UAH

The University of Alabama in Huntsville

Biological Safety Manual

The Office of Environmental Health and Safety

April 2003

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BIOLOGICAL SAFETY MANUAL

Office of Environmental Health and Safety
Laboratory Safety Committee
The University of Alabama in Huntsville
Huntsville, AL 35899

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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 information resources and consulting that will lead to the safest possible research, work, and learning environment. Input from the University Community is imperative for the achievement of this goal. Please direct any comments and suggestions to improve the Biosafety Program to the Office of Environmental Health and Safety.

Biological Safety Hazard Policy Statement

The University of Alabama in Huntsville is committed to full compliance with federal, state, and local laws and regulations pertaining to the safe management of biological agents. The University Institutional Biosafety Committee is charged with administrative responsibility for the UAH Biological Safety Program. The Director of Environmental Health and Safety is the University Biological Safety Officer with responsibility for oversight of the Biosafety Program. The director is responsible (1) for developing and maintaining University policies related to the purchase, receipt, storage, transportation, use, and disposal of biologically hazardous materials and (2) for identifying potential environmental and safety concerns. Colleges, departments, or other units utilizing biologically hazardous materials are responsible for the appropriate receipt, handling, use, and storage of biological materials within UAH facilities. Furthermore it is the responsibility of the Principal Investigator to adhere to the guidelines of the Biological Safety Manual and to ensure those persons entering into his/her laboratory are aware of the hazards. Colleges, departments, or other units may develop policies and procedures for safely utilizing and managing biohazardous materials within their units, but these policies are subject to review by the University Biosafety Committee and must be at least as stringent as University policies.

The Director of Environmental Health and Safety has overall responsibility for monitoring compliance with federal, state, and local regulations, and is responsible for identification of units within The University that may not be complying fully with regulations. The Director is responsible for providing notification of non-compliance to the units involved and for providing consultation regarding changes necessary to comply with regulations. When units fail to make necessary changes to comply with regulations, the Director is responsible for reporting such non-compliance to the Institutional Biosafety Committee (IBC). The IBC has administrative authority to revoke permission to use biologically hazardous materials within UAH facilities.

Executive Summary

The Office of Environmental Health and Safety (OEHS) and the Institutional Biosafety Committee (IBC) has developed this Biological Safety Manual for use at The University of Alabama in Huntsville with the following goals in mind:

- Protect students and personnel from exposure to infectious agents.
- Prevent environmental contamination.
- Provide an environment for high quality research and teaching while maintaining a safe workplace.
- Comply with applicable federal, state, and local guidelines, regulations, and standards.

This manual provides safety guidelines, policies, and procedures for the use and manipulation of biologically hazardous materials. Although the thorough implementation of the manual's procedures is the responsibility of the principle investigator (PI) or Professor, its success depends largely on the combined efforts of laboratory personnel, students, and administrative support. Planning for the successful implementation of biological safety controls must be part of every research proposal and laboratory activity that involves the use of biohazardous materials.

In general, the handling and manipulation of biological agents and toxins, including recombinant DNA molecules, requires the use of various precautionary measures depending upon the material(s) involved. This manual will provide assistance in the evaluation, containment, and control of biohazards. However, this document is not all-inclusive and it is imperative that all parties working with these materials seek additional advice and/or training when necessary. The OEHS and the IBC are available to assist in this endeavor.

All laboratories in which biohazardous materials are stored and/or used must maintain a copy of this document on hand at all times. Copies may be obtained by contacting the OEHS at 2352. If procedures currently in practice in your laboratory do not comply with those in this manual, please make the necessary changes. Compliance inspections will be conducted to ensure all policies and procedures herein are adopted throughout the UAH campus. Inspections will be based upon the safety checklists found in the appendices.

Disclaimer

This Biosafety Program was prepared for use on The University of Alabama in Huntsville (UAH) campus. It is provided as a means of presenting health and safety regulations and standards for use with biological materials and as a guideline to illustrate standard, accepted practices for the handling and disposition of biologically hazardous materials. The author nor The University of Alabama in Huntsville warrants its completeness or correctness.

UAH Emergency Phone Numbers

Hazardous Chemical/Substance Spill **6911**

Personal Injury **Contact Supervisor or call** **6911**
Employees and students must complete injury report
Available from the Office of Public Safety (OPS)
Return the form to the OPS

Personal Exposure **Contact Supervisor or call** **6911**
An incident where a person has been exposed to a hazardous material that is an inhalation or skin absorption hazard and no immediate physical indications are noted

Radioisotope spills **2352**

Biological spills **2352**

Material Safety Data Sheets (MSDS's)
Available in the lab or retrieve from www.uah.edu/admin/Fac/oehs

Office of Environmental Health and Safety **2352**

Office of Environmental Health and Safety
Johnson Research Center room 106
The University of Alabama in Huntsville
824-2352 fax 824-6668
oehs@email.uah.edu

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 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 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)
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 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
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)	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
Facilities: (Secondary Barriers)	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

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)	Agents that are associated with **human** disease but are rarely serious and for which preventative or therapeutic interventions are often available
Risk Group 3 (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)
Risk Group 4 (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.

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. 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 BNML 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 7 1, 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 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 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 BEPA 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 places 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 0-rings. Before use, inspect tubes, 0-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 are 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 bad.

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.

<u>Transportation in-between buildings or locations on and off campus roads:</u>

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 defines 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 defines 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 Myobacterium 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
Myobacteria	*Mycobacterium tuberculosis* *M. bovis*
Bacterial Spores	*Bacillus subtilis* *Clostridium sporogenes*
MOST RESISTANT	

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 Custodial Services 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 wasted

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 stearothernophilus*) 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/MSC 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/MSC 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/MSC 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 Plan

UAH is committed to protecting its employees from risks associated with exposure to bloodborne pathogens through implementation of its Exposure Control Plan (ECP). 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 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. As outlined in the ECP, 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 engineers 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 covered under the ECP need to 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 must be provided with Hepatitis B vaccination free of charge. The specific requirements and responsibilities of Principal Investigators, laboratory supervisors, health care managers, employees and other are outlined in the ECP. Please consult this plan for further information. Copies are available from the OEHS.

Appendix A

Select/Restricted Agents
Select Agent Registration

Select/Restricted Agents

The following list was compiled utilizing information from the Department of Health and Human Services (DHHS) and the Department of Agriculture regulatory guidelines. As mandated under the Public Health Security and Bioterrorism Preparedness and Response Act the use of any one or more of these agents requires a notification be sent to the Centers for Disease Control and Prevention (CDC).

Additional Information:

7 CFR 331 (Severe threat to plant health or marketability)
9 CFR 121 (Overlap Agents which pose a severe threat to human and animal health)
42 CFR 72 (CDC select agents and overlap agents)

http://www.access.gpo.gov/nara/cfr/index.html. This web site allows the user access to government publications by keyword, key terms, or citation.

DHHS Select Agents

- Crimean-Congo Haemorrhagic Fever Virus
- Ebola Viruses
- Lassa Fever Virus
- Marburg Virus
- Rickettsia Prowazekii
- Rickettsia Rickettsii
- South American Haemorrhagic Fever Viruses
- Tick-borne Encephalitis Complex Viruses
- Variola Major Virus
- Viruses causing Hantavirus Pulmonary Syndrome
- Yellow Fever Virus
- Yersinia Pestis
- Abrin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Tetrodotoxin

USDA-HHS Overlap Agents

- Bacillus Anthracis
- Brucella Abortus
- Brucella Melitensis
- Brucella Suis
- Burkholderia (Pseudomonas) Mallei
- Burkholderia (Pseudomonas) Pseudomallei
- Clostridium Botulinum
- Coccidioides Immitis
- Coxiella Burnetii
- Eastern Equine Encephalitis Virus
- Equine Morbillivirus (Hendra Virus)
- Francisella Tularensis
- Rift Valley Fever Virus
- Venezuelan Equine Encephalitis Virus
- Aflatoxins
- Botulinum Toxins
- Clostridium Perfringens Epsilon Toxin
- Shigatoxin
- Staphylococcal Enterotoxin
- T-2 Toxin

USDA High Consequence Livestock Pathogens and Toxins

- African Horse Sickness Virus
- African Swine Fever Virus
- Akabane Virus
- Avian Influenza Virus (Highly Pathogenic)
- Blue Tongue Virus (Exotic)
- Bovine Spongiform Encephalopathy Agent
- Camel Pox Virus
- Classical Swine Fever Virus
- Cowdria Ruminantium (Heartwater)
- Foot and Mouth Disease Virus
- Goat Pox Virus
- Japanese Encephalitis Virus
- Lumpy Skin Disease Virus
- Malignant Catarrhal Fever Virus
- Menangle Virus
- Mycoplasma Capricolum/M>F> 38/M. Mycoides Capri (Contagious Caprine Pleuropneumonia Agent)
- Mycoplasma Mycoides Mycoides (Contagious Bovine Pleuropneumonia Agent)
- Newcastle Disease Virus (Exotic)
- Nipah Virus
- Peste Des Petits Ruminants Virus
- Rinderpest Virus
- Sheep Pox Virus
- Swine Vesicular Disease Virus
- Vesicular Stomatitis Virus

Instructions for completing this form:

Direct any questions you may have concerning the completion of this form to the OEHS at 2352.

1. All recipients of this form must complete boxes #1-6.
2. Review the agents listed in box #7 and check each agent or toxin used or possessed by your laboratory. For each agent checked, check the appropriate descriptive category or categories, if known. Definitions are listed below.
3. Provide the information in boxes #8-12. Check all boxes that apply.
4. If your laboratory does not possess any agents on this list, provide only the information requested in boxes #13-15.
5. Do not report quantities of agents or toxins.

Definitions of Categories:

Viable: Capable of replication on its own, in cell culture, or in an appropriate host.

Recombinant organism, Nucleic acid, or Genetic elements from agent include any of the following:

- Nonviable agents
- Full-length nucleic acid from any of the viruses on the list. For Variola major virus (Smallpox), any segment that exceeds 100 nucleotides in length.
- Natural or synthetic nucleic acids from bacteria, fungi, or viruses on the list that encode for either a functional toxin or virulence factor sufficient to cause disease, or natural or synthetic nucleic acid that encodes for a functional toxin of any of the toxins listed, if: (1) expressed in vivo; (2) in an expression vector or host chromosome; or (3) in a carrier plasmid.

Altered USDA or FDA approved vaccine strains: Vaccine strains that have been modified from their original licensed, approved or registered forms.

Further explanation and the relevant regulations for this requirement can be found on the OEH&S web site: http://www.uah.edu/admin/oehs.

Notification of Possession of Select Agents or High Consequence Livestock Pathogens and Toxins

1. Principal Investigator:	
2. Department:	
3. Laboratory Room Number(s):	
4. Building:	
5. Phone: FAX:	6. E-mail:

7. Check "X" for each agent or Toxin Used or Possessed by Your Lab:	Viable	Recombinant Organism, Nucleic Acid or Genetic Element from Agent	Altered USDA or FDA Approved Vaccine Strains	Registered with HHS Select Agent Program
HHS Select Agents				
☐ CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS				
☐ EBOLA VIRUSES				
☐ LASSA FEVER VIRUS				
☐ MARBURG VIRUS				
☐ *RICKETTSIA PROWAZEKII*				
☐ *RICKETTSIA RICKETTSII*				
☐ SOUTH AMERICAN HAEMORRHAGIC FEVER VIRUSES				
☐ TICK-BORNE ENCEPHALITIS COMPLEX VIRUSES				
☐ VARIOLA MAJOR VIRUS (SMALLPOX VIRUS)				
☐ VIRUSES CAUSING HANTAVIRUS PULMONARY SYNDROME				
☐ YELLOW FEVER VIRUS				
☐ *YERSINIA PESTIS*				
☐ ABRIN				
☐ CONOTOXINS				
☐ DIACETOXYSCIRPENOL				
☐ RICIN				
☐ SAXITOXIN				
☐ TETRODOTOXIN				
USDA-HHS Overlap Agents				
☐ *BACILLUS ANTHRACIS*				
☐ *BRUCELLA ABORTUS*				
☐ *BRUCELLA MELITENSIS*				
☐ *BRUCELLA SUIS*				
☐ *BURKHOLDERIA (PSEUDOMONAS) MALLEI*				
☐ *BURKHOLDERIA (PSEUDOMONAS) PSEUDOMALLEI*				
☐ *CLOSTRIDIUM BOTULINUM*				
☐ *COCCIDIOIDES IMMITIS*				
☐ *COXIELLA BURNETII*				
☐ EASTERN EQUINE ENCEPHALITIS VIRUS				

❑ EQUINE MORBILLIVIRUS (HENDRA VIRUS)				
❑ *FRANCISELLA TULARENSIS*				
❑ RIFT VALLEY FEVER VIRUS				
❑ VENEZUELAN EQUINE ENCEPHALITIS VIRUS				
❑ AFLATOXINS	[redacted]			
❑ BOTULINUM TOXINS				
❑ *CLOSTRIDIUM PERFRINGENS* EPSILON TOXIN				
❑ SHIGATOXIN				
❑ STAPHYLOCOCCAL ENTEROTOXIN				
❑ T-2 TOXIN				
USDA High Consequence Livestock Pathogens and Toxins				
❑ AFRICAN HORSE SICKNESS VIRUS				
❑ AFRICAN SWINE FEVER VIRUS				
❑ AKABANE VIRUS				
❑ AVIAN INFLUENZA VIRUS (HIGHLY PATHOGENIC)				
❑ BLUE TONGUE VIRUS (EXOTIC)				
❑ BOVINE SPONGIFORM ENCEPHALOPATHY AGENT				
❑ CAMEL POX VIRUS				
❑ CLASSICAL SWINE FEVER VIRUS				
❑ COWDRIA RUMINANTIUM (HEARTWATER)				
❑ FOOT AND MOUTH DISEASE VIRUS				
❑ GOAT POX VIRUS				
❑ JAPANESE ENCEPHALITIS VIRUS				
❑ LUMPY SKIN DISEASE VIRUS				
❑ MALIGNANT CATARRHAL FEVER VIRUS				
❑ MENANGLE VIRUS				
❑ *MYCOPLASMA CAPRICOLUM/M.F 38/M.MYCOIDES CAPRI* (CONTAGIOUS CAPRINE PLEUROPNEUMONIA AGENT)				
❑ *MYCOPLASMA MYCOIDES MYCOIDES* (CONTAGIOUS BOVINE PLEUROPNEUMONIA AGENT)				
❑ NEWCASTLE DISEASE VIRUS (EXOTIC)				
❑ NIPAH VIRUS				
❑ PESTE DES PETITS RUMINANTS VIRUS				
❑ RINDERPEST VIRUS				
❑ SHEEP POX VIRUS				
❑ SWINE VESICULAR DISEASE VIRUS				
❑ VESICULAR STOMATITIS VIRUS				

8. Type of Work Performed by Laboratory:	☐ Diagnostic Work	☐ Vaccine Development	☐ Research	☐ Use in animals
	☐ Large Scale Production	☐ Teaching	☐ Storage Only (No current work)	☐ Other (Specify):

9. List all USDA Veterinary Permit Numbers for Importation and Transportation of Controlled Materials and Organisms and Vectors Numbers (if applicable):
I hereby certify that I am the designated Responsible Party or Principal Investigator for the laboratory listed above, and that the information supplied on this form is to the best of my knowledge accurate and truthful. I understand that a false statement on any part of this form could result in a fine up to $500,000 or imprisonment of up to five years, or both for each violation (18 USC1001; 18 USC 3559.3571)
10. Signature of Principal Investigator:

11. Print Name:	12. Date:

DECLARATION OF NON-POSSESSION: THIS LABORATORY DOES NOT POSSESS AN AGENT ON THIS LIST.
I hereby certify that I am the designated Responsible Party or Principal Investigator for the laboratory listed above, and that the information supplied on this form is to the best of my knowledge accurate and truthful. I understand that a false statement on any part of this form could result in a fine up to $500,000 or imprisonment of up to five years, or both for each violation (18 USC1001; 18 USC 3559.3571)
13. Signature of Principal Investigator:

14. Print Name:	15. Date:

Return this form to:
Environmental Health and Safety
Johnson Research Center 106

Appendix B

Standard Microbiological Practices – Checklists, Biosafety Levels 1-3

Laboratory Biosafety Levels (BL-1 through BL-3)

The following practices and procedures apply to projects and laboratories not utilizing recombinant DNA (rDNA) molecules. The biosafety level requirements for rDNA can be found in Appendix C.

Biosafety Level 1 (BL-1)

Biosafety level 1 practices, safety equipment, and facilities are appropriate for projects with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. This would apply to undergraduate and secondary educational training as well as teaching laboratories. BL-1 is appropriate for agents assigned to RG-1.

Biosafety Level 2 (BL-2)

Biosafety Level 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in experiments conducted on the open bench, provided the potential for producing splashes or aerosols is low. BL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. Please refer to the section on Bloodborne Pathogens for more information on the UAH occupational program. In general, BL-2 is appropriate for agents assigned to RG-2, unless specific procedures and tasks require a higher level of containment.

Biosafety Level 3 (BL-3)

Biosafety Level 3 practices, safety equipment, and facilties are appplicable to slinical, diagnostic, teaching, research, or production facilities in which work is done with the indigenous or exotic agents with a potential for respiratory transmission (e.g. mycobacterium tuberculosis), and which may cause serious and potential lethal infection. Primary hazards to personnel working with these agents relate to autoinnoculation, ingestion, and exposure to infectious aerosols.

All of the following practices and procedures are listed in the form of checklists allowing for self audits by laboratory personnel and annual safety inspections by the OEHS.

Biosafety Level 1 (BL-1)

Standard Microbiological Practices (BL-1)

Yes, No, N.A. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI) or laboratory supervisor (supervisor) when experiments are in progress.

Yes, No, N.A. Persons wash their hands after they handle viable materials and animals, after removing gloves and before exiting the laboratory.

Yes, No, N.A. Eating, drinking, smoking, handling contact lenses and applying cosmetics are not permitted in the work area (e.g., laboratory) See the UAH Laboratory Safety Manual. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

Yes, No, N.A. Mouth pipetting is prohibited; mechanical pipetting devices are used.

Yes, No, N.A. All procedures are performed carefully to minimize the creation of splashes or aerosols.

Yes, No, N.A. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, N.A. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the laboratory are packaged in accordance with applicable local, state and federal regulations, before removal from the facility.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 890-6482 for more information.

Safety Equipment (BL-1)

Yes, No, N.A. Special containment equipment is generally not required for manipulations of agents assigned to BL-1.

Yes, No, N.A. Laboratory coats, gowns, or uniforms are worn to prevent contamination or soiling of street clothes.

Yes, No, N. A- Gloves are available and are worn.

Yes, No, N.A. Protective eyewear is worn to protect from unanticipated splashes of microorganisms or other hazardous materials to the face.

Laboratory Facilities (BL-1)

Yes, No, N.A. Each laboratory contains a sink and hand soap for hand washing.

Yes, No, N.A. Safety eyewash and shower is available.

Yes, No, N.A. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

Yes, No, N.A. Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, N.A. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Biosafety Level 2 (BL-2)

Standard Microbiological Practices (BL-2)

Yes, No, N.A- Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI) or laboratory supervisor when experiments are in progress.

Yes, No, N.A. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

Yes, No, N.A. Eating, drinking, smoking, handling contact lenses, and applying cosmetics is not permitted in the work area (e.g., laboratory). See the UAH Laboratory Safety Manual. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

Yes, No, N.A. Mouth pipetting is prohibited; mechanical pipetting devices are used.

Yes, No, N. A- All procedures are performed carefully to minimize the creation of splashes or aerosols.

Yes, No, N.A. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, N.A. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 824-6482 for more information.

Special Practices (BL-2)

Yes, No, N.A. Access to the laboratory is limited or restricted by the laboratory supervisor or PI when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at higher risk of acquiring infections. The laboratory supervisor or PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Yes, No, N.A. The laboratory supervisor or PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory or animal rooms.

Yes, No, N.A. All areas operating at BL-2 have the universal biohazard symbol (Figure 1) attached to the main entrance door(s). When the infectious agent(s) in use in the laboratory require special provisions for entry (e.g., immunization), a hazard warning sign incorporating the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infectious agent lists the name and telephone number of the laboratory supervisor or other responsible person(s), and indicates the special requirements for entering the laboratory (Figure 2).

Yes, No, N.A. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., Hepatitis B vaccine, PPD test).

Yes, No, N.A. When appropriate for the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility. Contact the UAH OEHS for assistance at 6875 or 2171.

Yes, No, N.A. UAH Biosafety Manual is in use. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures outlined in the Biosafety Manual.

Yes, No, N.A. Laboratory personnel receive appropriate training through the laboratory supervisor or PI on the potential hazards associated with the work, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes

Yes, No, N.A. High degree of precaution is taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N. A- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal, rather, they must be carefully placed in conveniently located, approved, "Sharps" containers. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Yes, No, N.A. Syringes, which re-sheathe the needle, needle-less systems, and other safe devices, should be used when appropriate.

Yes, No, N.A. Contaminated broken glassware is *not* handled directly by hand, but is removed by mechanical means such as a brush and dustpan, tongs, or forceps and disposed of in "Sharps' containers. When sharps containers are 2/3 full, the OEHS is contacted for pick-up and disposal. For more information refer to the Biohazardous Waste section.

Yes, No, N.A. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. For more information refer to the Transportation of Biological Materials section.

Yes, No, N.A. Laboratory equipment and work surfaces are decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is completed, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated and accompanied by the UAH Equipment Release Form (Appendix M) before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Yes, No, N.A Spills and accidents, which result in overt exposures to infectious materials, are immediately reported to the laboratory supervisor or PI. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Yes, No, N.A Animals are not permitted in the lab unless they are part of the work being performed.

Safety Equipment (Primary Barriers, BL-2)

Yes, No, N.A. Properly maintained biological safety cabinets (BSC), preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

Yes, No, N.A. Procedures with a potential for creating infectious aerosols or splashes are conducted. 'These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

Yes, No, N.A. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

Yes, No, N.A. All BSCs are certified annually according to National Sanitation Foundation (NSF) Standard 49, by NSF certified personnel.

Yes, No, N.A. Face protection (goggles, mask, face shield or other splatter guards) is used for protection against splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside the BSC.

Yes, No, N.A. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). All protective clothing is either disposed of in the

laboratory or disinfected and laundered at UAH; it should never be taken home by personnel.

Yes, No, N.A. Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs, the hand will be protected after the contaminated glove is removed.

Yes, No, N.A. Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory. Disposable gloves are not washed or reused.

Laboratory Facilities (Secondary Barriers, BL-2)

Yes, No, N.A. Each laboratory contains a sink and disinfectant soap for hand washing.

Yes, No, N.A. The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.

Yes, No, N.A. Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, N.A. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, N.A. If the laboratory has windows that open, they are fitted with fly screens.

Yes, No, N.A. A method for decontamination of infectious or regulated laboratory wastes is available (e.g. autoclave, chemical disinfection, incinerator, or other approved decontamination system).

Yes, No, N.A. An eyewash and safety shower is readily available.

Biosafety Level 3 (BL-3)

Standard Microbiological Practices (BL-3)

Yes, No, N.A. Access to the laboratory or containment room is limited or restricted at the discretion of the Principal Investigator (PI), laboratory supervisor or facility manager when experiments are in progress.

Yes, No, N.A. Persons wash their hands after they handle infectious materials and animals, after removing gloves, and before leaving the laboratory.

Yes, No, N.A. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the laboratory or containment room (see the UAH Laboratory Safety Manual). Persons who wear contact lenses in these areas should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

Yes, No, N.A. Mouth pipetting is prohibited; mechanical pipetting devices are used.

Yes, No, N.A. All procedures are performed carefully to minimize the creation of splashes or aerosols.

Yes, No, N.A. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, N.A. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 890-6482 for more information.

Special Practices (BL-3)

Yes, No, N.A. Laboratory doors are kept closed when experiments are in progress.

Yes, No, N.A. The laboratory supervisor, PI or facility manager controls access to the laboratory and containment rooms, restricting access to persons whose presence is required for program or support purposes. For example, persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Persons who are at increased risk of acquiring infection or for whom infection may b unusually hazardous are not allowed in the laboratory or animal rooms. The laboratory supervisor/PI in cooperation with the facility manager has the responsibility for assessing each circumstance and determining who may enter or work in the laboratory or the containment room(s).

Yes, No, N.A. The laboratory supervisor/PI in cooperation with the facility manager or PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.

Yes, No, N.A. When infectious materials or infected animals are present in the laboratory or containment room, a biohazard warning sign, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory supervisor or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for immunizations, respirators, or other personal protective measures. All animal rooms require an OEHS approved Animal Hazard Control Form attached to the main access door(s).

Yes, No, N.A. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine, PPD test).

Yes, No, N.A. Baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens are collected periodically, depending on the agents handled or the function of the facility.

Yes, No, N.A. The UAH Biosafety manual is utilized. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures as outlined in the Biosafety manual.

Yes, No, N.A. Laboratory personnel receive appropriate training through the laboratory supervisor or PI on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

Yes, No, N.A. The laboratory supervisor or PI is responsible for insuring that before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory supervisor or other competent scientist proficient in safe microbiological practices and techniques.

Yes, No, N.A. A high degree of precaution is taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N.A. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles are not bent, sheared, broken, recapped, removed from

disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located, approved, "Sharps" containers. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Yes, No, N.A. Syringes, which re-sheathe the needle, needle-less systems, and other safe devices, are used when appropriate.

Yes, No, N.A. Contaminated broken glassware is not handled directly by hand, and is removed by mechanical means such as a brush and dustpan, tongs, or forceps and disposed of in "Sharps" containers. Sharps containers 2/3 full are of and picked-up by the OEHS. For more information refer to the Biohazardous Waste section.

Yes, No, N.A. All manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment room. No work in open vessels is conducted on the open bench.

Yes, No, N.A. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated and accompanied by the UAH Equipment Release Form (Appendix H) before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean up.

Yes, No, N.A. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. For more information refer to the Transportation of biological Materials section.

Yes, No, N.A. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories or animal rooms are decontaminated before disposal or reuse.

Yes, No, N.A. Spills of infectious materials are contained, decontaminated and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material.

Yes, No, N.A. Spills and accidents, which result in overt or potential exposures to infectious materials, are immediately reported to the laboratory supervisor or PI and the OEHS. Appropriate medical evaluation surveillance and treatment are provided and written records are maintained.

Yes, No, N.A. Animals not involved in the work being performed are not permitted in the lab.

Safety Equipment (Primary Barrier & BL-3)

Yes, No, N.A. Properly maintained biological safety cabinets (BSC's) are used (Class II or III) for all manipulations of infectious materials. NSF certified personnel must certify all BSCs annually according to NSF Standard 49.

Yes, No, N.A. Outside of a BSC, appropriate combinations of personal protective equipment are used (e.g., special protective clothing, masks, gloves, face protection, or respirators), in combination with physical containment devices (e.g., centrifuge safety cups, sealed centrifuge rotors, or containment caging for animals).

Yes, No, N.A. The equipment listed above is used for manipulations of cultures and on those clinical or environmental materials which may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs, and necropsy of infected animals.

Yes, No, N.A. Face protection (goggles and mask, or face shield) is worn for manipulations of infectious materials outside of a biological safety cabinet.

Yes, No, N.A. Respiratory protection is worn when aerosols cannot be safely contained (i.e., outside of a biological safety cabinet), and in rooms containing infected animals. If respiratory protection is required, all personnel involved need to be included in the UAH Respiratory Protection Program.

Yes, No, N.A. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn in, and not worn outside, the laboratory. Reusable laboratory clothing is decontaminated before being laundered.

Yes, No, N.A. Gloves are worn when handling infected animals and when hands may contact infectious materials and contaminated surfaces or equipment. Disposable gloves should be discarded when contaminated, and never washed for reuse.

Laboratory Facilities (Secondary Barriers, BL-3)

Yes, No, N.A. The laboratory is separated from areas, which are open to unrestricted traffic flow within the building. Passage through two sets of self-closing doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. A clothes change room (shower optional) may be included m the passageway.

Yes, No, N.A. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Yes, No, N.A. The interior surfaces of walls, floors, and ceilings are water-resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontamination.

Yes, No, N.A. Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate. heat.

Yes, No, N.A. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, N.A. Windows in the laboratory are closed and sealed.

Yes, No, N.A. A method for decontaminating all laboratory wastes is available, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method).

Yes, No, N.A. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air from "clean" areas into the laboratory toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building, and is discharged to the outside with filtration and other optional treatment. The outside exhaust must be dispersed away from occupied areas and air intakes. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper.

Yes, No, N.A. The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II or Class III biological safety cabinets is discharged directly to the outside or through the building exhaust system. If the HEPA-filtered exhaust air from Class II or III biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system. Exhaust air from Class II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months according to NSF Standard 49.

Yes, No, N.A. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory.

Yes, No, N.A. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent, which are routinely maintained and replaced as needed.

Yes, No, N.A. An eyewash facility and shower is readily available.

Animal Biosafety Levels (ABL 1-3)

The following three combinations of practices, safety equipment and facilities are used for experiments on animals infected with agents, which produce, or may produce, human infection. They provide increasing levels of protection to personnel and to the environment and are recommended as minimal standards for activities involving infected laboratory animals. These practices and procedures apply to animal projects not involving recombinant DNA molecules. The biosafety level requirements for rDNA animal research are listed in Appendix C.

These three combinations, designated Animal Biosafety Levels (ABL) 1 to 3, describe animal facilities and practices applicable to work on animals infected with agents assigned to the corresponding Risk Groups 1-3. Work with Risk Group 4 agents or projects requiring ABL 4 are not permitted at UAH.

All of the following practices and procedures are listed in form of a checklist allowing for self-audits by laboratory personnel and safety inspection through the OEHS.

Animal Biosafety Level 1

Standard Practices (ABL-1)

Yes, No, N.A. Access to the animal facility is limited or restricted at the discretion of the laboratory or animal facility director/PI.

Yes, No, N.A. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

Yes, No, N.A. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.

Yes, No, N.A. All procedures are carefully performed to minimize the creation of aerosols. Work surfaces are decontaminated after use or after any spill of viable materials.

Yes, No, N.A. Doors to animal rooms open inward, are self-closing and are kept closed when experimental animals are present.

Yes, No, N.A. All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leak-proof, covered containers.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 824-6482 for more information.

Special Practices (ABL-1)

Yes, No, N.A. The laboratory or animal facility director in coordination with the PI limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In

general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.

Yes, No, N.A. The laboratory or animal facility director in coordination with the PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization) may enter the animal room.

Yes, No, N.A. Bedding materials form animal cages are removed in such a manner as to minimize the creation of aerosols, and are disposed of in compliance with applicable institutional or local requirements.

Yes, No, N.A. Cages are washed manually or in the cage washer. Temperature of final rinse water in a mechanical washer should be 180°F.

Yes, No, N.A. Laboratory coats, gowns, or uniforms are worn in the animal facility. It is recommended that laboratory coats worn in the facility not be worn in other areas.

Yes, No, N.A. The UAH Biosafety manual is utilized. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures outlined in the Biosafety manual.

Safety Equipment (Primary Barrier & ABL 2)

Special containment equipment is not required for animals infected with agents assigned to Biosafety level 1.

Animal Facilities (Secondary Barriers, ABL-1)

Yes, No, N.A. The animal facility is designed and constructed to facilitate cleaning and housekeeping.

Yes, No, N.A. A hand-washing sink is available in the room where infected animals are housed.

Yes, No, N.A. If the animal facility has windows that open, they are fitted with fly screens.

Yes, No, N.A. Exhaust air is discharged to the outside without being recirculated to other rooms, and it is recommended, but not required, that the direction of airflow in the animal facility is inward.

Yes, No, N.A. An autoclave for use in decontaminating infectious laboratory waste is available in the building with the animal facility.

Animal Biosafety Level 2

Standard Practices (ABL-2)

Yes, No, N.A. Access to the animal facility is limited or restricted at the discretion of the laboratory or animal facility director/PI.

Yes, No, N.A. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

Yes, No, N.A. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use is not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.

Yes, No, N.A. All procedures are carefully performed to minimize the creation of aerosols. Work surfaces are decontaminated after use or after any spill of viable materials.

Yes, No, N.A. Doors to animal rooms open inward, are self-closing and are kept closed when experimental animals are present.

Yes, No, N.A. All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 890-6482 for more information.

Special Practices (ABL-2)

Yes, No, N.A. The animal facility manager in coordination with the PI limits access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room.

Yes, No, N.A. The animal facility manager in coordination with the PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization) may enter the animal room.

Yes, No, N. A- All areas operating at ABL-2 have the universal biohazard symbol attached to the main entrance door(s). When the infectious agent(s) in use in the animal room requires special entry provisions (e.g., the need for immunizations and respirators) an OEHS approved, Animal Hazard Control Form (Appendix G) is posted on the access door to the animal room. This form identifies the infectious agent(s) in use, lists the name and telephone number of the project supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room as well as any other necessary procedures and practices.

Yes, No, N.A. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

Yes, No, N.A. When appropriate, depending upon the agents handled, baseline serum samples from animal care and other at-risk personnel may be collected and stored. Additional serum samples may be collected periodically depending on the agents handled or the function of the facility. The decision to establish a serologic surveillance program must take into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program should provide for the testing of serum samples at each collection interval and the communication of results to the participants.

Yes, No, N.A. The UAH Biosafety manual is utilized. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures outlined in the Biosafety manual.

Yes, No, N.A. Laboratory personnel receive appropriate training through the PI on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

Yes, No, N.A. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N.A. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located approved,

"Sharps" containers. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Yes, No, N.A. Syringes, which re-sheathe the needle, needle-less systems, and other safe devices, are used when appropriate.

Yes, No, N.A. Broken glassware is not handled directly by hand, but is removed by mechanical means such as a brush and dustpan, tongs, or forceps and disposed of in Sharps containers. The OEHS is contacted for pick-up and disposal of sharps containers when they are 2/3 full. For more information refer to the Biohazardous Waste section.

Yes, No, N.A. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping. For more information refer to the Transportation of Biological Materials section.

Yes, No, N.A. Cages are appropriately decontaminated, preferably by autoclaving, before they are cleaned and washed. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Yes, No, N.A. Spills and accidents, which result in overt exposures to infectious materials, are immediately reported to the laboratory supervisor or PI. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Yes, No, N.A. Animals not involved in the work being performed are not permitted in the lab.

Safety Equipment (Primary Barrier ABL-2)

Yes, No, N.A. Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) are used whenever procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, in inoculation of animals, and manipulations of high concentrations or large volumes of infectious materials.

Yes, No, N.A. Appropriate face/eye and all personnel entering animal rooms wear respiratory protection housing nonhuman primates.

Yes, No, N.A. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.

Yes, No, N.A. Special care is taken to avoid skin contamination with infectious materials. Gloves are worn when handling infected specimens and when skin contact with infectious materials is unavoidable.

Animal Facilities (Secondary Barriers, ABL-2)

Yes, No, N.A. The animal facility is designed and constructed to facilitate cleaning and housekeeping.

Yes, No, N.A. A hand-washing sink is available in the room where infected animals are housed.

Yes, No, N.A. If the animal facility has windows that open, they are fined with fly screens.

Yes, No, N.A. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.

Yes, No, N.A. Exhaust air is discharged to the outside without being recirculated to other rooms, and it is recommended, but not required, that the direction of airflow in the animal facility is inward.

Yes, No, N.A. An autoclave that can be used for decontaminating infectious laboratory waste is available in the building with the animal facility.

Animal Biosafety Level 3

Standard Practices (ABL-3)

Yes, No, N.A. Access to the animal facility is limited or restricted at the discretion of the laboratory or animal facility manager or director/PI.

Yes, No, N.A. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

Yes, No, N.A. Eating & drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in animal rooms. Persons who wear contact lenses in animal rooms should also wear goggles or a face shield.

Yes, No, N.A. All procedures are carefully performed to minimize the creation of aerosols.

Yes, No, N.A. Work surfaces are decontaminated after use or after any spill of viable materials.

Yes, No, N.A. Doors to animal rooms open inward, are self-closing and are kept closed when experimental animals are present.

Yes, No, N.A. All wastes from the animal room are appropriately decontaminated, preferably by autoclaving, before disposal. Infected animal carcasses are incinerated after being transported from the animal room in leak-proof, covered containers.

Yes, No, N.A. An insect and rodent control program is in effect. Contact Facilities and Operations at 824-6482 for more information.

Special Practices (ABL-3)

Yes, No, N.A. The facility manger in coordination with the PI or other responsible person restricts access to the animal room to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. Persons who are at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal room. Persons at increased risk may include children, pregnant women, and persons who are immunodeficient or immunosuppressed. The facility manager has the final responsibility for assessing each circumstance and determining who may enter or work in the facility.

Yes, No, N.A. The facility manger in coordination with the PI establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization) may enter the animal room.

Yes, No, N.A. All areas operating at ABL-3 have the universal biohazard symbol (Figure 1) attached to the main entrance door(s) and when the infectious agent(s) in use requires special entry provisions (e.g., the need for immunizations and respirators) an OEHS approved Animal Hazard Control Form (Appendix G) is

posted on the access door to the animal room. This form identifies the infectious agent(s) in use, lists the name and telephone number of the project supervisor or other responsible person(s), and indicates the special requirements for entering the animal room as well as any other necessary procedures and practices.

Yes, No, N.A. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

Yes, No, N.A. Baseline serum samples from all personnel working in the facility and other at-risk personnel maybe collected and stored. Additional serum samples may be collected periodically and stored. If initiated, the serum surveillance program must take into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program should provide for the testing of serum samples at each collection interval and the communication of results to the participants.

Yes, No, N.A. The UAH Biosafety manual is in use. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures outlined in the Biosafety manual.

Yes, No, N.A. Laboratory personnel receive appropriate training through the PI and the facility manager on the potential hazards associated with the work involved the necessary precautions to prevent exposures, exposure evaluation procedures and emergency procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

Yes, No, N.A. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubas, and scalpels. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N.A. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located approved, "Sharps" containers. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

Yes, No, N.A. Syringes, which re-sheathe the needle, needle-less systems, and other safe devices, should be used when appropriate.

Yes, No, N.A. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps and disposed of in Sharps containers. Sharps containers 2/3 full are picked-up by the OEHS for further processing/disposal. For more information refer to the Biohazardous Waste section.

Yes, No, N.A. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport or shipping. For more information refer to the Transportation of Biological Materials section.

Yes, No, N.A. Cages are appropriately decontaminated, preferably by autoclaving, before they are cleaned and washed. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated and accompanied by a UAH Equipment Release Form (Appendix H) before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Yes, No, N.A. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

Yes, No, N.A. Baseline serum samples from all personnel working in the facility and other at-risk personnel maybe collected and stored. Additional serum samples may be collected periodically and stored. If initiated, the serum surveillance program must take into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program should provide for the testing of serum samples at each collection interval and the communication of results to the participants.

Yes, No, N.A. The UAH Biosafety manual is utilized. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures outlined in the Biosafety manual.

Yes, No, N.A. Laboratory personnel receive appropriate training through the PI and the facility manager on the potential hazards associated with the work, the necessary precautions to prevent exposures, exposure evaluation procedures and emergency procedures. Personnel receive annual updates, or additional training as necessary for procedural or policy changes.

Yes, No, N.A. A high degree of precaution is taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

Yes, No, N.A. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located approved, "Sharps" containers. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination preferably by autoclaving.

Yes, No, N.A. Syringes, which re-sheathe the needle, needle-less systems, and other safe devices, should be used when appropriate.

Yes, No, N.A. Broken glassware is not handled directly by hand, but is removed by mechanical means such as a brush and dustpan, tongs, or forceps and disposed of in Sharps containers. Sharps containers 2/3 full are picked-up by the OEHS for further processing/disposal. For more information refer to the Biobazardous Waste section.

Yes, No, N.A. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling processing, storage, transport or shipping. For more information refer to the Transportation of Biological Materials section.

Yes, No, N.A. Cages are appropriately decontaminated, preferably by autoclaving, before they are cleaned and washed. Equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, after work with infectious materials is completed, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated and accompanied by a UAH Equipment Release Form (Appendix H) before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

Yes, No, N.A. Spills and accidents, which result in overt exposures to infectious materials, are immediately reported to the laboratory supervisor, facility manger (if applicable) and the OEHS. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Yes, No, N.A. All wastes from the animal room are autoclaved before disposal. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leak-proof covered containers.

Yes, No, N.A. Animals not involved in the work being performed are not permitted in the lab.

Yes, No, N.A. Personal protective equipment is used for all activities involving manipulations of infectious materials or infected animals.

Yes, No, N.A. Personnel entering the animal room wear wrap-around or solid-front gowns or uniforms. Front-button laboratory coats are unsuitable. Protective gowns should be appropriately contained until decontamination or disposal has been performed.

Yes, No, N.A. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.

Yes, No, N.A. Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms housing non-human primates.

Yes, No, N.A. Boots, shoe covers, or other protective footwear, and disinfectant foot-baths are available and used when necessary.

Yes, No, N.A. Physical containment devices and equipment appropriate for the animal species are used for all procedures and manipulations of infectious materials or infected animals.

Yes, No, N.A. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar flow cabinets), solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems.

Animal Facilities (Secondary Barriers, ABL-3)

Yes, No, N. A- The animal facility is designed and constructed to facilitate cleaning and housekeeping, and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or other activities may also be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the animal room.

Yes, No, N.A. The interior surfaces of walls, floors, and ceilings are water-resistant so that they may be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.

Yes, No, N.A. A foot, elbow, or automatically operated hand-washing sink is provided in each animal room near the exit door.

Yes, No, N.A. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and a HEPA filter.

Yes, No, N.A. If floor drains are provided, they are protected with liquid traps that are always filled with water or disinfectant.

Yes, No, N.A. Windows in the animal room are non-operating and sealed.

Yes, No, N.A. Animal room doors are self-closing and are kept closed when infected animals are present.

Yes, No, N.A. An autoclave for decontaminating wastes is available, preferably within the animal facility. Materials are transferred to the autoclave in a covered leak-proof container whose outer surface has been decontaminated.

Yes, No, N.A. A non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to provide for directional flow of air into the animal room. The exhaust air is discharged directly to the outside and clear of occupied areas and air intakes. Exhaust air from the room can be discharged without filtration or other treatment. Personnel must periodically validate that proper directional airflow is maintained.

Yes, No, N.A. The HEPA filtered exhaust air from Class I or Class 11 biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the device is tested and certified at least every 12 months. If the HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the performance of either the cabinet or building exhaust system.

Appendix C

Recombinant DNA Standard Microbiological Practices – Checklists, Biosafety Levels 1-4

Recombinant DNA – Laboratory Biosafety Levels

This section specifies physical containment for standard laboratory procedures involving recombinant DNA as outlined by the NIH guidelines, and defines RDNA Biosafety Levels one through three. These biosafety level requirements are slightly different from those in the previous chapter, which are based on the BMBL. All laboratory experiments involving recombinant DNA must adhere to the NIH guidelines and the biosafety level requirements listed therein.

Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices. Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA receive adequate instruction (see, Responsibilities of Principle Investigator). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards has an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principle Investigator insures that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. The department in which the research is being conducted must provide serological monitoring, when appropriate.

The following practices and procedures are provided in the form of a checklist to allow for self-audits and determination of appropriate safety precautions, facilities and procedures for each biosafety levels. The Office of Environmental Health and Safety utilizes this list when conducting annual safety audits.

IMPORTANT:

In order to stay in *compliance with the NIH Guidelines on recombinant DNA, always refer to the most current edition for up dates and changes in procedures and requirements. Contact the OEHS at 2352 for more information.*

RDNA - Biosafety Level 1 (BL-1)

Standard Microbiological Practices (BL-1)

Yes, No, NA	Access to the laboratory is limited or restricted at the discretion of the Principal Investigator (PI) when experiments are in progress.
Yes, No, NA	Work surfaces are decontaminated once a day and after any spill of viable material.
Yes, No, NA	All contaminated liquid or solid wastes are decontaminated before disposal.
Yes, No, NA	Mechanical pipetting devices are used; mouth pipetting is prohibited.

Yes, No, NA Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. See the UAH Lab Safety Manual for mandatory lab safety guidelines. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Yes, No, NA Persons wash their hands: (I) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (II) before exiting the laboratory.

Yes, No, NA All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, NA In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) are provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Special Practices (BL-1)

Yes, No, NA Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container, which is closed before being removed from the laboratory.

Yes, No, NA An insect and rodent control program is in effect. Contact Facilities and Operations at 824-6482 for more information.

Containment Equipment (BL-1)

Yes, No, NA Special containment equipment is generally not required for manipulations of agents assigned to BL-1.

Laboratory Facilities (BL-1)

Yes, No, NA The laboratory is designed so that it can be easily cleaned.

Yes, No, NA Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, NA Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, NA Each laboratory contains a sink for hand washing.

Yes, No, NA If the laboratory has windows that open they are fitted with fly screens.

RDNA - Biosafety Level 2 (BL-2)
Standard Microbiological Practices (BL-2)

Yes, No, NA Access to the laboratory is limited or restricted by the Principal Investigator (PI) when work with organisms containing recombinant DNA molecules is in progress.

Yes, No, NA Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, NA All contaminated liquid or solid wastes are decontaminated before disposal.

Yes, No, NA Mechanical pipetting devices are used; mouth pipetting is prohibited.

Yes, No, NA Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. See the UAH Laboratory Safety Manual for mandatory laboratory safety guidelines. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Yes, No, NA Persons wash their hands: (I) after handling materials involving organisms containing recombinant DNA molecules and animals, and (II) when exiting the laboratory.

Yes, No, NA All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, NA Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Special Practices (BL-2)

Yes, No, NA Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container, which is closed before being removed from the laboratory.

Yes, No, NA The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Yes, No, NA The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Yes, No, NA All areas operating at BL-2 need to have the universal biohazard symbol (Figure 1) attached to the main entrance door(s). When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Yes, No, NA An insect and rodent control program is in effect. Contact Facilities and Operations at 824-6482 for more information.

Yes, No, NA Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, and administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Yes, No, NA Animals not involved in the work being performed are not permitted in the laboratory.

Yes, No, NA Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Yes, No, NA All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Yes, No, NA Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in an approved sharps container and transferred to the OEHS for appropriate disposal.

Yes, No, NA Spills and accidents, which result in overt exposures to organisms containing recombinant DNA molecules, are immediately reported to the OEHS, Institutional Biosafety Committee and NIWORDA. Reports to NIWORDA is sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Yes, No, NA When appropriate, depending upon the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Yes, No, NA The UAH Biosafety Manual is utilized. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures as outlined in the Biosafety Manual.

Containment Equipment (BL-2)

Yes, No, NA Biological safety cabinets (Class I or II) or other appropriate personal protective or physical containment devices are used whenever:

Yes, No, NA Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Yes, No, NA High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Laboratory Facilities (BL-2)

Yes, No, NA The laboratory is designed so that it can be easily cleaned.

Yes, No, NA Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate heat. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, NA Each laboratory contains a sink for hand washing.

Yes, No, NA If the laboratory has windows that open, they are fitted with fly screens.
Yes, No, NA An autoclave for decontaminating laboratory wastes is available.

RDNA - Biosafety Level 3 (BL-3)

Standard Microbiological Practices (BL-3)

Yes, No, NA Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, NA All contaminated liquid or solid wastes are decontaminated before disposal.

Yes, No, NA Mechanical pipetting devices are used; mouth pipetting is prohibited.

Yes, No, NA Eating, drinking, smoking, storing food, and applying cosmetics is not permitted in the work area. See the UAH Laboratory Safety Manual for mandatory safety guidelines.

Yes, No, NA Persons wash their hands: (I) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (II) when exiting the laboratory.

Yes, No, NA All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, NA Persons under 16 years of age are not admitted to the laboratory.

Yes, No, NA If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with

experiments requiring BL-3 level physical containment, they are conducted in accordance with all BL-3 level laboratory practices.

Special Practices (BL-3)

Yes, No, NA Laboratory doors are kept closed when experiments are in progress.

Yes, No, NA Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container, which is tightly closed and sealed before being removed from the laboratory.

Yes, No, NA The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Yes, No, NA The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures may enter the laboratory or animal rooms.

Yes, No, NA All areas operating at BL-3 need to have the universal biohazard symbol (Figure 1) attached to the main entrance door(s). When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Yes, No, NA All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Yes, No, NA The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean up.

Yes, No, NA An insect and rodent program is in effect. Contact Facilities and Operations at 824-6842 for more information.

Yes, No, NA Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Yes, No, NA Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when hand contact with infectious materials is unavoidable.

Yes, No, NA Molded surgical masks or respirators are worn in rooms containing experimental animals.

Yes, No, NA Animals and plants not related to the work being conducted are not permitted in the laboratory.

Yes, No, NA Laboratory animals held in a BL-3 area are housed in partial-containment caging systems, such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices must include minimally, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel must shower on exit from areas where these devices are required

Yes, No, NA All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Yes, No, NA Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Yes, No, NA Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in an approved sharps container and transferred to the OEHS for disposal.

Yes, No, NA Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the OEHS, Institutional Biosafety Committee, and NIH/ORDA. Reports to NIWORDA must be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Yes, No, NA Baseline serum samples for all laboratories and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Yes, No, NA The UAH Biosafety Manual is utilized. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures as outlined in the Biosafety Manual.

Alternative Selection of Containment Equipment (BL-3)
Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL-3 laboratory using containment equipment specified for the BL-2 level of physical containment.

Containment Equipment (BL-3)

Yes, No, NA Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Laboratory Facilities (BL-3)

Yes, No, NA The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-door clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Yes, No, NA The interior surfaces of walls, floors, and ceilings are water-resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

Yes, No, NA Bench tops are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, NA Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Yes, No, NA Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Yes, No, NA Windows in the laboratory are closed and sealed.

Yes, No, NA Access doors to the laboratory or containment module are self-closing.

Yes, No, NA An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Yes, No, NA A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Yes, No, NA The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or budding exhaust system.

RDNA - Animal Biosafety Levels

This section specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. This appendix applies to animal research activities with the following modifications:

This section supersedes the requirements on RDNA Laboratory Biosafety Levels when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. The animals covered in this section include but are not limited to cattle, swine, sheep, goats, horses, and poultry.

General Considerations

The containment levels required for research involving recombinant DNA associated with or in animals are based on classification of experiments in Section III of the NIH Guidelines on recombinant DNA. For the purpose of animal research, four levels of containment are established. These are referred to as BL1 -Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following section.

When an animal covered by this section contains recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass is disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency. In addition, a permanent record of the experimental use and disposal of each animal or group of animals is maintained.

IMPORTANT:

In order to stay in compliance with the NIH Guidelines on recombinant DNA, always refer to the most current edition for updates and changes in procedures and requirements.

Biosafety Level 1 - Animal (BL1-N)

Standard Practices (BL1-N)

Animal Facility Access (BL1-N)

Yes, No, NA The containment area is locked.

Yes, No, NA Access to the containment area is limited or restricted when experimental animals are being held.

Yes, No, NA The containment area is patrolled or monitored at frequent intervals.

Other (BL1-N)

Yes, No, NA All genetically engineered neonates are permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Yes, No, NA A double barrier is provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Yes, No, NA The containment area is in accordance with state and Federal laws and animal care requirements.

Animal Facilities (BL1-N)

Yes, No, NA Animals are confined to securely fenced areas or are in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Biosafety Level 2 - Animal (BL2-N)

Standard Practices (BL2-N)

Animal Facility Access (BL2-N)

Yes, No, NA The containment area is locked.

Yes, No, NA The containment area is patrolled or monitored at frequent intervals.

Yes, No, NA The containment building is controlled and has a locking access.

Yes, No, NA The Animal Facility Director or PI has established policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Yes, No, NA Animals of the same or different species that are not involved in the work being performed are not permitted in the animal area.

Decontamination and Inactivation (BL2-N)

Yes, No, NA Contaminated materials that are decontaminated at a site away from the laboratory are placed in a closed durable leak-proof container prior to removal from the laboratory.

Yes, No, NA Needles and syringes are promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Signs (BL2-N)

Yes, No, NA All areas operating at BL2-N need to have the universal biohazard symbol (Figure 1) attached to the main entrance door(s). When the infectious agent(s) in use in the animal room requires special entry provisions (e.g., the need for immunizations and respirators) an OEHS/IBC approved Animal Hazard Control Form (Appendix G) is posted on the access door to the animal room. This form indicates: (I) the agent (II) the animal species, (III) the name and telephone number of the Animal Facility Director or other responsible individual, and (IV) any special requirements for entering the laboratory, as well as any other necessary procedures and practices.

Protective Clothing (BL2-N)

Yes, No, NA Laboratory coats, gowns, smocks, or uniforms are worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, and administrative offices), protective clothing is removed and kept in the work entrance area.

Yes, No, NA Special care is taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves are worn when handling experimental animals and when hand contact with an infectious agent is unavoidable.

Records (BL2-N)

Yes, No, NA Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules is reported immediately to the OEHS, Institutional Biosafety Committee, NEH/ORDA, and other appropriate authorities when applicable.

Reports to the NEWORDA must be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 208927010, (301) 496-9838. Medical evaluation, surveillance, and treatment must be provided as appropriate and written records maintained. If necessary, the area is appropriately decontaminated.

Yes, No, NA When appropriate and giving consideration to the agent handled, baseline serum samples are collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Transfer of Materials (BL2-N)

Yes, No, NA Biological materials removed from the animal containment area in a viable or intact state are transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container labeled with the universal biohazard symbol and accompanied with the appropriate information (e.g., container content emergency contact). All containers, primary and secondary, are disinfected before removal from the animal facility. Advance approval for transfer of material is obtained from the OEHS. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction. For more information refer to the Transportation of Biological Materials section.

Other (BL2-N)

Yes, No, NA All genetically engineered neonates are permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Yes, No, NA Needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution is used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles must not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes are promptly placed in an approved sharps container and transferred to the OEHS for disposal.

Yes, No, NA Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Yes, No, NA Eating, drinking, smoking, and applying cosmetics are not permitted in the work area.

Yes, No, NA Individuals who handle materials and animals containing recombinant DNA molecules are required to wash their hands before exiting the containment area.

Yes, No, NA A double barrier is provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Yes, No, NA The containment area is in accordance with state and Federal laws and animal care requirements.

Yes, No, NA The UAH Biosafety Manual is utilized. Personnel are advised of special hazards and required to read and follow instructions on practices and procedures as outlined in the Biosafety Manual.

Animal Facilities (BL2-N)

Yes, No, N.A- Animals are contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Yes, No, NA Surfaces are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, NA The animal containment area is designed for easy cleaning.

Yes, No, NA Windows that open are fitted with fly screens.

Yes, No, NA An autoclave is available for decontamination of laboratory wastes.

Yes, No, NA If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas are appropriately screened (52 mesh). All perimeter joints and openings are sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Biosafety Level 3 - Animals (BL3-N)

Standard Practices (BL3-N)

Animal Facility Access (BL3-N)

Yes, No, NA The containment area is locked.

Yes, No, NA The containment area is patrolled or monitored at frequent intervals.

Yes, No, NA The containment building is controlled and has a locking access.

Yes, No, NA The Animal Facility Director or PI has established policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) enter the laboratory or animal rooms.

Yes, No, NA Animal room doors, gates, or other closures are kept closed when experiments are in progress.

Decontamination and Inactivation (BL3-N)

Yes, No, NA The work surfaces of containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is completed. Where feasible, plastic-backed paper toweling is used on nonporous work surfaces to facilitate clean up.

Yes, No, NA All animals are euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Yes, No, NA Needles and syringes are promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Yes, No, NA Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval is required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Yes, No, NA Liquid effluent from containment equipment sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers are decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes is monitored with a recording thermometer. The effectiveness of the heat decontamination process system is revalidated every 30 days with an indicator organism.

Signs (BL3-N)

Yes, No, NA All areas operating at BL3-N need to have the universal biohazard symbol (Figure 1) attached to the main entrance door(s). When the infectious agent(s) in use in the animal room requires special entry provisions (e.g., the need for immunizations and respirators) an OEHS/IBC approved Animal Hazard Control Form (Appendix G) is posted on the access door to the animal room. This form

indicates: (I) the agent, (II) the animal species, (III) the name and telephone number of the Animal Facility Director or other responsible individual, and (IV) any special requirements for entering the laboratory, as well as any other necessary procedures and practices.

Protective Clothing (BL3-N)

Yes, No, NA Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) is worn in the animal area. Clothing is not worn outside the animal containment area and is decontaminated before laundering or disposal. Personnel shower before exiting the BL3-N area and donning personal clothing. Special care is taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves are worn when handling experimental subjects and when skin contact with an infectious agent is unavoidable.

Yes, No, NA Appropriate respiratory protection is worn in rooms containing experimental animals.

Records (B3-N)

Yes, No, NA Documents regarding experimental animal use and disposal is maintained in a permanent record book.

Yes, No, NA Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA is reported immediately to the OEHS, Institutional Biosafety Committee, NIWORDA, and other appropriate authorities (if applicable). Reports to the NIWORDA must be sent to the Office of Recombinant DNA Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010, (301) 496-9838. Medical evaluation, surveillance, and treatment must be provided departmentally as appropriate and written records maintained. If necessary, the area must be appropriately decontaminated.

Yes, No, NA When appropriate and giving consideration to the agent handled, baseline serum samples are collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Transfer of Materials (BL3-N)

Yes, No, NA Biological materials removed from the animal containment area in a viable or intact state are transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container labeled with the universal biohazard symbol and accompanied with the appropriate information (e.g., container content, emergency contact). All containers, primary and secondary, are disinfected before removal from the animal facility. Advance approval for transfer of material is obtained from the OEHS. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated

or incapable of reproduction. For more information refer to the Transportation of Biological Materials section.

Yes, No, NA Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval is required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Other (BL3-N)

Yes, No, NA All genetically engineered neonates are permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemical assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Yes, No, NA Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Yes, No, NA Eating, drinking, smoking, and applying cosmetics are not permitted in the work area.

Yes, No, NA Individuals who handle materials and animals containing recombinant DNA molecules are required to wash their hands before exiting the contaminated area.

Yes, No, NA Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3 -N practices.

Yes, No, NA Animal holding areas are cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Yes, No, NA All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, NA A double barrier is provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Yes, No, NA The containment area is in accordance with state and Federal laws and animal care requirements.

Yes, No, NA All animals are euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Yes, No, NA Personnel are required to shower before exiting the BL3-N area and wearing personal clothing.

Yes, No, NA Animals of the same or different species that are not involved in the work being performed are not permitted in the animal area.

Yes, No, N.A- Needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution is used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles must not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes are promptly placed in an approved sharps container and transferred to the OEHS for disposal.

Yes, No, NA The UAH Biosafety Manual is utilized. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures as outlined in the Biosafety Manual.

Animal Facilities (BL3-N)

Yes, No, NA Animals are contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Yes, No, NA The interior walls, floors, and ceilings are impervious to water and resistant to acids, bases, organic solvents, and moderate heat to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) are sealed.

Yes, No, NA Windows in the animal facility remain closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Yes, No, NA An autoclave, incinerator, or other effective means to decontaminate animals and waste are available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Yes, No, NA If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod; the interior work area is screened with at least 52 mesh. All perimeter joints and openings are sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Yes, No, NA Access doors to the containment area are self-closing.

Yes, No, NA The animal area is separated from all other areas. Passage through two sets of doors is the basic requirement for entry into the animal area from access corridors or other contiguous areas. A double-door clothes change room equipped with integral showers and airlock physically separates the animal containment area from access corridors and other laboratories or areas.

Yes, No, NA Liquid effluent from containment equipment sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers is decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes is monitored with a recording thermometer. The effectiveness of the heat decontamination process system is revalidated minimally every 30 days with an indicator organism

Yes, No, NA An exhaust air ventilation system is provided. This system must create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and is dispersed away from occupied areas and air intakes. Personnel verify that the direction of the airflow (into the animal room) is proper.

Yes, No, NA If the agent is transmitted by aerosol, then the exhaust air must pass through a high efficiency particulate air (HEPA) filter.

Yes, No, NA Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Yes, No, NA In lieu of open housing in the special animal room, animals held in a BL3-N area are housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Yes, No, NA Each animal area contains a foot, elbow, or automatically operated sink for hand washing. The sink is located near the exit door.

Yes, No, NA Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

RDNA - Plant Biosafety Levels

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

The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose). In addition, the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Definitions:

The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

IMPORTANT:

In order to stay in compliance with the NIH Guidelines on recombinant DNA, always refer to the most current edition for updates and changes in procedures and requirements.

RDNA - Biosafety Level 1 - Plants (BL1-P)

Standard Practices (BL1-P)

Greenhouse Access (BLI-P)

Yes, No, NA — Access to the greenhouse is limited or restricted, at the discretion of the Greenhouse Director/PI, when experiments are in progress. Prior to entering the greenhouse, personnel are required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures are performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Records (BL1-P)

Yes, No, NA — A record is kept of experiments currently in progress in the greenhouse facility.

Decontamination and Inactivation (BL1-P)

Yes, No, NA Experimental organisms are rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Control of Undesired Species and Motile Macroorganisms (BL1-P)

Yes, No, NA A program is implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and federal laws.

Yes, No, NA Arthropods and other motile macroorganisms are housed in appropriate cages. If microorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions are taken to prevent escape from the greenhouse facility.

Concurrent Experiments Conducted in the Greenhouse (BL1-P)

Yes, No, NA Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment provided that all work is conducted in accordance with BL 1-P greenhouse practices.

Facilities (BL1-P)

Greenhouse Design (BL1-P)

Yes, No, NA The greenhouse floor is composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Yes, No, NA Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

RDNA - Biosafety Level 2 - Plants (BL2-P)

Standard Practices (BL2-P)

Greenhouse Access (BL2-P)

Yes, No, NA Access to the greenhouse is limited or restricted, at the discretion of the Greenhouse Director/PI, to individuals directly involved with the experiments when they are in progress.

Yes, No, NA Personnel are required to read and follow instructions on BL2-P practices and procedures. All procedures are conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Records (BL2-P)

Yes, No, NA A record is kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Yes, No, NA A record is kept of experiments currently in progress in the greenhouse facility.

Yes, No, NA The Principal Investigator reports all greenhouse accidents involving the inadvertent release or spill of microorganisms to the OEHS, Institutional Biosafety Committee, NIH/ORDA and other appropriate authorities immediately when applicable. Documentation of any such accident is prepared and maintained.

Decontamination and Inactivation (BL2-P)

Yes, No, NA Experimental organisms are rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Yes, No, NA Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments are made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Control of Undesired Species and Motile Macroorganisms (BL2-P)

Yes, No, N.A. A program is implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Yes, No, NA Arthropods and other motile macroorganisms are housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions are taken to prevent escape from the greenhouse facility.

Concurrent Experiments Conducted in the Greenhouse (BL2-P)

Yes, No, NA Experiments involving other organisms that require a containment level lower than BL2-P are conducted in the greenhouse concurrently with experiments that require BL2-P containment. This work is conducted in accordance with BL2-P greenhouse practices.

Signs (BL2-P)

Yes, No, NA A sign is posted indicating that a restricted experiment is in progress. The sign indicates the following: (I) the name of the responsible individual, (II) the plants in use, and (III) any special requirements for using the area.

Yes, No, NA If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence is indicated by a sign posted on the greenhouse access doors.

Yes, No, NA If there is a risk to human health, a sign is posted incorporating the universal biosafety symbol.

Transfer of Materials (BL2-P)

Yes, No, NA Materials brought into or removed from the greenhouse facility in a viable or intact state containing experimental microorganisms are transferred in a closed non-breakable container.

Greenhouse Practices Manual (BL2-P)

Yes, No, N.A. A greenhouse practices manual is available. This manual provides (I) advice personnel of the potential consequences if appropriate practices are not followed, and (II) outlines plans to be implemented in the event of the unintentional release of organisms.

Facilities (BL2-P)

Greenhouse Design (BL2-P)

Yes, No, N.A. The greenhouse floor is composed of impervious material. Concrete is recommended. Gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil.

Yes, No, NA Windows and other openings in the walls and roof of the greenhouse facility are open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms. However, screens are required to exclude small flying animals (e.g., arthropods and birds).

Autoclavcs (BL2-P)

Yes, No, NA An autoclave is available for the treatment of contaminated greenhouse materials.

Supply and Exhaust Air Ventilation Systems (BL2-P)

Yes, No, NA If intake fans are used, measures are taken to minimize the ingress of arthropods. Louvers or fans are constructed in a manner that allows opening only when the fan is in operation.

Other (BL2-P)

Yes, No, N. A. BL2-P greenhouse containment requirements are satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing requirements.

RDNA - Biosafety Level 3 - Plants (BL3-P)

Standard Practices (BL3-P)

Greenhouse Access (BL3-P)

Yes, No, NA Authorized entry into the greenhouse is restricted to individuals who are required for program or support purposes. The Greenhouse Director is responsible for assessing each circumstance and determining who is authorized to enter the greenhouse facility.

Yes, No, NA Prior to entering the greenhouse, personnel are required to read and follow instructions on BL3-P practices and procedures. All procedures are conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Records (BL3-P)

Yes, No, NA A record is kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Yes, No, NA A record is kept of experiments currently in progress in the greenhouse facility.

Yes, No, NA The Principal Investigator reports all greenhouse accidents involving the inadvertent release or spill of microorganisms to the OEHS, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately when applicable. Documentation of any such accident is prepared and maintained.

Decontamination and Inactivation (BL3-P)

Yes, No, NA All experimental materials are sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal except those that are to remain in a viable or intact state for experimental purposes. This includes water that comes in contact with experimental microorganisms and contaminated supplies and materials.

Control of Undesired Species and Motile Macroorganisms (BL3-P)

Yes, No, NA A program is implemented to control undesired species (e.g., weed, rodent or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Yes, No, NA Arthropods and other motile macroorganisms are housed in appropriate cages. When appropriate to the organism, experiments are conducted within cages designed to contain the motile organisms.

Concurrent Experiments Conducted in the Greenhouse (BL3-P)

Yes, No, NA Experiments involving organisms that require a containment level lower than BL3-P are conducted in the greenhouse concurrently with experiments that require BL3-P containment. All work is conducted in accordance with BL3-P greenhouse practices.

Signs (BL3-P)

Yes, No, NA A sign is posted that indicates a restricted experiment is in progress. The sign incorporates the following information: (I) the name of the responsible individual, (II) the plants in use, and (III) any special requirements for using the area.

Yes, No, NA Presence of organisms that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems is indicated on a sign posted on the greenhouse access doors.

Yes, No, NA A risk to human health is indicated by a sign with the universal biosafety symbol posted on the access doors.

Transfer of Materials (BL3-P)

Yes, No, NA Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state are transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector is present within the effective dissemination distance of propagates of the experimental organism the surface of the secondary container is decontaminated. Decontamination is accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has been demonstrated to effectively inactivate the experimental organism.

Greenhouse Practices Manual (BL3-P)

Yes, No, NA A greenhouse practices manual is prepared. This manual provides (I) advice to personnel of the potential consequences if such practices are not followed, and (II) outline plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Protective Clothing (BL3-P)

Yes, No, NA Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) is worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Yes, No, NA Protective clothing is removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Other (BL3-P)

Yes, No, NA Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Yes, No, NA All procedures are performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Facilities (BL3-P)

Greenhouse Design (BL3-P)

Yes, No, NA The greenhouse floor is composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Yes, No, NA Windows are closed and sealed. All glazing is resistant to breakage (e.g., double-pane-tempered glass or equivalent).

Yes, No, NA The greenhouse is a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry is passage through two sets of self-closing locking doors.

Yes, No, NA The greenhouse facility is surrounded by a security fence or protected by equivalent security measures.

Yes, No, NA Internal walls, ceilings, and floors are resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) are sealed.

Yes, No, NA Bench tops and other work surfaces have seamless surfaces that are impervious to water and resistant to acids, bases, organic solvents, and moderate heat.

Yes, No, NA The greenhouse contains a foot, elbow, or automatically operated sink that is located near the exit door for hand washing.

Autoclaves (BL3-P)

Yes, No, NA An autoclave is available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Supply and Exhaust Air Ventilation Systems (BL3-P)

Yes, No, NA Individual supply and exhaust air ventilation systems are provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Yes, No, NA The exhaust air from the greenhouse facility is filtered through high efficiency particulate air (HEPA) filters and discharged to the outside. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters are 80-85% average efficiency by the American Society of Heating, Refrigeration, and Air

Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans are equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow is interlocked to assure inward (zero) airflow at all times.

Other (BL3-P)

Yes, No, NA BL3-P greenhouse containment requirements are satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing requirements.

Yes, No, NA Vacuum lines are protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Biological Containment Practices (Plants)

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Biological Containment Practices (Plants)

One or more of the following procedures may prevent effective dissemination of plants by pollen or seed:

(I) Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity;

(II) Remove reproductive structures by employing male sterile strains or harvest the plant material prior to the reproductive stage;

(III) Insure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or

(IV) Insure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Biological Containment Practices (Plants and Microorganisms)

One or more of the following procedures can prevent effective dissemination of microorganisms beyond the confines of the greenhouse:

(I) Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces;

(II) Insure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated;

(III) Conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection;

(IV) Use viruses and other microorganisms or their genomes that has known arthropod or animal vectors, in the absence of such vectors;

(V) Use microorganisms that have an obligate association with the plant; or

(VI) Use microorganisms that are genetically disabled to survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Biological Containment Practices (Plants and Macroorganisms)

Using one or more of the following procedures can prevent effective dissemination of arthropods and other small animals:

(I) Use non-flying, flight-impaired, or sterile arthropods;

(II) Use non-motile or sterile strains of small animals;

(III) Conduct experiments at a time of year that precludes the survival of escaping organisms;
(IV) Use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or
(V) Prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

Appendix D

Risk Groups 2,3,and 4

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

Risk Group 2 Bacterial Agents	Risk Group 2 Fungal Agents	Risk Group 2 Parasitic Agents	Risk Group 2 Viruses
Acinetobacter baumanni	Blastomyces dermatitidis	Ancylostoma human hookworms including A. duodenale, A. ceylanicum	Adenoviruses, human – all types
Actinobacillus	Cladosporium bantianum, C. (Xylohypha) trichoides	Ascaris including Ascaris lumbricoides suum	Alphaviruses (Togaviruses) – Group A Arboviruses
Actinomyces pyogenes	Cryptococcus neoformans	Babesia including B. divergens, B. microti	-Eastern equine encephalomyelitis virus - Venezualan equine encephalomyelitis vaccine strain TC-83, Western equine encephalomyelitis virus
Aeromonas hydrophila	Dactylaria galopava (Ochoconis gallopavum)	Brugia filaria worms including B. malayi, B. timori	Arenaviruses -Lymphocytic choriomeningitis virus, Tacaribe virus complex
Amycolata autotrophica	Epidermophyton	Coccidia	Bunyaviruses -Bunyamwera virus, Rift Valley fever virus vaccine strain MP-12
Archanobacterium haemolyticum	Exophiala (Wangiells) dermatitidis	Cryptosporidium including C. parvum	Calciviruses
Arizona hinshawii	Fonsecaea pedrosoi	Cysticercus cellulosae	Coronaviruses
Bacillus anthracis	Microsporum	Echinococcus including E. granulosis, E. multilocaris, E. vogeli	Flaviviruses – Group B Arboviruses -Dengue virus serotypes 1, 2, 3 & 4 -Yellow fever virus vaccine strain 17D
Bartonella henselae, B. quintana, B. vinsonii	Paracoccidioides brazilliensis	Entamoeba histolytica	Hepatitis A, B, C, D, & E viruses
Bordetella including B. pertussis	Penicillium marneffei	Enterobius	- Herpesviruses – except Herpesvirus simiae (Monkey B virus), cytomegalovirus, Epstein Barr virus, Herpes Simplex 1 & 2, Herpes zoster, Human Herpes virus types 6 & 7
Borrelia recurrentis, B. burgdorferi	Sporothrix schenckii	Fasciola including F. gigantica, F. hepatica	Orthomyoxoviruses - Influenza type A, B & C, Other tick borne orthomyoxoviruses

Risk Group 2 Bacterial Agents	Risk Group 2 Fungal Agents	Risk Group 2 Parasitic Agents	Risk Group 2 Viruses
Campylobacter coli, C. fetus, C. jejunji		Heterophyes	Paramyxoviruses -Newcastle disease virus, measles virus, mumps virus, parainfluenza viruses types 1, 2, 3 & 4, Respiratory synctial virus
Chlamydia psittaci, C. trachomatis, C. pnuemoniae		Hymenolepis including H. diminuta, H. nana	Parvoviruses - Human parvovirus (B19)
Clostridium botulinum, Cl. Chauvoei, Cl. Haemolyticum, Cl. Histolyticum, Cl. Novyi, Cl. Septicum, Cl. Tetani		Isospora	Picornavirus - Coxsackie viruses types A & B, Echoviruses (all types), Polioviruses (all types, wild and attenuated), Rhinoviruses (all types)
Corynbacterium diptheriae, C. pseudotuberculosis, C. renale		Laishmania including L. braziliensis, L. donovani, L. ethiopia, L. majoria, L. mexicana, L. peruvania, L. tropica	Pox viruses – all types except Monkey-pox virus, and restricted pox virus including Alastrim, Smallpox, Whitepox
Dermatophilus congolensis		Loa Loa filaria worms	Reoviruses – all types including Coltivirus, human Rotavirus, & Orbivirus
Edwardsiella tarda		Microsporidium	Rhabdoviruses - Rabies virus (all strains), Vesicular stomatitis virus
Erysipelothrix rhusiopathiae		Naegleria fowleri	Togaviruses - Rubivirus (rubella)
Eschericia Coli, all enteropathogenic, enterotoxigenic, enteroinvasiv, and strains bearing K1 antigen, including E. coli O157:H7		Necator human hookworms including N. americanus	
Haemophilus ducreyi, H. influenzae		Onchocerca filaria worms including, O. volvulus	
Helicobacter pylori		Plasmodium including simian species, P. cynomologi, P. falciparum, P. malariae, P. ovale, P vivax	
Klebsiella – all species except K. oxytoca (RG1)		Sarcocystisi including S. sui hominis	

Risk Group 2 Bacterial Agents	Risk Group 2 Fungal Agents	Risk Group 2 Parasitic Agents	Risk Group 2 Viruses
Legionella including L. pneumophila		Schistosoma including S. haematobium, S. intercalatum, S. japanicum, S. mansoni, S. mekongi	
Leptospira interrogans – all serotypes		Strongyloides including S. stercoralis	
Listeria		Taenia solium	
Moraxella		Toxocara including T. canis	
Mycobacterium (except those in RG3) including: M. avium complex, M. asiaticum, M. bovis BCG vaccine strain, M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi		Toxoplasma including T. gondii	
Mycoplasma, except M. mycoides and M. agalacticiae which are restricted animal pathogens		Trichinell spiralis	
Neisseria gonorrhoeae, N. meningitidis		Trypanosoma including T. brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi	
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N.transvalensis		Wuchereria bancrofti filaria worms	
Rhodococcus equi			
Salmonella including S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi, A, B, C, S. typhi, S. typhimurium			
Shigella including S. boydii, S. dysenteriae, type 1, S. flexneri, S. sonne			
Sphaerophorus necrophorus			
Staphylococcus aureus			
Streptobacillus moniliformis			
Streptococcus including S. pneumoniae, S. pyogenes			

Risk Group 2 Bacterial Agents	Risk Group 2 Fungal Agents	Risk Group 2 Parasitic Agents	Risk Group 2 Viruses
Treponema pallidum, T. carateum			
Vibrio cholerae, V. parahemolyticus, V. fulnificus			
Yersinia enterocolitica			

Risk Group 3 – RG3 agents are associated with serious or lethal human disease for which preventative or therapeutic interventions *may* be available.

Risk Group 3 Bacterial Agents	Risk Group 3 Fungal Agents	Risk Group 3 Parasitic Agents	Risk Group 3 Viruses and Prions
Bartonella	Coccidioides immitis (sporulating cultures; contaminated soil)	None	Alphaviruses (togaviruses) – Group A Arboviruses – Semliki Forest virus, St. Louis encephalitis virus, Venezualan equine encephalomyelitis virus (except TC-83)
Brucella including B. abortus, B. canis, B. suis	Histoplasma capsulatum, H. capsulatum var. duboisii		Arenaviruses – Flexal, Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Burkholderia (pseudomonas) mallei, B. pseudomallei			Bunyaviruses – Hantaviruses including Hantaan virus, Rift Valley fever virus
Coxiella burnetii			Flavivviruses (Togaviruses) – Group B Arboviruses – Japenese encephalitis virus, yellow fever virus
Francisella tularensis			Poxviruses – Monkeypox virus
Mycobacterium bovis, M. tuberculosis			Prions – Transmissible spongioform encephalopathies (TME)
Pasteurella multocida type B – buffalo and other virulent strains			Retroviruses – Human immunodeficiency virus (HIV) type 1 & 2, Human T cell lymphotrophic virus (HTLV) types 1 & 2, Simian immunodeficiency virus (SIV)
Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R. siberica, R. tsutsugamushi, R. typhi			Rhabdoviruses – Vesicular stomatitis virus
Yersinia pestis			

Risk Group 4 – RG 4 agents are likely to cause serious or lethal human disease for which preventable or therapeutic interventions are *not usually* available.

RG 4 Bacterial Agents	RG 4 Fungal Agents	RG 4 Parasitic Agents	RG 4 Viral Agents
None	None	None	Arenaviruses – Guanarito virus, Lassa virus, Junin virus, Machupo virus, Sabia
			Bunyaviruses – Crimean-congo hemorrhagic fever virus
			Filoviruses – Ebola virus, Marburg virus
			Flaviviruses (Togaviruses) – Group B Arboviruses – Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasaner Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
			Herpesvirus (alpha) – Herpesvirus simiae (Herpes B or Monkey B virus)
			Paramyoxyviruses – Equine morbilliviruses
			Hemorrhagic fever agents and viruses not yet defined

Appendix E

Recombinant DNA Registration Form

The University of Alabama in Huntsville
Office of Environmental Health Safety

RECOMBINANT DNA PROJECT REGISTRATION

Instructions: Complete this form for projects that are not exempt from NIH Guidelines.
Questions can be directed to the Office of Environmental Health & Safety (OEHS) at 824-2352.
Send completed forms to the OEHS at JRC 106.

I

Name (Principal Investigator/Title): _____

email address: _____

Title of Project: _____

Campus/Business Phone No.: _____

Location of Project (Building and Room): _____

UAH Department, Center or Institute: _____

Other personnel involved in this project: _____

_____

II

1. The Biosafety Containment Level(BSL) to be used during this project is:
   BSL1 ☐ **BSL2** ☐ **BSL3** ☐
2. What will be the sources of DNA? (species, clone bank, etc.) _____

_____

3. Do you plan to propagate the recombinant? ☐ **Yes** ☐ **No**
4. Host(s) to be used: _____
5. If virus source, is it more than 2/3 viral? ☐ **Yes** ☐ **No**
6. Is helper virus used? ☐ **Yes** ☐ **No** _____
7. Specific vector, phage, or plasmid to be used: _____
8. The vectors will be:
   ☐ constructed in my lab
   ☐ purchased from vendor, specify _____
   ☐ obtained elsewhere, specify _____
9. If the project calls for the use of viral vectors, describe the vector, its construction and frequency of recombination.

_____

_____

_____

10. Is the vector propagation defective? ☐ **Yes** ☐ **No**

If yes, how? _____

11. Are plant or animal cells exposed to the recombinant? ☐ **Yes** ☐ **No**

If yes, give the name, abbreviation, and source of each cell line to be used.

_____

_____

12. Will the studies include deliberate attempts to obtain expression of a foreign gene? ☐ **Yes** ☐ **No**

If yes, what protein(s)? _____

13. Have you read and do you agree to comply with the NIH guidelines for shipment and handling recombinant DNA? ☐ **Yes** ☐ **No**
14. The resulting recombinants or products are toxic to vertebrates. ☐ **Yes** ☐ **No**

IV

The signature of the investigator indicates that he/she is familiar with UAH safety procedures and policies relevant to this project and has incorporated safety training, practices and procedures into laboratory routines.

Signature: _____ **Date:** _____

Appendix F

Animal Hazard Sign Template

WARNING

Animal Hazard Control Area

Permission to enter must be obtained from the Principle Investigator (PI).

PI: _____ **Phone No.:** _____

Animal Species: _____

Biohazardous Agent: _____

Entrance Requirements: _____