL and OSHA's Laboratory Safety Standard 29 CFR 1910.1450. A copy of the most current list of select/restricted agents and toxins covered under this rule is included in Appendix A. A notification form must be submitted to the OEHS prior to shipping, receiving, transferring or working with select agents in any way. The notification form can be found in Appendix A.

5. Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:

United Nations: Recommendations of the Committee of Experts on the Transportation of Dangerous Goods

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

International Air Transport Association (IATA): Dangerous Goods Regulations

U.S. Department of Transportation (DOT): 40 CFR Parts 171-178

U.S. Public Health Service: 42 CFR Part 72

U.S. Postal Service: 39 CFR Part III

U.S. Department of Labor, OSHA: 29 CFR 1910.1030

6. Importation permits are required for certain infectious agents, biological materials and animals as outlined in the U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms and vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APIUS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

#### **IV. Practices and Procedures**

##### *Routes of Infection*

The general laboratory procedures outlined in this manual address issues related to laboratory-acquired infections and provide for guidance in handling infectious or potentially infectious materials.

When working in a biological research environment it is not unreasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and subsequently overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth

Eating, drinking and/or smoking in the laboratory.

Mouth pipetting.

Transfer of microorganisms to mouth by contaminated fingers or articles.

2. Through the skin  
Accidental inoculation with a hypodermic needle, other sharp instruments or glass.

Cuts, scratches.

Passive absorbance (chemical diffusion).

3. Through the lungs

Inhalation of airborne microorganisms.

Most of the laboratory-acquired infections reported in the literature point to accidents during work with some type of infectious agent. These include spillage, splashes and accidents involving needles or other sharp objects.

#### **A. Administrative Controls**

##### *Laboratory Safety Procedures*

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 sign 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 sign is at the main entrance door(s) to laboratories and animal rooms, on equipment like refrigerators, incubators, transport containers, and/or lab benches. Signs 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 agents 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 attend the biosafety training offered by the OEHS.

### *Bloodborne Pathogen Program*

In accordance with OSHA requirements, UAH has established an 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.

### *Infectious Agents 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 by the OEHS. A copy of the Select Agent Notification Form may be found in Appendix A.

### *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 – Additional Requirements for Facilities Transferring or Receiving Select Agent and the Homeland Security and Bioterrorism Response Act). A list of these select/restricted agents is included in Appendix A. The OEHS must be notified of activities involving these agents. The Principal Investigator, Professor or laboratory supervisor shall notify the OEHS by completing the Select Agent Notification Form found in Appendix A. Please contact the OEHS for further information.

### *Institutional Biosafety Committee*

The IBC has been established to meet the requirements mandated in the NIH Guidelines for

Research Involving Recombinant DNA Molecules. In addition, the IBC is involved in the oversight of all projects involving infectious agents (RG-2 and 3) and certain toxins at UAH.

## **B. Engineering Controls**

### *Biological Safety Cabinets (BSCs)*

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II, and III have been developed to meet various research and clinical needs. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. Biological safety cabinets must not be confused with other laminar flow devices or “clean benches”, in particular, horizontal flow cabinets that direct air towards the operator should never be used for handling infectious, toxic or sensitizing materials.

Laboratory personnel must be trained in the correct use and maintenance of BSCs to ensure that personnel and product protection (where applicable) is maintained. Before selecting any BSC for purchase, contact the OEHS for a work specific assessment and for selection criteria.

#### 1. Class I Biosafety Cabinet

This is a ventilated cabinet for personnel protection with an unrecirculated inward airflow away from the operator. This unit is fitted with a HEPA filter to protect the environment from discharged agents. A Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection (e.g., sterility).

#### 2. Class II Biosafety Cabinet

This is a ventilated cabinet for personnel, product and environmental protection, which provides inward airflow and HEPA-filtered supply and exhaust air. The Class II cabinet has four designs depending on how much air is recirculated and/or exhausted and if the BSC is hard-ducted to the ventilation system or not. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes.

#### 3. Class III Biosafety Cabinet

A Class III cabinet is a totally enclosed ventilated cabinet, which is gas-tight, and maintained under negative air pressure (0.5 inches water). The supply air is HEPA filtered and the exhaust air has two HEPA filters in series. Work is performed in the cabinet by the use of attached rubber gloves. Biological safety cabinets, when properly used in research and teaching activities involving the manipulation of biohazardous agents, are effective in containing and controlling particulates and aerosols and complement good laboratory practices and procedures. The correct location, 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. The OEHS web site has links to these materials.

### *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.
- 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.

### *Safety Equipment*

For a comprehensive listing of safety equipment required in the laboratory refer to Prudent Practices for Handling Hazardous Chemicals in Laboratories.

#### 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 LSM.

## C. Personal Protective Equipment

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. Personal protective equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The following PPE is recommended for regular use.

### Face Protection

Splash goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face.

### Laboratory Clothing

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization in the event it becomes contaminated. At a minimum, a laboratory coat should be worn in all laboratories working at a BL-2. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered (Department facilities). Personnel must not take laboratory clothing home.

### 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 6875.

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, telephones, etc.

### Respirators

For certain protocols and projects, additional PPE like respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection must contact UAH for assistance in selection of proper equipment and training in its usage. Personnel wearing respirators need to be included in the UAH Respiratory Protection Program.

## **D. Recommended Work Practices**

### *Pipettes and Pipetting Aids*

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench. Use the following precautions:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse. Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet while in use.

### *Syringes and Needles*

Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette. Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe. When using syringes and needles with biohazardous or potentially infectious agents:

- 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 resuspending 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:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters. (For more information, call the OEHS).
- Work in a BSC when re-suspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air

from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.

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

### *Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers*

The use of any of these devices results in considerable aerosol production. Blending, cell disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

#### *Safety Blenders*

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

#### *Lyophilizers and Ampoules*

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible. 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 pen, 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 pre-sterilized 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 plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended. Continuous flame gas burners should not be used in BSCs. These burners can

produce turbulence that disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

### *Laundry*

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hampers (labeled or color coded) or in biohazardous bags. Clothing must be sterilized prior to being laundered. Appropriate PPE must be worn by employees who handle contaminated laundry.

### *Housekeeping*

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter – surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Properly disposing of chemicals and wastes – old and unused chemicals should be disposed of promptly and properly. Refer to The UAH Hazardous Waste Management Plan for more information.
- Providing a workplace that is free of physical hazards – aisles and corridors should be free of tripping hazards.
- 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 6875 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.

Continued

- 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 containers with lids before autoclaving.
- Reopen area to general use only after spill clean up and decontamination is

complete.

- Inform all personnel and laboratory supervisor about the spill and successful clean up as soon as possible.

### **Spill Inside the Biological Safety Cabinet (BL-2, RG-2)**

**Have a complete biological spill kit ready to go before you start the cleanup.**

- Wear lab coat, safety goggles and gloves during clean up.
- Allow cabinet to run during clean up.
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.
- Wipe up spillage and disinfectant with disposable paper towels.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean up.
- Expose non-autoclavable materials to disinfectant, 10 minutes contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for further processing by laundry (UAH or department).
- Run cabinet at least 10 minutes after cleanup and before resuming work.
- Inform all users of the BSC as well as the laboratory supervisor about the spill and successful clean up as soon as possible.

### **Spill Outside the Laboratory, During Transport on Campus**

Always transport biohazardous materials in an unbreakable well-sealed primary container placed inside a leak-proof, closed and unbreakable secondary container, labeled with the biohazard symbol (plastic cooler, bio-specimen pack, etc.). Should a spill of RG-2 material occur in the public, contact the OEHS at 6875. 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 6875 for assistance.

## **E. General Guidelines and Policies**

### *Biological Risk Assessment*

The assessment of risk is an essential element of safety in the laboratory. For most situations, guidelines, rules and regulations have clearly defined the procedures and practices to be followed in order to achieve safety in the work place. However, the newly isolated agent or toxin, or the new procedure, never before employed, need further evaluation. Questions concerning the appropriate safety equipment, training and waste disposal need to be addressed as well as safe procedures and practices. Something is considered safe when the risk associated with it is judged to be acceptable. However, since individual judgment involves both personal and social values, opinions on what is safe or not can vary significantly. In order to find a common ground for an acceptable risk

assessment, the “rule of reason” needs to be applied. The following factors should be considered for the determination of reasonableness:

1. Custom of usage (or prevailing professional practice): Many laboratory procedures involve the maintenance of sterility and cleanliness. These procedures are commonly considered safe, since adverse effects would have been obvious over time. However, because a procedure has been used for many years do not necessarily imply that it is safe. The best example is mouth pipetting, which was used for centuries and finally considered a very dangerous procedure and habit.
2. Best available practice, highest practicable protection, and lowest practicable exposure: It should be common practice in the microbiological laboratory to use the best available procedures with the highest level of protection. This not only provides for a safe work environment but also fosters excellence in scientific conduct.
3. Degree of necessity or benefit: The common question is, are the benefits worth the risk? There is no need to use a human pathogen causing severe gastroenteritis in a teaching laboratory when principal microbiological practices can be taught with an organism that is not considered to be infectious.
4. No detectable adverse effects: This can be a very weak criterion since it involves uncertainty or even ignorance.
5. Principal knowledge: Many times, existing procedures are modified, involving the same or similar toxic chemicals or agents. For that reason, similar safety procedures should be applied. If new agents are isolated, we need to ask what we know about the close relatives. Many agents of known etiologic character are already categorized in risk groups allowing for the selection of the appropriate biosafety level. New isolates from infected animals or humans with known infectious relatives warrant at a minimum the same level of protection.

Taking the above mentioned factors, as well as others, into consideration will allow for a reasonable approach to a new challenge. The OEHS is available to assist in this process and should be contacted for questions concerning radiation, chemical and biological safety. Once a risk assessment is completed, the results should be communicated to everyone involved in the 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 Class I cell lines and handled at Biosafety Level 1. Appropriate tests should confirm this assessment.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until

shown to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level 2. Studies involving suspensions of IRV prepared from T-cell lines must be handled at BL-3.

Recent product recalls for bovine serum have raised the awareness of potential Bovine Spongiform Encephalopathy (BSE) or TSE (Transmissible Spongiform Encephalopathy) contamination of those sera. For more information on testing and purity of bovine serum used in your laboratory, contact your supplier.

#### *Guidelines for Preventing the Transmission of Tuberculosis*

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30-year downward trend. Recently, drug resistant strains of mycobacterium tuberculosis have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died. In October 1994, CDC published its Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at risk for exposure to tuberculosis. For more information, contact the UAH OEHS at 6875. Investigators intending to work with Mycobacterium sp. in the laboratory must register with the OEHS by using the Select Agent Registration Form (Appendix A). Propagation and/or manipulation of mycobacterium tuberculosis and M. bovis cultures in the laboratory or animal room must be performed at BL-3 and require IBC approval.

#### *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 IBC;
- 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 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 will 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 RDNA; infectious or transmissible agents; human blood, body fluids or tissues; toxins; carcinogenic, mutagenic, teratogenic chemicals; or physically hazardous chemicals (reactive, explosive, etc.) must be submitted as part of the Project or Recombinant DNA Registration to the OEHS for review prior to initiation of the research.

All areas housing research animals are required to have an Animal Hazard Sign (Appendix F)

posted on the main entrance door if the project involves any of the hazards listed above. It is the responsibility of the Principal Investigator to establish this sign listing all relevant entry, animal care and emergency procedures. The OEHS as well as the appropriate animal Facility Supervisor will assist the PI in this process.

#### *Transportation of Biological Materials On and Off Campus*

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and endangerment of the public and the environment.

#### Transportation in-between buildings or locations on and off campus roads:

Diagnostic and clinical specimens, infectious materials and recombinant DNA molecules need to be packaged in a sealed, leak-proof primary container (e.g., glass tube), which is securely positioned in a secondary leak-proof and closeable container (e.g., cooler, ice chest) containing a clearly visible biohazard symbol on the outside. A list of contents as well as emergency information (e.g., PI phone number) needs to accompany the material (e.g., attached to the cooler in a plastic pouch). The use of private cars for the transportation of such materials on or off campus is highly discouraged. University vehicles are available upon request through the individual departments. In case of an emergency (e.g., car accident) make all police and safety personnel aware of the presence of biohazardous materials and contact the OEHS at 6875.

#### Transportation and shipment via carrier off campus:

The shipment of diagnostic and clinical specimens, biological products, infectious agents and recombinant DNA molecules is regulated by national and international transportation rules. This includes 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 heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

### *Liquid Chemicals Used as Disinfectants*

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- Nature of surface being disinfected – Porous or smooth. The more porous and rough the surface, the longer a disinfectant will need to be effective.
- Number of microorganisms present – Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms – Microbial agents can be classified according to increasing resistance to disinfectants and heat (see Table 3)
- Presence of organic material – The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature – Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are discussed below:

#### Alcohols:

Ethyl or isopropyl alcohol in concentration of 70% to 90% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores. Concentrations above 90% are less effective.

#### Formalin:

Formalin is 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. Formaldehyde is a human carcinogen and creates respiratory problems at low

levels of concentration.

### Glutaraldehyde:

This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehydes are irritating to the eyes, nasal passages and upper respiratory tract. They should be used always in accordance with the instructions on the label and the appropriate personal protective equipment.

### Phenol and Phenol Derivatives:

Phenol based disinfectants come in various concentrations ranging mostly from 5% to 10%. These derivatives including phenol have an odor, which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacterium tuberculosis*, fungi and lipid-containing viruses. They are not active against spores or non-lipid viruses.

### Quaternary Ammonium Compounds (Quats):

Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. Quats 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 cleaning.

### Halogens (Chlorine and Iodine):

Chlorine-containing solutions have broad-spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Diluted solutions may be kept for extended periods if kept in a closed container and protected from light. However, it is recommended to use freshly prepared solutions for spill cleanup purposes. Excess organic materials inactivate chlorine-containing disinfectants. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilants since they inactivate bacteria spores. Iodine has similar properties to chlorine. Iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

### Vapors and Gases:

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like biological safety cabinets. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

**Radiation:**

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. Ultraviolet (UV) radiation is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms. LTV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.

Table 3  
Increasing Resistance to Chemical Disinfectants

LEAST RESISTANT	EXAMPLES
Lipid or medium size viruses	Herpes Simplex Virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Human Immunodeficiency virus
Vegetative bacteria	Pseudomonas aeruginosa Staphylococcus aureus Salmonella choleraesuis
Fungi	Trichophyton sp. Cryptococcus sp. Candida sp.
Nonlipid or Small viruses	Poliovirus Coxsackievirus Rhinovirus
Mycobacteria	Mycobacterium tuberculosis M. bovis
Bacterial Spores	Bacillus subtilis Clostridium sporogenes
<b>MOST RESISTANT</b>	

**G. Biohazardous Waste**

At UAH, the term biohazardous waste is used to describe different types of waste that might include infectious agents. Currently, the following waste categories are all considered to be biohazardous waste:

- **Medical waste:** Defined as any solid waste, which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals.

• **Medical waste includes:**

- (a) Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
- (b) Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.
- (c) Pathological waste: defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
- (d) Sharps: Defined as needles, syringes, scalpels, and intravenous tubing with needles attached regardless of whether they are contaminated or not.
- (e) Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

• **Regulated biological wastes include:**

- (a) Liquid or semi-liquid blood or other potentially infectious materials;
- (b) Contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed;
- (c) Items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling;
- (d) Contaminated sharps which includes any contaminated object that can penetrate the skin;
- (e) Pathological and microbiological wastes containing blood or other potentially infectious materials.

• **Laboratory waste and regulated waste** as defined in the Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) and the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories.

The CDC/NIH Guidelines cover contaminated waste that is potentially infectious or hazardous for humans and animals. The same is true for the NIH Guidelines on recombinant DNA, which also covers contaminated waste potentially infectious or hazardous for plants.

*General Labeling, Packaging and Disposal Procedures*

Currently, biohazardous waste is to be decontaminated before leaving UAH. Most of the waste can be autoclaved prior to disposal. The responsibility for decontamination and proper disposal of biohazardous waste lies with the producing facility (e.g., laboratory and department). The OEHS assists only in the disposal of sharps and pathological waste including animal carcasses.

All biohazardous waste needs to be packaged, contained and located in a way that protects and prevents the waste from release at any time at the producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents in the air must be prevented.

**No biohazardous waste can be stored for more than 90 days at UAH.**

If not stated otherwise (see below), most biohazardous waste will be disposed of in biohazard bags. Currently, UAH requires the use of orange biohazard bags that include the biohazard symbol and a built-in heat indicator with the word "AUTOCLAVED." Bags that meet these requirements are available in various sizes at laboratory supply stores. All waste disposed of in these bags is to be

autoclaved until the waste is decontaminated. The built-in heat indicator will turn dark. 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 opaque (black) plastic non-biohazard bags that are leak-proof. These opaque bags can be put in the local picked up by custodial services. Biohazardous waste that has been successfully decontaminated 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. For more information contact the UAH OEHS at 824-6875.

### ***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 work "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 labeled with a UAH Hazardous Materials Pick Up Tag or a UAH Hazardous Materials Label. 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 3 (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 building 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.

Contact the OEHS at 6875 for animal waste removal. Ensure that each bag/container is labeled as to the contents. 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. Alternatively, put the opaque plastic bag inside a biohazard bag. Biohazardous Wastes must be disposed of through the OEHS. Call 2352 to request a pick-up. If no known viable pathogens are present, mark the waste as non-infectious 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 waste contaminated with hazardous chemicals or radioisotopes with uncontaminated waste. Pathological wastes containing radioactive materials shall also be labeled with a radioactive waste tag for pick-up by the OEHS.

### Human Waste

Collect human pathological waste in leak-proof containers labeled with the words "Medical Waste". Human pathological waste shall be cremated or buried in a cemetery. Small pieces of tissue and fluids shall be disposed of by grinding and flushing down a sanitary sewer or incineration.

### Department or Facility Specific Waste Procedures

If required, departments or facilities may establish biohazardous waste procedures that are more stringent than the above listed procedures. A written copy of these procedures should be made available to the OEHS prior to initiation.

### Decontamination of Biohazardous Waste by Autoclaving

Autoclaving is accepted as a safe and effective procedure for sterilization. There are numerous operating autoclaves on the UAH campus. To ensure that any biohazardous waste created by the UAH community is properly decontaminated, each autoclave should be tested annually for appropriate function. Biological and chemical tests are used to monitor the autoclave cycle inside the chamber. Ampoules with heat resistant spores (*Bacillus stearothermophilus*) are used to indicate

that adequate sterilization conditions are reached. A steam sterilization integrator strip is used to indicate pressure, moisture, and time.

### **Procedures:**

- All autoclaves used for decontamination need to be tested on at least an annual basis.
- Strong oxidizing material (chemicals) must not be autoclaved with organic material: Oxidizer + Organic Material + Heat = Possible Explosion
- All biohazardous waste must be placed in orange biohazard bags with a heat sensitive “Autoclaved” indicator.
- Prior to autoclaving, a biohazard bag containing waste must be kept closed to prevent airborne contamination and nuisance odors. However, when autoclaving, the bag must be open to allow the steam to penetrate. Upon removal of the bag from the autoclave, it should be closed and disposed of in an opaque (black) waste bag.
- It is recommended to add water to each biohazard before autoclaving.
- Autoclave biohazardous materials for at least 40 minutes at the standard 121°C/250°F and 15 PSI for a single bag and at least 60 minutes for a run with numerous bags.

## **H. Recombinant DNA Research**

As a condition for funding of recombinant DNA research, UAH must ensure that research conducted at or sponsored by UAH, irrespective of the source of funding, complies with the most current (NIH) Guidelines for Research Involving Recombinant DNA Molecules. At UAH, the responsibility for ensuring that recombinant DNA activities comply with all applicable guidelines rests with the institution and the Institutional Biosafety Committee (IBC) acting on its behalf. Before experiments involving recombinant DNA begin, the Principal Investigator (PI) must submit a Registration for Recombinant DNA Research to the OEHS. When the research is regulated by the NIH guidelines, the OEHS will submit the Recombinant DNA Registration to the IBC. A copy of the UAH Registration for Recombinant DNA Research is located in Appendix E of this manual.

All recombinant DNA research proposals require the PI to make an initial determination of the required level of physical and biological containment. For that reason, the NIH has developed six categories (III-A to III-F) addressing different types of RDNA research.

If the proposed research falls within section III-A of the NIH Guidelines, the experiment is considered a “Major Action”. This includes experiments involving human gene transfer experiments. As a result, the experiment cannot be initiated without submission of relevant information to the Office of Recombinant DNA Activities (ORDA) at NIH. In addition, the proposal has to be published in the Federal Register for 15 days, it needs to be reviewed by the RAC, and specific approval by the NIH has to be obtained. The containment conditions for such an experiment will be recommended by the RAC and set by the NIH at the time of approval. The proposal requires IBC approval before initiation.

If the proposed research falls within section III-B, the research cannot be initiated without submission of relevant information on the proposed experiment to NIH/ORDA (for exceptions see the guidelines). Experiments covered in III-B include the cloning of toxic molecules. The containment conditions for such experiments will be determined by NIH/ORDA in consultation

with ad hoc experts. Such experiments require Institutional Biosafety Committee (IBC) approval before initiation. Please refer to the guidelines for more specifics.

Section III-C, covers experiments with human subjects. These experiments require IBC approval and NIH/ORDA registration before initiation.

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 the time the experiment is initiated. The IBC reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required.

Section III-F experiments are exempt and a registration with the IBC is not required.

For every recombinant DNA research proposal (except for exempt experiments, such as III-F), the following information must be submitted to the IBC as part of the Registration Document for Recombinant DNA Research:

- Description of the proposed research
- Host strain(s) used, (include genus, species, and parent strain).
- Source of DNA/RNA sequences (include genus, species, gene name and abbreviation, and the function of the gene, if known).
- Recombinant plasmid(s)/vectors used.
- Will there be an attempt to obtain a foreign gene? (If yes, identify the gene and gene function)
- Will this project require large-scale fermentation (> 10 liters) of organisms containing recombinant DNA molecules?
- Will the project require the release of organisms containing recombinant DNA into the environment?
- The containment conditions that will be implemented as specified in the NIH Guidelines.
- Will the project involve the use of transgenic plant or animal species? (If so, identify them).
- Will there be any attempt to transfer recombinant DNA molecules in vivo to plant or animal systems (other than tissue culture)?

The descriptions must provide sufficient information about the experiments so that reference to other documents is not required.

#### Accident, Spill and Disposal Procedures

A spill contingency plan must be described and implemented. This plan must provide for the containment as well as the safe clean up and decontamination of any spilled recombinant DNA material. Disposal methods must also be documented (refer to the UAH Hazardous Waste Management Plan).

#### Precautionary Medical Practices

Describe the reasons for using any medical monitoring of your personnel (e.g., immunization,

baseline serum sampling). This description should include the specific test used, frequency, and actions to be taken upon receipt of test results.

### 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 Registration Document. The Principal Investigator (PI) in charge of the recombinant DNA project must then sign and date the document. The statement must say:

- “I agree to fully comply with the NIH requirements pertaining to the shipment, transfer, and accident reporting for recombinant DNA materials. I agree to abide by all provisions of the most current NIH Guidelines. I have carefully reviewed and accept the responsibilities for Principal Investigators described in the NIH Guidelines. The information above is accurate and complete.”

### Responsibility of the Principal Investigator (PI) for Recombinant DNA Research

The Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research. Please refer to the most recent edition of the NIH Guidelines for Research Involving Recombinant DNA Molecules for more information.

### General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

1. Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation until that research or the proposed modification there of has been approved by the Institutional Biosafety committee and has met all other requirements of the NIH Guidelines;
2. Determine whether experiments are covered by Section III-E, Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation, and that the appropriate procedures are followed;
3. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer, and the IBC, NIH/ORDA, and other appropriate authorities within 30 days. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSB 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838;
4. Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH/ORDA (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSB 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838);
5. Be adequately trained in good microbiological or biochemical techniques;
6. Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination; and
7. Comply with shipping requirements for recombinant DNA molecules.

### Submissions by the Principal Investigator to the NIH/ORDA

The Principal Investigator shall:

1. Submit information to NIH/ORDA for certification of new host-vector systems;
2. Petition NIH/ORDA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the NIH Guidelines;
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;
4. Petition NIH/ORDA for determination of containment for experiments requiring case-by-case review; and
5. Petition NIH/ORDA for determination of containment for experiments not covered by NIH Guidelines.

#### Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines;
2. Select appropriate microbiological and biochemical practices and techniques to be used for the research;
3. Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, In-B, HI-C, IH-D, or III-E to the Institutional Biosafety Committee for review and approval or disapproval; and
4. Remain in communication with the Institutional Biosafety Committee throughout the duration of the project.

#### Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

1. Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
2. Instruct and train laboratory staff in: (1) the practices and techniques required to ensure safety, and (2) the procedures for dealing with accidents; and
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

#### Responsibilities of the Principal Investigator Duties the Conduct of Research

The Principal Investigator shall:

1. Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
2. Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer, the Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSB 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838;
3. Correct work errors and conditions that may result in the release of recombinant DNA materials;

4. Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics);
5. Comply with annual data reporting and adverse event reporting requirements for NIH- and FDA-approved human gene transfer experiments.

## **I. Bloodborne Pathogens Program and Exposure Control**

UAH is committed to protecting its employees from risks associated with exposure to bloodborne pathogens through implementation of exposure control procedures. These procedures follow the guidelines established by the Department of Public Health Occupational Health Standards Commission as adopted from the rules issued by the U.S. Occupational Safety and Health Administration in December 1991 (29 CFR 1910.1030). All employees at UAH that have a reasonable anticipated risk for exposure to bloodborne pathogens need to be included in the Bloodborne Pathogens Program. These employees need to be identified and provided with the appropriate means to safely conduct their individual jobs. The following principles must be followed when employees are potentially exposed to bloodborne pathogens:

- Minimize all exposure to bloodborne pathogens;
- Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to bloodborne pathogens;
- Routinely employ “Universal Precautions” when exposure to blood or potentially infectious materials is anticipated.

All employees requiring exposure control must attend an initial training class on bloodborne pathogens as well as an annual refresher course. Classes are offered on a regular basis at the UAH. In addition, employees in a high risk category for exposure to Hepatitis B must be provided a vaccination free of charge. Contact the OEHS if any further information is required.