UAMS

BIOSAFETY MANUAL

Department of
Occupational
Health and Safety

Second Edition
April 2019

DISCLAIMER

This biosafety manual was prepared for use by faculty, staff, students, volunteers, visitors, etc. at the University of Arkansas for Medical Sciences (UAMS). It is provided as a means of presenting regulations and standards pertaining to safely performing work with biohazardous or potentially biohazardous materials. Neither the author nor UAMS warrants its completeness or correctness. Any discrepancies noted should be brought to the attention of the UAMS Biological Safety Officer in the Department of Occupational Health & Safety.

EMERGENCY INFORMATION

	Telephone
Principal Investigator/Supervisor_____	_____
Building Coordinator_____	_____
Environmental Services_____	_____
UAMS Police (On Campus)	501-686-7777
Fires on campus	501-686-5333
Little Rock Police	911
Occupational Health and Safety Office Office Hours: Mon-Fri 7:30 am – 4:00 pm Location: Central Bldg. G154	501-686-5536
Campus Operations Call Center	501-526-0000

WHEN REPORTING A BIOHAZARD EMERGENCY

1) State that this is a Biohazard Emergency
2) Give your name
3) Give your location (Building and Room)
4) Give the phone number you are using
5) Describe the nature of the emergency
6) Report personal injury or threat of injury

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Section 1: Introduction

This section contains general information about the University of Arkansas for Medical Sciences (UAMS) Biosafety Program. Included in this section are the biosafety program purpose, biohazardous material definition, biosafety program information, campus policies, roles and responsibilities, and related Occupational Health and Safety (OH&S) programs.

1.1 Manual Purpose

The UAMS Biosafety Manual was prepared to outline the policies and procedures of the UAMS Biosafety Program. It is intended to accomplish the following goals:

- Protect personnel from exposure to infectious agents
- Prevent environmental contamination
- Provide an environment for high quality research while maintaining a safe work place
- Comply with applicable federal, state, and local requirements

The biosafety manual provides campus-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of laboratory supervisors and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used. In general, the handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of various precautionary measures depending on the materials involved. This manual will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary. Occupational Health & Safety (OH&S) as well as the Institutional Biosafety Committee (IBC) are available to assist in this endeavor.

1.2 Biohazardous Material Definition

Biohazardous materials include infectious or etiologic (disease causing) agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant or synthetic nucleic acid molecules (rDNA), and any materials potentially containing infectious agents or biohazards.

Biohazardous agents may include *but are not limited to*:

- Certain bacteria, fungi, viruses, rickettsiae, spirochetes, protozoa, parasites
- Recombinant or synthetic nucleic acid molecules
- Listed Select Agents & Toxins (exempt & non-exempt quantities)
- Cultured human or animal cells and the potentially infectious agents these cells may contain
- Viroids and prions
- Other infectious agents as outlined in laws, regulations, or guidelines.

1.2.1 Other Hazards

Other hazards not considered biohazardous may be present in the laboratory setting. It is the responsibility of the PI to ensure laboratory workers are trained on all risks present, as well as proper procedures for safe handling of all hazardous materials. Other hazards may include chemicals or nanoparticles, both of which require safety protocols to be submitted as part of the Biosafety Protocol to the IBC. These protocols must be reviewed and approved by the IBC before research is initiated.

1.3 UAMS Biosafety Program

The purpose of the UAMS Biosafety Program is to minimize the health risk to faculty, staff, students, and the public by identifying, evaluating, and controlling potential exposure to biohazardous materials used in research and teaching activities at UAMS. The UAMS Biosafety Program is administered by the Biological Safety Division of the Occupational Health & Safety Department. Oversight of the program is provided by the Institutional Biosafety Committee (IBC). More information about the UAMS Biosafety Program can be found on the OH&S website: http://www.uams.edu/campusop/depts/ohs/divisions.aspx?listid=bio.

1.4 Campus Policies

Policy number 11.4.08, the campus Laboratory Safety Policy, states:

- All UAMS employees, students, volunteers, and visitors will follow the UAMS Laboratory Safety Manual developed by the Department of Occupational Health & Safety.
- Primary responsibility for ensuring safe conduct and conditions in the laboratory or research area resides with the principal investigator (PI).
- The UAMS Laboratory Safety Manual should be used in conjunction with the UAMS Biosafety Manual, which provides specific biosafety information.

Other applicable policies can be found on the OH&S webpage (http://www.uams.edu/campusop/depts/ohs/divisions.aspx?listid=bio), or under Policies and Procedures in the UAMS Compliance 360 database.

1.5 Roles and Responsibilities

This section will define six categories for roles and responsibilities; individuals may fall into more than one of these categories:

1. Principal Investigators and Supervisors
2. Biohazard Workers
3. Ancillary Workers (Non-Biohazard Qualified Personnel)
4. Deans, Directors, Administrators, and Department Heads
5. Occupational Health & Safety
6. UAMS Institutional Biosafety Committee (IBC)

1.5.1 Principal Investigators and Supervisors

The principal investigator (PI) is defined in UAMS Campus Policy 11.4.08 as the individual who has authority over the work being conducted and the staff in the laboratory. Principal investigators and supervisors have primary responsibility for safety when work is conducted with biohazardous materials. Their responsibilities include, but are not limited to:

- Full compliance with the *NIH Guidelines* in the conduct of recombinant or synthetic nucleic acid molecule research or Biosafety Level 2 or 3 containment research (see *NIH Guidelines* Section IV-B-7).
- Submit the initial biosafety protocol and any subsequent amendments or renewals to the IBC for review and approval prior to initiating recombinant or synthetically derived nucleic acid molecule or Biosafety Level 2 or 3 containment research activities.
- Include all persons who use biohazardous materials in their work in the biosafety protocol and ensure these persons receive appropriate training. All training must be documented by the PI or supervisor.
- Report any significant problems, violations of the *NIH Guidelines*, any research-related accidents and illnesses, any spill of biohazardous material, equipment or facility failure, and/or any breakdown in procedure that could result in potential exposure of laboratory personnel and/or the public to biohazardous material to the Biological Safety Officer.
- Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken.
- Ensure the integrity of the physical containment and biological containment equipment and procedures.
- Be adequately trained in good microbiological practices.
- Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination.
- Comply with shipping requirements for biological material or recombinant or synthetic nucleic acid molecules.

1.5.2 Biohazard Workers

Biohazard workers are persons who work under the supervision of a principal investigator or supervisor. Most of these employees work with biohazardous material on a daily basis. Their responsibilities include, but are not limited to:

- Complete all necessary training required for biohazard workers.
- Report all accidents, biohazard exposures, work related illnesses, hazardous circumstances, and incidents to their supervisor in a timely manner.
- Know and follow all proper protocols and procedures for acquisition, use, storage, and disposal of biohazardous materials.
- Know how to respond to emergencies involving biohazardous materials.
- Be familiar with and use Personal Protective Equipment (PPE) appropriately for safety.

1.5.3 Ancillary Workers

Ancillary workers are persons who work in areas containing biohazardous material, but who do not normally work directly with these biohazardous materials. Examples of ancillary workers include custodial staff, IT, maintenance staff, delivery personnel, police officers, and visitors/contractors. Their responsibilities include, but are not limited to:

- Take precautions to avoid disturbing biohazardous materials.
- Report releases and/or other unsafe conditions involving biohazardous materials to their supervisor.
- Use proper Personal Protective Equipment (PPE) as directed for safety.

- Request assistance from the supervisor, PI, or OH&S when uncertain about risks related to biohazardous materials.

1.5.4 Deans, Directors, Administrators, and Department Heads

Deans, directors, administrators, and department heads have the following responsibilities (not all inclusive):

- Be familiar with and provide safety leadership, and ensure compliance regarding NIH, CDC, UAMS IBC, and the UAMS Biosafety Program guidelines.
- Assure health surveillance program in maintained for personnel engaged in research at UAMS.
- Assist in data collection of all significant violations, releases, spills, injuries, illnesses or other emergencies related to biohazardous materials use and ensure information is reported to NIH by OH&S.
- Complete and submit NIH annual report.
- Identify funding sources when needed to correct safety hazards and ensure that appropriate facilities are available to control biohazards.

1.5.5 Occupational Health & Safety

Occupational Health & Safety is the campus administrative unit that oversees the Biosafety Program. Specific responsibilities of OH&S include, but are not limited to:

- Develop safety and security guidelines for the campus community so that biohazardous materials are used in compliance with government regulations.
- Maintain databases of information related to the UAMS Biosafety Program.
- Provide or arrange appropriate training programs and staff to meet the IBC and campus community needs.
- Periodic monitoring or auditing of areas where biohazardous materials are used or stored to assure that program guidelines are followed.
- Advise and assist the campus community on biosafety matters such as emergency response, personnel contamination, investigation of incidents, and reporting.
- Assist in arrangement for the proper shipping and transportation of biohazardous materials.
- Serve as the liaison with regulatory agencies such as CDC, NIH, DOT, and Department of Agriculture.

1.5.6 Institutional Biosafety Committee (IBC)

The UAMS Institutional Biosafety Committee (IBC) oversees the campus Biosafety Program. Specific responsibilities include, but are not limited to:

- Review and evaluate investigator-generated safety protocols for the proposed use of biohazardous agents or rDNA and approve or disapprove the protocols based on that review.
- Periodically report to the Safety Coordinating Committee and make recommendations regarding biohazards that may exist or arise on the UAMS campus.
- Provide a campus forum for addressing issues involving biohazardous materials and assist in resolving problems.

1.6 Related Occupational Health & Safety Programs

The UAMS Department of Occupational Health & Safety provides comprehensive environmental and occupational safety services to minimize health and safety impacts to the campus and the greater Little Rock community. OH&S accomplishes this through training, consultation, and partnership with members of the campus community as well as with local, state, and federal agencies. Training and guidance are offered on a variety of issues that pose potential hazards to people and the environment. These issues include, but are not limited to:

- Chemical Safety
- Driver Safety/Insurance
- Environmental Programs
- Fire Safety
- Industrial Hygiene
- Laser Safety
- Life Safety
- Radiation Safety
- Respirator Safety
- Safety Awareness for Employees (S.A.F.E.) Program

For more information about these programs, contact OH&S at 501-686-5536 or visit the OH&S website: http://www.uams.edu/campusop/depts/ohs/.

Section 2: Training and Information Resources

This section details training and information resources to assist the UAMS campus community in working safely with biohazardous materials. Resources to aid in the classification of biohazardous materials are also included in this section.

2.1 Training Policies

All persons working with biohazardous materials must have appropriate training. Good microbiological and laboratory practices are essential for a safe work environment. Training should 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

It is the responsibility of the Principal Investigator to administer and document training required for their laboratory personnel. See Appendix A for more details.

2.2 Biosafety Courses Offered by OH&S

The UAMS Department of OH&S offers the following training courses for those involved with biohazardous materials:

- Basics of Biosafety Training
- Centrifugation Hazards
- Difference Between Respirators and Surgical Masks
- Emergency Response in a Biological Laboratory
- Mammalian Cell Culture
- Practicing Safe Science in a Laboratory
- Zoonoses Training
- Bloodborne Pathogens
- Chemical/Laboratory Safety Refresher Training
- DOT-IATA Shipping Infectious Substances
- Laboratory Glassware Washing
- NIH Guidelines for Recombinant DNA Research
- UAMS Waste Handling and Disposal Procedures

More information about these courses can be obtained by calling OH&S at 501-686-5536 or by visiting the Campus Operations Training Center website at http://uams.edu/campusop/depts/traincenter.aspx?ListID=1#viewlist.

2.3 Resources for the Classification of Biohazardous Materials

Proper classification of biohazardous materials is essential for the risk assessment process. The resources listed below can assist users in identifying and understanding risk factors associated with the specific biohazardous material used. In addition to the resources below, please refer to

Section 4 Biological Risk Assessment and Risk Management, Section 5 Biosafety Levels, and Section 6 Animal Biosafety Levels for more information.

Resources for Infectious Agents and Biological Toxins

- University of Arkansas for Medical Sciences Department of Occupational Health & Safety Biological Safety website: http://www.uams.edu/campusop/depts/ohs/divisions.aspx?listid=bio

- Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) Guidelines: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition: https://www.cdc.gov/biosafety/publications/bmbl5/

- CDC Select Agents Regulation (42 CFR 73.0) Federal Select Agent Program: http://www.selectagents.gov/regulations.html

- American Biological Safety Association Biosafety Links: https://absa.org/links/

- NIH Office of Science Policy (OSP) Dual Use Research of Concern: https://osp.od.nih.gov/biotechnology/dual-use-research-of-concern/

- U.S. Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030): http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

- U.S. Public Health Service (USPHS) Foreign Quarantine (42 CFR 71): CDC Importation Permits for Etiologic Agents: http://www.cdc.gov/od/eaipp/

Resources for Recombinant or Synthetic Nucleic Acid Molecules

- *NIH Guidelines for Research involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*): http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

- USDA Introduction of Genetically Engineered Organisms Regulations (7 CFR 340) http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/biotechnology

Resources for Animal Use or Animal Products

- UAMS Institutional Animal Care and Use Committee http://inside.uams.edu/iacuc/?_ga=2.182126499.56095839.1551364986-1824008387.1501507901 (accessible on campus only)

- Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) Overview of governmental regulations regarding use of animals: http://www.aaalac.org/resources/usregs.cfm

- U.S. Department of Agriculture (USDA) Import-Export Regulations (7 CFR): http://www.aphis.usda.gov/import_export/index.shtml

- Occupational Health and Safety in Care and Use of Research Animals (National Research Council): http://www.nap.edu/catalog/4988.html

- Guide for the Care and Use of Agricultural Animals in Research and Teaching Third Edition, Federation of Animal Science Societies (January 2010): https://www.aaalac.org/about/ag_guide_3rd_ed.pdf

2.4 Other Biosafety Information Resources

- American Biological Safety Association Resources https://absa.org/links/

- American Industrial Hygiene Association (AIHA) Publications and Resources http://www.aiha.org/publications-and-resources/pages/default.aspx

- Laboratory Health & Safety Committee Technical Topics-Biosafety (AIHA) http://www.aiha.org/get-involved/volunteergroups/labHSCommittee/Pages/Technical-Topics---Biosafety.aspx

- National Sanitation Foundation (NSF)/ANSI Standard 49-2000 (Laminar Flow) Class II Biosafety Cabinetry http://www.nsf.org http://www.nsf.org/Certified/Biosafety-Certifier

- CDC and NIH Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets 3rd Edition (9/2007) https://www.hsdl.org/?abstract&did=1878

- CDC Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories http://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Section 3: Rules, Regulations, and Guidelines Governing Use of Biohazards and rDNA Molecules

This section includes a summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents, and recombinant or synthetic nucleic acid molecules (rDNA).

3.1 CDC/NIH Biosafety in Microbiological and Biomedical Laboratories

Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th edition. In 1984, the CDC/NIH published the first edition 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. UAMS is using the BMBL as the basis for this biosafety manual.

3.2 NIH Guidelines

National Institute of Health (NIH): *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* address the safe conduct of research that involves 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 many times since then. Included in the *Guidelines* is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed rDNA research using the *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.*

3.3 The Select Agent Rule

Department of Health and Human Services (HHS - *42CFR 73): Possession, Use, and Transfer of Select Agents and Toxins*. In 2002, HHS published a set of rules that require facilities and institutions to be registered and approved in order to possess, use, or transfer certain biological agents and toxins. HHS requires UAMS to comply with the BMBL (see above) and OSHA's Laboratory Safety Standard 29 CFR 1910.1450. A copy of the most current list of restricted agents and toxins covered under this rule can be found: https://www.selectagents.gov/

3.4 Bloodborne Pathogens Standard

Occupational Safety and Health Administration Bloodborne Pathogens Standard. In 1992, the Occupational Safety and Health Administration (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. It applies to staff with reasonably anticipated exposure to blood or other potentially infectious materials (including human cell lines) during the course of their work. https://www.osha.gov/SLTC/bloodbornepathogens/standards.html

3.5 Department of Transportation Shipment of Untreated Waste

Department of Transportation Title 49 regulations apply to all untreated biohazardous waste that is shipped off-site for treatment and disposal by the UAMS Biohazard Waste Pickup Team. Employees who prepare biohazardous waste for collection by the waste pickup team must complete mandatory OH&S training before offering shipments and annually thereafter. https://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title49/49tab_02.tpl

3.6 Packaging, Shipment and Transportation

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
  https://www.unece.org/?id=3598

- International Civil Aviation Organization (ICAO)
  Technical Instructions for the Safe Transport of Dangerous Goods by Air
  https://www.icao.int/safety/dangerousgoods/pages/technical-instructions.aspx

- International Air Transport Association (IATA)
  Dangerous Goods Regulations
  https://www.iata.org/publications/dgr/Pages/index.aspx

- U.S. Department of Transportation
  49 CFR Parts 171-178

- U.S. Postal Service
  39 CFR Part 111

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

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

Importation permits are required for certain infectious agents, biological materials and animals as outlined in U.S. Public Health Service 42 CFR Part 71, Foreign Quarantine. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or

vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

3.7 U.S. Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC)

Dual use research of concern is defined by the NIH Office of Science Policy as:

> life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security (*USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern*, p. 6-7)

Research that is considered dual use research of concern (DURC) should be evaluated for potential risks, as well as benefits, to ensure that risks are managed appropriately, and benefits realized. Oversight of DURC includes both the U.S. Government and institutional processes. The U.S. Government (USG) has limited the scope of the *Policy for Institutional Oversight* as well as the *March 2012 DURC Policy* to include a well-defined set of criteria involving 15 agents and toxins and seven categories of experiments (see Table 1). UAMS reserves the right to consider other categories of research for DURC potential and may expand oversight to other types of research if deemed appropriate, though such expansion would not be subject to oversight as articulated in the USG Policy.

This policy applies to USG departments and agencies that fund or conduct life sciences research, institutions within the United States that both receive USG funds to conduct or sponsor research and conduct or sponsor research that involves one or more of the 15 agents and toxins listed in Table 1, even if the research is not funded by USG.

Table 1. USG Scope of Research Requiring Oversight

Agents and Toxins	1. Avian influenza virus (highly pathogenic) 2. *Bacillus anthracis* 3. Botulinum neurotoxin 4. *Burkholderia mallei* 5. *Burkholderia pseudomallei* 6. Ebola virus 7. Foot-and-mouth disease virus 8. *Francisella tularensis* 9. Marburg virus 10. Reconstructed 1918 Influenza virus 11. Rinderpest virus 12. Toxin-producing strains of *Clostridium botulinum* 13. Variola major virus 14. Variola minor virus 15. *Yersinia pestis*
Categories of Experiments	1. Enhances the harmful consequences of the agent or toxin 2. Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification 3. Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies 4. Increases the stability, transmissibility, or the ability to disseminate the agent or toxin 5. Alters the host range or tropism of the agent or toxin 6. Enhances the susceptibility of a host population to the agent or toxin 7. Generates or reconstitutes an eradicated or extinct agent or toxin listed above

Failure to comply with the USG Policy may result in suspension, limitation, or termination of USG funding, or loss of future USG funding opportunities consistent with existing regulations and policies governing USG funded research. Non-compliance may also subject UAMS to other potential penalties under applicable laws and regulations.

It is the responsibility of the Principal Investigator to notify the UAMS Institutional Biosafety Committee of research meeting DURC criteria. PIs must comply with all UAMS and USG policies and requirements for oversight of DURC. The Institutional Biosafety Committee will consider DURC as part of biosafety reviews.

More information and USG Policies can be found at https://osp.od.nih.gov/biotechnology/dual-use-research-of-concern/.

Section 4: Biological Risk Assessment

This section outlines the steps to be taken to complete a biological risk assessment as well as necessary steps for risk management. Routes of exposure are detailed in this section, also.

4.1 Biological Risk Assessment Overview

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a laboratory acquired infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of biosafety levels, work practices, safety equipment, and facility safeguards that can prevent laboratory acquired infections and minimize environmental contamination risk.

A risk assessment should always be conducted prior to initiating any work in a laboratory and is a key component of the Biosafety Protocol that must be submitted to the IBC for approval. The PI is responsible for identifying hazards, assessing risks associated with those hazards, and establishing protocols and standard procedures to minimize employee exposure to those risks. These protocols and procedures must be made available to all staff working in the laboratory.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. Capability of the laboratory staff to control hazards must also be considered. Their capability will depend on the training, technical proficiency, and good habits of all members of the laboratory, and the operational integrity of containment equipment and other facility safeguards.

4.1.1 Agent Hazards

The principal hazardous characteristics of an agent that should be considered include:

- Capability to infect and cause disease in a susceptible human or animal host
- Virulence as measured by the severity of disease
- Availability of preventive measures and effective treatments for the disease
- Probable routes of transmission of laboratory infection, including mucous membrane exposure, percutaneous exposure, ingestion, and inhalation of infectious aerosols
- Infective dose
- Stability in the environment
- Host range
- Its endemic nature
- Origin of the agent

Classification of Infectious Agents on the Basis of Hazard (Risk Groups)

Risk groups (RG) are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human etiological agents based on hazard to both

the individual and the community. There are four risk groups. Determining the risk group of a biological agent can be part of the biological risk assessment and helps in assigning the correct biosafety level for containment. The WHO and NIH risk groups correlate to, but are not equivalent to, biosafety levels. 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 may require BL-3 conditions, while some RG-3 agents may be safely manipulated at BL-2 under certain conditions. Consultation with the Biological Safety Officer is available for help determining risk group classification of agents. Examples of risk group agents can be found in the *NIH Guidelines*, Appendix B: Classification of Human Etiologic Agents on the Basis of Hazard (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Toc446948380).

Table 2. Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research Involving rDNA (2002)	WHO Laboratory Biosafety Manual 3rd Edition (2004)
Risk Group 1 (RG-1)	Agents not associated with disease in healthy adult humans.	A microorganism unlikely to cause human or animal disease. (No or low individual and community risk)
Risk Group 2 (RG-2)	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. (Moderate individual risk; low community risk)
Risk Group 3 (RG-3)	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk, but low community risk).	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. (High individual risk; low community risk)
Risk Group 4 (RG-4)	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. (High individual and community risk)

Hazards of Genetically-Modified Agents
When conducting a risk assessment of genetically modified agents, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to consider the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Sometimes, important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced, and the biosafety level assignment should be made conservatively. Once more information is available another risk assessment should be completed.

The *NIH Guidelines* are the key reference in assessing risk and establishing an appropriate biosafety level for work involving recombinant or synthetic nucleic acid molecules (rDNA). These guidelines specify appropriate practices and procedure for research involving constructing and/or handling rDNA molecules as well as organisms and viruses that contain rDNA. These guidelines should be followed for any work which involves rDNA; more information can be found in Section 7 of this manual.

Hazards of Cell Cultures
Human and animal cells and tissues have the potential to harbor latent infectious agents and personnel who handle these materials are at risk for possible exposure. Human and other primate cells should be handled using BL-2 practices and containment. All work should be performed in a biosafety cabinet, and all material should be decontaminated by autoclaving or disinfection before discarding.

Potential laboratory hazards associated with human cells and tissues include bloodborne pathogens, such as hepatitis B virus (HBV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), human T-cell lymphotropic virus (HTLV), Epstein-Barr virus (EBV), human papillomavirus (HPV), and cytomegalovirus (CMV), as well as agents such as *Mycobacterium tuberculosis* which may be present in human lung tissue. Other primate cells and tissues also present risks to laboratory personnel. OSHA's Bloodborne Pathogen Standards should be applied to all work in the laboratory involving human blood, tissues, body fluids, and primary cell lines. Non-human primate (NHP) and other mammalian cells, blood, lymphoid, and neural tissues should also be considered potentially hazardous.

4.1.2 Laboratory Procedure Hazards/Routes of Infection
Investigations of laboratory acquired infections have identified five principal routes of laboratory transmission. These routes of transmission of laboratory infection is a hazardous characteristic that should be considered in the risk assessment. The predominant probable routes of transmission in the laboratory are outlined in Table 3 below.

Table 3. Routes of Infection

Route of Transmission	Examples of Activities/Practices that may Result in Transmission
Mucous Membranes	• Splashes of infectious material into the eyes, nose, or mouth • Transfer of microorganisms to eyes, nose, or mouth by contaminated fingers
Percutaneous Exposure	• Accidental inoculation with a needle, other sharp instrument, or glass • Cuts, scratches
Ingestion	• Eating, drinking or smoking in the laboratory • Mouth pipetting • Transfer of microorganisms to mouth by contaminated hands or articles
Inhalation	• Inhalation of airborne microorganisms • Aerosolized solid material (spores, dust, particulate matter, etc.) • Liquid material (mists, sprays, coughing, sputum, etc.) • Technical processes (blending, grinding, sonicating, lyophilizing, sawing, centrifuging, etc.)
Animal Bites & Scratches	• Bites and/or scratches from handling laboratory animals

The general laboratory procedures outlined in this manual address these issues and provide guidance in handling infectious or potentially infectious materials.

4.1.3 Hazards Associated with Work Practices, Safety Equipment and Facility Safeguards

Laboratory workers are the first line of defense for protecting themselves, others in the laboratory, and the public from exposure to hazardous or potentially hazardous materials. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment. A risk assessment should identify any potential deficiencies in the practices of the laboratory workers. Factors affecting the competencies and experience of laboratory personnel should be considered in the risk assessment, such as:

- Worker experience (less experienced employees may be at higher risk)
- Genetic predisposition and/or nutritional deficiencies, immune/medical status (e.g., underlying illness, use of immunosuppressive drugs, allergies, etc.)
- Education, training, competence
- Stress, fatigue, mental status, excessive workload
- Perception, attitude, adherence to safety precautions

In addition, safety equipment should be maintained and kept in proper working order to provide a high degree of protection for the laboratory worker. Safety equipment that is not working properly is hazardous, therefore training in the correct use of equipment, proper procedure, routine inspections, and potential malfunctions of equipment is essential.

Facility safeguards help prevent the accidental release of hazardous agents from the laboratory. Consideration of facility safeguards is an integral part of the risk assessment. A biological safety professional, building and facilities staff, and the IBC should help assess the facility's capability to provide appropriate protection for the planned work, and recommend changes as necessary. More information about facility safeguards such as biological safety cabinets can be found in Section 5 of this manual (Biosafety Levels).

4.2 Recommended Approach to Biological Risk Assessment

This section describes a five-step approach to biological risk assessment that gives structure to the risk assessment process.

1. Identify agent hazards and perform an initial assessment of risk.
   Consider the principal hazardous characteristics of the agent according to the WHO and NIH Risk Group classifications (see Table 2).
2. Identify laboratory procedure hazards.
   The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate aerosols or droplets, and the use of sharps. Procedures involving animals can also present a number of hazards, such as bites and scratches. The risk assessment should identify specific hazards associated with laboratory procedures.
3. Determine the appropriate biosafety level and select additional precautions indicated by the risk assessment.
   More information on biosafety levels can be found in Section 5 of this manual.
4. Evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.
5. Review the risk assessment with a biosafety professional, subject matter expert, or the IBC.
   A review of the risk assessment and selected safeguards by knowledgeable individuals is always beneficial, and is sometimes required by regulatory or funding agencies, as is the case with the *NIH Guidelines*.

Risk assessment is the basis for the safeguards developed by the CDC, the NIH, and the microbiological and biomedical community to protect the health of laboratory workers, the public, and the environment from the risks associated with the use of hazardous or potentially hazardous biological materials in laboratories.

Section 5: Principles of Biosafety

This section outlines basic principles of biosafety and methods to contain biological hazards. Included in this section is basic lab practices and techniques, an overview of safety equipment, recommended work practices, and engineering and administrative controls. Understanding the fundamental principles of biosafety will lead to a safer work environment for all UAMS personnel.

5.1 Lab Practices and Techniques

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. All laboratory personnel, students, and visitors should respect the safety and health hazards associated with biohazardous materials and equipment in use and must always follow the general safety guidelines listed in this section.

5.1.1 Standard Microbiological Practices

The CDC *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* outlines standard microbiological practices that are highly recommended for all researchers to observe in addition to agent-specific procedures.

Standard Microbiological Practices are accepted as the initial standard of practice and are built upon depending upon the specific materials used. The standard practices are listed below.

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name, telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

5.2 Safety Equipment

Safety equipment includes primary barriers and personal protective equipment, such as biological safety cabinets, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. Also included are items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

5.2.1 Biological Safety Cabinets

Biological safety cabinets, or 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, Class II, and Class 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. BSCs must not be confused with other laminar flow devices or "clean benches;" in particular, horizontal flow cabinets with

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 biological safety cabinets to ensure that personnel, environment and product protection (where applicable) are maintained. Before selecting any BSC for purchase, contact the Biological Safety Officer for a work specific assessment and selection criteria. Selection of the correct biological safety cabinet is based on the classification of the agent, the associated biosafety level for the particular agent, and chemicals which will be used in the laboratory.

Table 4. Classes of Biological Safety Cabinets

Class	Operation and Uses
Class I	This is a ventilated cabinet for personnel protection with a non-recirculating inward airflow away from the operator. This unit is fitted with a HEPA filter to protect the environment from discharged agents. Suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection (e.g., sterility).
Class II	This is a ventilated cabinet for personnel, product and environmental protection which provides vertical airflow and HEPA-filtered supply and exhaust air. For use with low to moderate risk biological agents.
Class II, Type A	Recirculated 70% of the air inside the cabinet; exhausts 30% into room after filtration. 75 fpm average face velocity. Do not use with volatile radionuclides or toxic chemicals.
Class II, Type B1	Recirculated 30% of the air inside the cabinet and exhausts the remainder to the outside of the building. Maintains 100 fpm average face velocity. All contaminated ducts are under negative pressure. May be used with minute amounts of volatiles.
Class II, Type B2	Referred to as Total Exhaust; no recirculation. 100% exhausted outside after filtration. Maintains 100 fpm average velocity. Contaminated ducts are under negative pressure. Suitable where volatile toxic chemicals and radionuclides are required.
Class III (Glove Box)	A totally enclosed, gas-tight ventilated cabinet which maintains negative air pressure. Used to work with highly infectious, carcinogenic, or hazardous materials. All operations are conducted through rubber gloves attached to entry portals.

Certification of Biological Safety Cabinets

When used properly, biological safety cabinets 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. All BSCs used for RG-2 or RG-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 re-certification are mandatory if the cabinet is relocated or after major repairs, filter changes, etc.

Safe and Effective Use of Biological Safety Cabinets

1. General:
    a. Make sure the BSC is certified (NSF sticker) when it is installed or after it is moved or repaired, and annually thereafter. Check the magnehelic gauge or electronic controls regularly for any indication of a problem.
    b. Understand how your cabinet works.
    c. Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you, and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
    d. Plan your work.
    e. Minimize the storage of materials in and around the BSC.
2. Operation:
    a. Before using, wipe work surface with 70% alcohol or other disinfectant suitable for the agent(s) in use. Wipe off each item you need for your procedures before placing it inside the cabinet.
    b. DO NOT place any objects over the front air intake grille. DO NOT block the exhaust grille.
    c. Segregate contaminated items from clean items. Work from "clean to dirty."
    d. Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. DO NOT use vertical pipette discard canisters on the floor outside the cabinet.
    e. Move arms slowly when removing or introducing new items into the BSC.
    f. If you use a piece of equipment that creates air turbulence in the BSC (such as a micro-centrifuge or blender), place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
    g. Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
    h. Clean up spills in the cabinet immediately. Wait 10 minutes before resuming work.
3. Upon Completion of Procedures:
    a. Remove all materials and wipe all interior surfaces with 70% alcohol or other disinfectant suitable for the agent(s) in use.
    b. The cabinet blower should be left on for at least five minutes to purge the air.
    c. Turn on the UV light.
    d. Remove lab coat, gloves and other PPE and wash hands thoroughly before leaving the laboratory.

5.2.2 Personal Protective Equipment

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 devices and safety equipment as well as training in the proper use of those devices and equipment, must be provided to all employees under the appropriate circumstances. Employees have the responsibility of properly using the equipment.

Personal protective equipment should be used in combination with engineering controls, such as biological safety cabinets, and work practice controls, such as using good microbiological practices. The extent and kind of clothing and equipment to be selected for any particular activity depends upon the research activities and risk levels associated with the research.

Minimum PPE Requirements
PPE requirements to work in any UAMS laboratory includes: full length lab coat, fully enclosed shoes, safety glasses/goggles, and gloves.

Laboratory Clothing
Laboratory clothing protects the wearer, the experiment, and the environment against contamination. This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or personal 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 even it becomes contaminated. Additional criteria for selecting clothing include: comfort, appearance, closure types and locations, antistatic properties, and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposable clothing should be available for visitors, maintenance and service workers in the event it is required.

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.

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 required a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required.

Respirators
For certain protocols and projects, additional PPE such as respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection must be enrolled in the UAMS Respiratory Protection Program and should contact OH&S for assistance in selection of proper equipment and training in its usage.

5.2.3 Laboratory Equipment
This section describes different types and proper use of laboratory safety equipment. Where necessary, laboratory equipment should be marked with the biohazard symbol or the word biohazard to alert personnel of the potential for exposure. Equipment which may be contaminated with blood or potentially infectious materials must be decontaminated prior to servicing.

Laboratory safety equipment must be used according to manufacturer guidelines. All best practices and procedures to minimize exposure must be followed. Appropriate training is the responsibility of the department in charge of the laboratory. Engineering and Operations is responsible for the maintenance of equipment installed by the University as part of the facility (e.g., fume hoods, safety showers, eyewashes, sinks, etc.).

All equipment must be inspected prior to use to ensure that electrical cords are not damaged, safety interlocks have not been compromised and no chemicals have been spilled in the chambers of the equipment.

Equipment may be modified as necessary for research purposes. However, Clinical Engineering (501-526-0000) MUST be contacted prior to the use of modified equipment to verify the electrical and mechanical safety of the equipment.

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 on the open bench.
- 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.
- 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 biological safety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet while in use.

Homogenizers, Shakers, Blenders, Sonicators

The greatest risk when using any of these techniques is the creation of aerosols. Blenders, sonicators, homogenizers, shakers, etc. should be used in a biological safety cabinet whenever possible when working with biohazardous material.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used the cleaning process, such as in dishwashers, animal cage washers, etc., all items should be sterilized prior to cleaning.

Safety blenders should be used where possible. Safety blenders 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 rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous materials. The use of glass blender jars is not recommended due to 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 a least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Domestic (kitchen) homogenizers are not sealed and release aerosols. Only equipment designed for laboratory use should be used as their construction minimizes or prevents such release. Homogenizers should be used in a biological safety cabinet when possible. Homogenizers used for Risk Group 3 microorganisms should always be loaded and reopened in the biological safety cabinet.

Disposable Loops, Needles, Cell Spreaders
The advantage of disposable transfer loops, needles, and cell spreaders is that they do not have to be sterilized and can therefore be used in biological safety cabinets where Bunsen burners or microincinerators would disturb the airflow. These loops, needles, and cell spreaders should be placed in disinfectant after use and discarded as contaminated waste.

5.3 Recommended Work Practices

This section covers recommended practices for working with laboratory equipment such as autoclaves, needles and sharps, centrifuges, lyophilizers, etc. All laboratory personnel, students, and visitors should respect the safety and health hazards associated with biohazardous materials and equipment in use, and must always follow the general safety guidelines listed here.

5.3.1 Autoclaving

Autoclaving is a sterilization process that utilizes pressurized steam to kill microorganisms. It is the preferred method for decontaminating regulated medical waste containing bacteria, viruses, and other biological waste, as well as sterilization of laboratory equipment, materials, media, and reagents. Personnel responsible for operating an autoclave must receive proper training on operational procedures prior to operation. Ensure any caps on containers have been loosened prior to autoclaving. Open the autoclave only after the temperature and pressure have been returned to normal. Any leakage or release of contaminated materials should be reported to the PI or laboratory supervisor immediately.

Basic operation procedures for autoclaving should always be followed:

- Always follow the manufacturer guidelines.
- Appropriate PPE is required when operating an autoclave including a lab coat, heat resistant gloves, and eye protection.
- Place items to be autoclaved into the chamber, then close and seal the door.
- Program the appropriate autoclave cycle for the item being autoclaved, then start the cycle.
- When the cycle is complete, wait for the pressure to drop to zero and the temperature to drop below 121° C before opening the door.
- Wearing appropriate PPE, open the door slowly and only slightly to allow steam to escape. Keep the door ajar for 5-10 minutes before opening fully.
- Check autoclave tape indicator for a color change and print-out from the recorder to ensure proper time and temperature were attained.

- Place autoclaved biohazardous waste bags in the appropriate biohazardous bins and shut the autoclave door.

Potential risks associated with using an autoclave include burns from residual steam, boiling liquid and spillage in the autoclave, hands and/or arms injury from contact with autoclave door, and injuries from pressure release/explosion. To avoid these risks always don proper PPE when operating the autoclave. Do not attempt to open the door while the autoclave cycle is running. Avoid standing directly in front of the door and avoid superheating liquids. If problems occur during the cycle, abort the cycle and report problems to the PI or laboratory supervisor immediately.

The following items or waste are not recommended for autoclaving:

- Radioactive materials
- Pathological waste
- Hazardous chemicals (flammable, corrosive, toxic, reactive)
- Household bleach
- Liquid in a sealed container
- Toxins
- Prions

5.3.2 Centrifuge Equipment

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

The majority of all centrifuge accidents result from user error. To avoid injury, the following steps should be followed:

- Do not use a centrifuge that has debris inside or material splashed on the sides.
- Ensure that centrifuge bowls and tubes are dry.
- Ensure that the spindle is clean.
- Use matched sets of tubes, buckets, or other equipment.
- Always use safety centrifuge cups to contain potential spills and prevent aerosols.
- Inspect tubes or containers for cracks or flaws before using them. DO NOT use any cracked or damaged tubes or containers.
- Avoid overfilling tubes or other containers.
- Ensure that the rotor is properly seated on the drive shaft.
- Make sure that tubes or containers are properly balanced in the rotor.
- Only check O-rings on the rotor if you are properly trained, otherwise you may harm the rotor.
- Apply vacuum grease in accordance to manufacturer guidelines.
- Do not exceed the rotor's maximum run speed.
- Close the centrifuge lid during operation. Do not open the lid until the centrifuge has come to a complete stop.
- Make sure the centrifuge is operating properly before leaving the area.

- If a spill occurs in the centrifuge during use, use appropriate decontamination and clean-up procedures for the spilled material(s). Report all accidents to the PI or laboratory supervisor immediately.

5.3.3 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 with a HEPA filter 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 open, nick the neck of the ampoule with a file, wrap it in a disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen can explode, 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.

5.3.4 Sharps, Needles, and Syringes

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.

When using sharps, needles, and/or syringes, follow these safety guidelines:

- 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 biological safety cabinet whenever possible.
    - Wear gloves.
    - Fill the syringe carefully to minimize air bubbles.
    - Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with 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, a mechanical device or the one-handed scoop method must be used.
- Always dispose of needle and syringe unit promptly into an approved sharps container.

- An appropriate sharps container must be kept close to the work area to avoid walking around with contaminated sharps. Do not overfill sharps containers.

5.4 Facility Design and Engineering Controls

Facility design and engineering controls contribute to laboratory workers' protection, provide barriers to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory.

5.4.1 Ventilation

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 space. There are two main types of ventilation controls:

1. General (Dilution Exhaust): A room or building-wide system which brings in air from outside and ventilates within. Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during work. General exhaust systems are inadequate for Risk Group 3 agents or BL-3 work.
2. Local Exhaust or Filtration: A ventilated, enclosed work space intended to capture, contain, and exhaust or filter harmful or dangerous fumes, vapors, or particulate matter. In the case of hazardous chemicals this includes a fume hood; for infectious agents BSCs should be used.

5.4.2 Other Engineering Controls

Other examples of engineering controls include biological safety cabinets, HEPA filters on vacuum lines, safety-engineered needles, centrifuge safety cups, mechanical pipetting aids, and sharps containers.

5.5 Administrative Controls

Administrative controls consist of written policies, safety training, standard operating procedures, signage requirements, and access restriction policies. These policies and procedures are in place to keep employees, visitors, and the public safe and should be followed at all times.

5.5.1 Access and Security

Access to biohazardous materials must be restricted to authorized personnel only. The control of biological hazards shall be maintained by securing and locking the laboratory when unattended by authorized personnel and during all off-hours. When transporting biohazardous materials between laboratories, materials must be properly packaged and must never be left unattended or unsecured.

5.5.2 Required Biohazard Warning Signs and Labels

Areas where biohazardous materials are used or stored require a biohazard sign or label. Anyone entering these areas must be made aware of the potential hazards. The biohazard symbol must be on all entries to biohazardous material laboratories, containers, biological safety cabinets, infectious waste containers, freezers, refrigerators and other equipment where blood and other potentially infectious materials are used or stored.

Biohazard Warning Signs
Warning signs posted in permanent areas should be visible and legible to all laboratory occupants and visitors. Temporary signs may be posed for less than one month on refrigerators, freezers, or entry doors. Temporary signs should be removed when the hazard no longer exists.

The biohazard warning door sign placed on all biohazard laboratory doors must include the assigned biosafety level (BL), name of the agent(s) in use, the name and phone number of the PI or laboratory supervisor, and entry and exit requirements. See Appendix B for a biohazard warning door sign template.

Biohazard Labels
Biohazard labels should be affixed to all biohazardous material in addition to the required area signs referred to above. Red or orange biohazard labels should be placed on containers, biological safety cabinets, and storage units, including refrigerators, freezers, incubators, waste containers, etc., that are used for biohazardous materials. Any contaminated equipment must be labeled as well.

5.5.3 Standard Operating Procedures (SOPs)
Standard Operating Procedures (SOPs) are comprised of procedural steps, safe work practices, safety information such as Safety Data Sheets (SDS), and emergency response procedures. They are prepared by the PI, laboratory supervisor, or designee. SOPs should be specific to the laboratory and include procedures such as bloodborne pathogen exposure control, biosafety manuals, laboratory specific training protocols, etc. PIs are required to maintain and annually review laboratory specific SOPs and laboratory personnel and students must adhere to the SOPs.

5.5.4 Other Administrative Policies
Biohazard Releases
If a biohazardous material is released and makes contact with skin, wash the area with soap and water immediately. If you suspect aerosolization of a biohazardous material, hold your breath and immediately exit the area. If emergency assistance is required, call 911. Any biohazard release incidents should be reported to the UAMS OH&S (501-686-5536). For more information on biohazard spill procedures, see Section 10.

Children and Unauthorized Persons
Due to the hazards in laboratory areas, it is imperative that we do not allow children under the age of sixteen (16) in any of the laboratories or common areas. Unauthorized persons are not permitted in laboratory areas. UAMS Administrative Guide policy 3.1.42 outlines the responsibilities for approval of job shadowing. Children sixteen and older must comply with all regulations for labs, including dress code, and are not to be left unattended.

Electrical
Access to electrical equipment such as plugs, switches, and electrical panels should be maintained at all times. Immediate access should never be prevented in case of emergency. Use of polarized and grounded receptacle outlets in general laboratory areas and Ground Fault Circuit Interrupters (GFCIs) in wet or outdoor locations is required. Cords must not run in aisles or corridors, through doors, walls, partitions, under rugs, or above suspended ceilings.

Emergency Eyewash and Safety Showers
Emergency eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where personnel perform tasks that might result in splashes of potentially infectious materials. Emergency eyewash stations and safety showers should be properly located and maintained. There should be no obstructions that might inhibit the use of this equipment.

Fire Extinguishers
Appropriate type fire extinguishers must be available, charged, and properly located where they are immediately accessible. There should be no obstructions that might inhibit the use of this equipment.

Food, Drink, Cosmetics, and Contact Lenses
Eating, drinking, and the application of cosmetics or contact lenses are prohibited in areas where biohazardous materials are used. Food may not be stored in the same refrigerator or freezer where biohazardous materials are stored. Food used for research must be labeled, "Not for Human Consumption."

Freezers and Refrigerators
All biohazardous or toxic materials stored in refrigerators or freezers must be labeled. Refrigerators and freezers should be checked and cleaned out regularly to remove any old specimens or broken ampoules, tubes, etc. containing infectious or toxic material. Rubber gloves must be worn during cleaning.

Hair
Long hair must be tied back to prevent possible contamination and injury.

Hand Hygiene
Hand washing facilities must be available. If a sink with warm running water is not immediately available, a 60-90% alcohol-based gel hand sanitizer should be used until the employee can wash hands in a sink. Employees should immediately wash hands with soap and water upon glove removal and on completion of tasks involving contact with human blood, body fluids, or other potentially infectious materials.

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 items 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, exits, and fire extinguishers must not be blocked.

- Chemicals and waste must be disposed of properly. Old and unused chemicals should be disposed of promptly and properly by contacting OH&S (501-686-5536) for chemical pickup.
- The workplace must be 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 avoidance of the creation of electrical hazards in wet areas.
- A minimum of 36 inches width must be maintained for laboratory aisles.
- Hallways may not be used as storage areas.
- All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

Open Flames in Biological Safety Cabinets
Open flames, such as Bunsen burners, should never be used in biological safety cabinets. Open flames inside a BSC disrupt the airflow, compromising protection of both the worker and the work. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in a BSC. Electric incinerators or disposable inoculating loops are acceptable alternatives.

Smoking
Smoking is prohibited on the UAMS campus. A burning cigarette, cigar, or pipe is a source of ignition for flammable solvents. The handling of chewing tobacco, cigarettes, cigars, or pipes from bench to mouth is a potential route of transmission for microorganisms and toxic materials. Wash hands before smoking whenever biohazardous materials have been handled.

Unattended Experiments
Avoid leaving experiments unattended. If biohazardous material operations are carried out with no one present, it is the responsibility of the worker to prevent accidental release in the event of an interruption in utility services. Appropriate arrangements should be made for periodic inspection of the operation. Unattended experiments must have posted identification providing a point of contact, what the hazard is and in what quantity, and when the experiment will be discontinued, as well as any instrument settings that must be maintained.

Standard Precautions
All blood or other potentially infectious materials (OPIM) will be considered infectious, regardless of the perceived status of the source individual. Standard precautions will be observed at UAMS to prevent contact with potentially infectious materials.

Section 6: Laboratory Biosafety Level Criteria

There are four biosafety levels that correspond to the four risk groups of microorganisms listed in Section 4 and Table 2. Biosafety levels are often abbreviated as BSL or BL; both are acceptable abbreviations. This manual will use the BL abbreviation. These biosafety levels (1-4) consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities deemed appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity.

The agents of minimal hazard are Biosafety Level 1 (BL-1), with the more dangerous microorganisms classified as Biosafety Level 4 (BL-4). Each biosafety level has different recommended practices, containment and facilities that build upon the recommendations for the level below. See Table 5: Summary of Recommended Biosafety Levels for Infectious Agents, adapted from CDC BMBL, 5th Edition.

It should be noted that CDC biosafety guidelines do not include protocols for work with recombinant DNA, while NIH guidelines do address containment for rDNA work. It is the responsibility of the Principal Investigator to determine which guidelines apply to their laboratory. More information about rDNA guidelines can be found in Section 8 of this manual.

6.1 Risk Groups and Biosafety Levels

Biosafety levels and risk groups correlate with each other, but should be differentiated. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with the mode of transmission, procedural protocols, experience of staff, and other factors in determining the BL in which the work will be conducted. The biosafety level for the organism(s) being used represent those conditions under which the agent ordinarily can be safely handled.

Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increased levels of containment.

6.2 Biosafety Level 1 (BL-1)

Biosafety level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy human adults, and of minimal potential hazard to laboratory personnel and the environment. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BL-1.

6.2.1 BL-1 Standard Microbiological Practices

The following standard microbiological practices must be followed for BL-1 labs:

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name, telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an

individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

6.2.2 BL-1 Special Practices

None required.

6.2.3 BL-1 Safety Equipment

Primary barriers and personal protective equipment required for BL-1:

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. Additionally, BL-1 workers should:
    a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

6.2.4 BL-1 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for BL-1:

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior should be fitted with screens.

6.3 Biosafety Level 2 (BL-2)

Biosafety level 2 (BL-2) builds upon BL-1 requirements. BL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BL-2.

6.3.1 BL-2 Standard Microbiological Practices

The following standard microbiological practices must be followed in the BL-2 lab:

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name, telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

6.3.2 BL-2 Special Practices

The following special practices are required for BL-2:

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.

9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

6.3.3 BL-2 Safety Equipment

Primary barriers and personal protective equipment required for BL-2:

1. Properly maintained BSCs, other appropriate personal protective equipment, or physical containment devices must be used whenever:
    a. Procedures with potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BL-2 workers should:
    a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

6.3.4 BL-2 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for BL-2:

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work areas must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if present, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily trafficked laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet has been tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system either by a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

6.4 Biosafety Level 3 (BL-3)

Biosafety level 3 (BL-3) applies to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. BL-3 laboratories require special engineering and design features. The following standard and special practices, equipment, and facility requirements apply to BL-3.

6.4.1 BL-3 Standard Microbiological Practices

The following standard microbiological practices must be followed in the BL-3 lab:

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below must always be taken with sharp items. These include:
    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
    b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name, telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an

individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

6.4.2 BL-3 Special Practices

The following special practices are required for BL-3:

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor must be used.

6.4.3 BL-3 Safety Equipment

Primary barriers and personal protective equipment required for BL-3:

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as a tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
4. Glove must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BL-3 laboratory workers should:
    a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

6.4.4 BL-3 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for BL-3:

1. Laboratory doors must be self-closing and have locks in accordance with institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that is can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation opening should be capable of being sealed to facilitate space decontamination.
    a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
    b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
    c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

    Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major

renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.
    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
    a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
    b. The laboratory exhaust air must not recirculate to any other area of the building.
    c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

    HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory wastes should be available in the facility, preferable within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).
12. Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. The laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.
15. The BL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented as least annually.

6.5 Biosafety Level 4 (BL-4)

Currently not at UAMS

Biosafety level 4 (BL-4) is required for work with dangerous and exotic agents that post a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BL-4 laboratories:

1. A *Cabinet Laboratory*—Manipulation of agents must be performed in a Class III BSC; and
2. A *Suit Laboratory*—Personnel must wear a positive pressure supplied air protective suit.

BL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

UAMS does not have Biosafety Level 4 capabilities. For further information on requirements for BL-4 work, see the CDC *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, Section IV.

6.6 Summary of Biosafety Level Criteria

The table below is a summary of biosafety level criteria adapted from the CDC BMBL, 5th Edition. Detailed information about each biosafety level can be found in the corresponding sections above.

Table 5. Summary of Recommended Biosafety Levels for Infectious Agents

Biosafety Level (BL)	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
BL-1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	• No primary barriers required • PPE: laboratory coats and gloves; eye, face protection as needed	Laboratory bench and sink required
BL-2	• Associated with human disease • Hazards: percutaneous injury, mucous membrane exposure, ingestion	BL-1 practice plus: • Limited Access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials • PPE: Laboratory coats, gloves, face & eye protection, as needed	BL-1 plus: • Autoclave available
BL-3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	BL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering	Primary barriers: • BSCs or other physical containment devices used for all open manipulations of agents • PPE: Protective laboratory clothing, gloves, face, eye and respiratory protection, as needed	BL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory • Entry through airlock or anteroom • Hand washing sink near laboratory exit
BL-4 **(not at UAMS)**	• Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments • Agents with a close or identical antigenic relationship to a BL-4 agent until data are available to redesignate the level • Related agents with unknown risk of transmission	BL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontamination on exit from facility	Primary Barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure suit	BL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in BMBL text

Section 7: Vertebrate Animal Biosafety Level Criteria

There are four recommended vertebrate animal biosafety levels. The recommendations below describe practices, safety equipment and facilities for experiments with animals infected with agents that cause, or may cause, human infection. The animal biosafety levels provide increasing levels of protection to personnel and to the environment and are recommended as minimal standards for activities involving infected laboratory animals. In general, the biosafety level recommended for working with biohazardous material *in vivo* and *in vitro* are comparable. The animal biosafety levels are abbreviated as ABL-1 to ABL-4.

It should be noted that these CDC animal biosafety guidelines do not include protocols for work with recombinant DNA in animals, while NIH guidelines do address containment for rDNA work. It is the responsibility of the Principal Investigator to determine which guidelines apply to their laboratory. More information about rDNA guidelines can be found in Section 8 of this manual.

7.1 Animal Biosafety Level 1 (ABL-1)

Animal Biosafety Level 1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

ABL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment of facility design may be required as determined by the risk assessment.

The following standard practices, safety equipment, and facility requirements apply to ABL-1.

7.1.1 ABL-1 Standard Microbiological Practices

The following standard microbiological practices must be followed in the ABL-1 lab:

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.
    a. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
    b. Prior to beginning a study, animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC); if the protocols involve any work with biohazardous material they must also be reviewed and approved by the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.).

a. Personnel must receive annual updates and additional training when procedures or policies change.
b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. An appropriate medical surveillance program is in place, as determined by the risk assessment. The need for animal allergy prevention program should be considered.
    a. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
    b. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
    c. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name, telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room
    a. Security-sensitive agent information should be posted in accordance with the institutional policy.
    b. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.
    a. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
    a. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
    b. Glove and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
    c. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
    d. Eye, face and respiratory protection should be used in room containing infected animals, as dictated by the risk assessment.

8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
    a. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
        i. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
        ii. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the worksite as possible.
        iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
        iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
        v. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
14. An effective integrated pest management program is required.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
    a. Decontaminate all potentially infectious materials before disposal using an effective method.

7.1.2 ABL-1 Special Practices

None required.

7.1.3 ABL-1 Safety Equipment

Primary barriers and personal protective equipment required for ABL-1:

1. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
2. Special containment devices or equipment may not be required as determined by appropriate risk assessment.
3. Protective laboratory coats, gowns, or uniforms may be required to prevent contamination of personal clothing.
    a. Protective outer clothing is not worn outside areas where infectious materials and/or animals are housed or manipulated. Gowns and uniforms are not worn outside the facility.
4. Protective eyewear is worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
    a. Persons having contact with non-human primates (NHPs) must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed.
5. Gloves are worn to protect hands from exposure to hazardous materials.
    a. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
    b. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    c. Gloves must not be worn outside the animal rooms.
    d. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
    e. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
6. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

7.1.4 ABL-1 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for ABL-1:

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
    a. Access to the animal facility is restricted.
    b. Doors to areas where infectious materials and/or animals are housed, open inward, and are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. The animal facility must have a sink for hand washing.
    a. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
    a. It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the Institute for Laboratory Animal Research's *Guide for Care and Use of Laboratory Animals*. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional flow.
    a. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

7.2 Animal Biosafety Level 2 (ABL-2)

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABL-1. ABL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals and the manipulation of pathogenic agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry

procedures; and 4) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

The following standard and special practices, safety equipment, and facility requirements apply to ABL-2.

7.2.1 ABL-2 Standard Microbiological Practices

The following standard microbiological practices must be followed in the ABL-2 lab:

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.
    a. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
    b. Prior to beginning a study, animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
    a. Consideration should be given to specific biohazards unique to the animal species and protocol in use.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazards or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.).
    a. Personnel must receive annual updates and additional training when procedures or policies change.
    b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by the risk assessment. The need for animal allergy prevention program should be considered.
    a. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
    b. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

    c. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name, telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.
    a. Security-sensitive agent information should be posted in accordance with the institutional policy.
    b. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.
    a. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
    a. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals.
    b. Glove and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
    c. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
    d. Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
    a. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
        i. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.

ii. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the worksite as possible.
iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
v. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
14. An effective integrated pest management program is required.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
    a. Decontaminate all potentially infectious materials before disposal using an effective method.

7.2.2 ABL-2 Special Practices

The following special practices apply to ABL-2 areas:

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.
    a. When appropriate, a base line serum sample should be stored.
2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.
    a. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.
3. Decontamination by an appropriate method (e.g., autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.
    a. A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.
    b. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or manipulated must be placed in a durable,

leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

c. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.

4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

7.2.3 ABL-2 Safety Equipment

Primary barriers and personal protective equipment required for ABL-2:

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
    a. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
    a. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.
    b. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. Eye and face protection (e.g., mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who

wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.

   a. Persons having contact with non-human primates (NHPs) must assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields, etc.) as appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.

4. Gloves are worn to protect hands from exposure to hazardous materials.
   a. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
   b. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
   c. Gloves must not be worn outside the animal rooms.
   d. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
   e. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
5. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

7.2.4 ABL-2 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for ABL-2:

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
   a. Access to the animal facility is restricted.
   b. Doors to areas where infectious materials and/or animals are housed, open inward, and are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.
   a. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.
   b. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
   a. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

    a. Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the Institute for Laboratory Animal Research's *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms.
    a. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washers should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
    a. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer guidelines. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.
    b. All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and have an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.

14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

7.3 Animal Biosafety Level 3 (ABL-3)

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission, and agents causing serious or potentially lethal disease. ABL-3 builds upon the standard and special practices, procedures, containment equipment, and facility requirements of ABL-2

The ABL-3 laboratory has special engineering and design features.

ABL-3 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in animal facility procedures, the handling of infected animals, and the manipulation of potentially lethal agents; 3) personnel must be supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations, and husbandry procedures; and 4) procedures involving the manipulation of infectious materials, or where aerosols or splashes may be created, must be conducted in BSCs or by use of other physical containment equipment.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and containment equipment. Employee occupational health programs must be implemented.

The following standard and special safety practices, safety equipment, and facility requirements apply to ABL-3.

7.3.1 ABL-3 Standard Microbiological Practices

The following standard microbiological practices must be followed in the ABL-3 lab:

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies.
    a. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review.
    b. Prior to beginning a study, animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
    a. Consideration must be given to specific biohazards unique to the animal species and protocol in use.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazards or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.).

a. Personnel must receive annual updates and additional training when procedures or policies change.
b. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.

4. An appropriate medical surveillance program is in place, as determined by the risk assessment. The need for animal allergy prevention program should be considered.
    a. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
    b. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
    c. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name, telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room.
    a. Security-sensitive agent information should be posted in accordance with the institutional policy.
    b. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
6. Access to the animal room is limited to the fewest number of individuals possible. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.
    a. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
    a. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. Double-glove practices should be used when dictated by risk assessment.
    b. Glove and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
    c. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.

    d. Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designated and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
    a. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
        i. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
        ii. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the worksite as possible.
        iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
        iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
        v. Equipment containing sharp edges and corners should be avoided.
12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.
13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/or animals are housed or are manipulated.
14. An effective integrated pest management program is required.
15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements.
    a. Decontaminate all potentially infectious materials before disposal using an effective method.

7.3.2 ABL-3 Special Practices

The following special practices apply to ABL-3 areas:

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.
    a. When appropriate, a base line serum sample should be stored.
2. All procedures involving the manipulation of infectious materials, handling of infected animals or the generation of aerosols must be conducted within BSCs or other physical containment devices when practical.
    a. When a procedure cannot be performed within a biosafety cabinet, a combination of PPE and other containment devices must be used.
    b. Restraint devices and practices are used reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications).
3. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in containment caging systems, such as solid wall and bottom cages covered with filter bonnets, open cages placed in inward flow ventilated enclosures, HEPA-filtered isolators and caging systems, or other equivalent primary containment systems.
4. Actively ventilated caging systems must be designed to prevent the escape of microorganisms from the cage. Exhaust plenums for these systems should be sealed to prevent escape of microorganisms if the ventilation system becomes static, and the exhaust must be HEPA filtered. Safety mechanisms should be in place that prevent the cages and exhaust plenums from becoming positive to the surrounding area should the exhaust fan fail. The system should also be alarmed to indicate operational malfunctions.
5. A method for decontaminating all infectious materials must be available within the facility, preferably within the areas where infectious materials and/or animals are housed or are manipulated (e.g., autoclave, chemical disinfection, or other approved decontamination methods).
    a. Consideration must be given to means for decontaminating routine husbandry equipment, sensitive electronic and medical equipment.
    b. Decontaminate all potential infectious materials (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) by an appropriate method before removal from the areas where infectious materials and/or animals are housed or manipulated.
    c. It is recommended that animal bedding and waste be decontaminated prior to manipulation and before removal from the areas where infectious materials and/or animals are housed or are manipulated, preferable within the caging system.
    d. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements.
6. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.
    a. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.

b. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

7. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

7.3.3 ABL-3 Safety Equipment

Primary barriers and personal protective equipment required for ABL-3:

1. Properly maintained BSCs and other physical containment devices or equipment, should be used for all manipulations for infectious materials and when possible, animals. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.
    a. The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.
    a. Personnel within the animal facility wear protective clothing, such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable PPE such as non-woven olefin cover-all suits, wrap-around or solid front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable.
    b. Disposable PPE must be removed when leaving the areas where infectious materials and/or animals are housed or manipulated. Scrub suits and uniforms are removed before leaving the animal facility.
    c. Disposable PPE and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face and respiratory protection. To prevent cross contamination, boots, shoe covers, or other protective footwear are used where indicated.
    a. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
4. Gloves are worn to protect hands from exposure to hazardous materials.
    a. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
    b. Procedures may require the use of wearing two pairs of gloves (double-glove).

c. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
d. Gloves must not be worn outside the animal rooms.
e. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
f. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

5. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

7.3.4 ABL-3 Laboratory Facilities

Laboratory facilities serve as secondary barriers. The following are required for ABL-3:

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
    a. Access to the animal facility is restricted.
    b. Doors to areas where infectious materials and/or animals are housed, open inward, and are self-closing, are kept closed when experimental animals are present, and should never be propped open.
    c. Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on the risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated.
    a. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.
    b. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant.
    a. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface.
    b. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases.
    c. Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials

and methods used to decontaminate the animal room must e based on the risk assessment.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
    a. Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.
5. External windows are not recommended; if present, all windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the Institute for Laboratory Animal Research's *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from "clean" areas and toward "contaminated" areas.
    a. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on-site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes.
    b. Personnel must verify that the direction of the airflow (into the animal room) is proper. It is recommended that a visual monitoring system device that indicates directional inward airflow be provided at the animal room entry. The ABL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

a. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer guidelines. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.
b. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room.
c. All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.
d. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

12. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious materials and waste before moving it to the other areas of the facility. If not convenient to areas where infectious materials and/or animals are housed or manipulated, special practices should be developed for transport of infectious materials to designated alternate location(s) within the facility.
13. Emergency eyewash and shower are readily available; location is determined by risk assessment.
14. The ABL-3 facility design and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to use. Facilities should be re-verified at least annually against these procedures as modified by operational experience.
15. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions, or other applicable federal, state or local regulations.

7.4 Animal Biosafety Level 4 (ABL-4)

Currently not at UAMS

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments; or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level. Animal care staff must have specific and thorough training in handling extremely hazardous, infectious agents and infected animals. Animal care staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and

laboratory design characteristics. All animal care staff and supervisors must be competent in handling animals, agents and procedures requiring ABL-4 containment. The animal facility director and/or laboratory supervisor control access to the animal facility within the ABL-4 laboratory in accordance with institutional policies.

There are two models for ABL-4 laboratories:

1. A *Cabinet Laboratory*—All handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs;
   and
2. A *Suit Laboratory*—Personnel must wear a positive pressure protective suit; infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust; and infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

ABL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

UAMS does not have Animal Biosafety Level 4 capabilities. For further information on requirements for BL-4 work, see the CDC *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, Section V.

7.5 Summary of Animal Biosafety Level Criteria

The table below is a summary of animal biosafety level criteria adapted from the CDC BMBL, 5th Edition. Detailed information about each biosafety level can be found in the corresponding sections above.

Table 6. Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals are Used

Animal Biosafety Level (ABL)	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
ABL-1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species • PPE: laboratory coats and gloves; eye, face protection as needed	Standard animal facility: • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
ABL-2	• Agents associated with human disease • Hazards: percutaneous injury, mucous membrane exposure, ingestion	ABL-1 practice plus: • Limited Access • Biohazard warning signs • "Sharps" precautions • Biosafety manual • Decontamination of all infectious wastes and animal cages prior to washing	ABL-1 equipment plus: • Containment equipment appropriate for animal species • PPE: Laboratory coats, gloves, face, eye and respiratory protection, as needed	ABL-1 plus: • Autoclave available • Hand washing sink available • Mechanical cage washer recommended • Negative airflow into animal and procedure rooms recommended
ABL-3	Indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure	ABL-2 practice plus: • Controlled access • Decontamination of laboratory clothing before laundering • Cages decontaminated before bedding is removed • Disinfectant foot bath as needed	ABL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • Class I, II, or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols • PPE: Appropriate respiratory protection	ABL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations and windows • Autoclave available in facility • Entry through ante-room or airlock • Negative airflow into animal and procedure rooms • Hand washing sink near exit of animal or procedure room
ABL-4 **(not at UAMS)**	• Dangerous/exotic agents which pose high individual risk of aerosol-transmitted laboratory infections that are frequently fatal, for which there are no vaccines or treatments • Agents with a close or identical antigenic relationship to a BL-4 agent until data are available to redesignate the level • Related agents with unknown risk of transmission	ABL-3 practices plus: • Entrance through change room where personal clothing is removed, and laboratory clothing is put on; shower on exit • All wastes are decontaminated before removal from the facility	ABL-3 Equipment plus: • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities	ABL-3 Facility plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in BMBL text

Section 8: Recombinant or Synthetic Nucleic Acid (rDNA) Research

Research involving recombinant or synthetic nucleic acid molecules (or rDNA) is regulated by the National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.* The NIH defines recombinant and synthetic nucleic acid as:

1. Molecules that A) are constructed by joining nucleic acid molecules and B) can replicate in a living cell (i.e., recombinant nucleic acids)
2. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e., synthetic nucleic acids)
3. Molecules that result from the replication of those described in (1) or (2) above

The NIH *Guidelines* apply to all recombinant or synthetic nucleic acid research within the United States that is within the category of research described in (1) or (2) below:

1. Research that is conducted at or sponsored by an institution that receives any support for recombinant or synthetic nucleic acid research from NIH, including research performed directly by NIH. An individual who receives support for research involving rDNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH *Guidelines*.
2. Research that involves testing in humans of materials containing recombinant or synthetic nucleic acids developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

As a condition for NIH funding of rDNA research, institutions must ensure that such research conducted at or sponsored by the institution, irrespective of the funding source, shall comply with the NIH *Guidelines.*

Note: The phrase "rDNA" is used throughout this document to refer to recombinant and synthetic nucleic acid molecules. This reflects changes in the March 2013 version of the NIH *Guidelines*. "Recombinant and synthetic nucleic acid molecules" and "rDNA" are used interchangeably in this document.

8.1 Responsibilities of the Principal Investigator

The responsibility for ensuring that recombinant DNA activities comply with all applicable NIH guidelines belongs to the Principal Investigator (PI). Before research involving rDNA begins, the PI must submit a Biosafety Protocol to the IBC and receive IBC approval.

When working with rDNA materials, effective biological safety programs involve selecting the appropriate biological containment method, including the physical containment facilities. It is the responsibility of the PI to assess the potential risk associated with the experiment and determine an appropriate host-vector system (biological containment) and physical containment to be used for the proposed experiment. The IBC will review this risk assessment.

Core responsibilities for Principal Investigators include:

- Identifying if their research is subject to the NIH *Guidelines*
- Determining which section of the NIH *Guidelines* are applicable to their work
- Proposing an appropriate biocontainment level (BL) to ensure their research is performed safely
- Obtaining authorization from the UAMS IBC prior to starting any research that is subject to the NIH *Guidelines*

Non-exempt recombinant DNA experiments, which must be registered and approved prior to initiation include experiments that involve:

- The deliberate transfer of a drug resistance trait to a microorganism
- Human gene transfer
- Cloning DNA or RNA encoding molecules lethal to vertebrates at an LD50 of <100ng/kg body weight
- Human or animal pathogens as host-vector systems
- Cloning of DNA or RNA from certain pathogens
- Recombinant DNA work in whole animals or plants
- Large-scale DNA work with more than 10 liters of culture

Principal Investigators are also responsible for ensuring their laboratory workers are trained in safe work practices and that everyone working in the laboratory is aware of the emergency response procedures that must be followed after an incident. Staff must also be alerted of the rationale for any special medical surveillance restrictions or immunizations. Once work has been initiated, PIs must also supervise and monitor staff for their adherence to safety protocols.

If the scope of research changes significantly, the PI must amend their Biosafety Protocol and submit it to the UAMS IBC, then await approval of the protocol change. It is also important that PIs report any significant problem, such as a violation of the NIH *Guidelines* or any significant research related accidents, exposures or illnesses to the UAMS Biological Safety Officer (501-686-5536).

For more information on responsibilities regarding work with rDNA, refer to the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, Section IV: Roles and Responsibilities.

8.1.1 Required Information for rDNA Research Proposal

For every recombinant DNA research proposal (except for exempt experiments) the following information must be submitted to the IBC as part of the Biosafety Protocol:

1. Host strain(s) used (include genus, species, and parent strain)
2. Source of DNA/RNA sequences (include genus, species, gene name and abbreviation, and the function of the gene, if known)
3. Recombinant plasmid(s)/vectors used, and source supplier provided
4. If there will be an attempt to obtain a foreign gene, identify the gene and gene function
5. Indicate if this project will require large-scale fermentation (≥ 10 liters) of organisms containing rDNA molecules

6. Indicate whether the project will require the release of organisms containing rDNA into the environment
7. The containment conditions that will be implemented as specified by the NIH *Guidelines*
8. If the project will involve the use of transgenic plant or animal species, you must identify them
9. Indicate whether there will be any attempt to transfer rDNA molecules *in vivo* to animal systems other than tissue culture

The descriptions provided in the Biosafety Protocol submitted to the IBC must provide sufficient information about the experiments so that reference to other documents is not required.

The Biosafety Protocol form is available on the Department of Occupational Health and Safety website, found here: http://www.uams.edu/campusop/depts/ohs/divisions.aspx?listid=bio.

8.2 rDNA Biosafety Level Criteria

The NIH *Guidelines* address physical and biological containment for rDNA research involving humans, animals, plants and large-scale use, including standard microbiological practices, special practices, containment equipment and laboratory facilities.

8.2.1 Physical Containment

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. There are four levels of physical containment, which are designated by the NIH as BL1, BL2, BL3, and BL4. The NIH *Guidelines* also allow for alternative selections of primary containment dependent on the level of biological containment provided by the host-vector system used in the experiment. For detailed information on the criteria for each containment level, see the NIH *Guidelines,* Appendix G-II: Physical Containment Levels.

8.2.2 Biological Containment

Experiments involving rDNA molecules, by their nature, lend themselves to biological containment. Natural barriers exist which limit either the infectivity of a vector or vehicle (plasmid, bacteriophage, or virus) to specific hosts or its dissemination and survival in the environment.

The vectors that provide the means for replication of the rDNA and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of rDNA molecules outside the laboratory.

To determine biological containment, the vector (plasmid, organelle, or virus) for the recombinant or synthetic nucleic acid molecule and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together.

For detailed information on the criteria for biological containment levels, see the NIH *Guidelines,* Appendix I: Biological Containment.

8.3 NIH Recombinant DNA Review Categories

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 (Section III-A to Section III-F) addressing different types of rDNA research.

Note: If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B or III-C shall be followed. If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the NIH *Guidelines*.

8.3.1 Section III-A: Experiments that Require IBC Approval, RAC Review, and NIH Director Approval Before Initiation

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 NIH Office of Science Policy. In addition, the proposal must be published in the Federal Register for 15 days of comment, it needs to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC), and specific approval by the NIH must be obtained. The containment conditions for such an experiment will be recommended by the NIH OSP and set by the NIH at the time of approval. The proposal requires IBC approval before initiation. For more details, see the NIH *Guidelines*, Section III-A.

8.3.2 Section III-B: Experiments that Require NIH OSP and IBC Approval Before Initiation

If the proposed research falls within Section III-B of the NIH *Guidelines*, the research cannot be initiated without submission of relevant information on the proposed experiment to NIH Office of Science Policy. Experiments covered in Section III-B include the cloning of toxic molecules. The containment conditions for such experiments will be determined by NIH OSP in consultation with *ad hoc* experts. Such experiments require IBC approval before initiation. For more details, see the NIH *Guidelines*, Section III-B.

8.3.3 Section III-C: Experiments that Require IBC and IRB Approvals and RAC Review (if applicable) Before Research Participant Enrollment

Section III-C covers experiments involving human subjects. These experiments require IBC and IRB (Institutional Review Board) approval as well as NIH registration before initiation.

Experiments Involving Human Gene Transfer

Experiments involving human gene transfer are covered in the NIH *Guidelines* under Section III-C.

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
    a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
c. Have the potential to replicate in a cell; or
d. Can be translated or transcribed.

No research participant shall be enrolled until the NIH protocol registration process has been completed. The NIH will make a determination in the protocol review whether a proposed human gene transfer experiment meets the requirements for selecting protocols for RAC review and discussion. For details regarding the process of public RAC review and discussion, see the NIH *Guidelines*, Section III-C-1. Approval from the UAMS IBC and IRB are also required for these experiments. For more details, see the NIH *Guidelines*, Section III-C.

8.3.4 Section III-D: Experiments that Require IBC Approval Before Initiation

Section III-D covers whole animal 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 Principal Investigator must submit a Biosafety Protocol and receive approval from the IBC for all experiments in this category prior to their initiation. Experiments in this section involving whole animals must also have approval from the UAMS IACUC (Institutional Animal Care and Use Committee) prior to initiation. For more details, including exceptions, see the NIH *Guidelines*, Section III-D.

8.3.5 Section III-E: Experiments that Require IBC Notice Simultaneous with Initiation

Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, and their subsections are considered in Section III-E. All such experiments may be conducted at BL1 containment. For experiments in this category, a Biosafety Protocol must be submitted by the PI and filed with the UAMS IBC at the time the experiment is initiated. The IBC will review and approve all such proposals, but IBC review and approval prior to initiation of the experiment is not required. Examples of experiments to be included in this section include those in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-E and may be conducted at BL1 containment. For more details, see the NIH *Guidelines*, Section III-E.

8.3.6 Section III-F: Exempt Experiments

Experiments that fall into Section III-F are considered exempt from the NIH *Guidelines* and require a UAMS Exempt Recombinant DNA Registration form to be completed and submitted to the IBC. This form can be requested from the IBC. Other federal and state standards of biosafety may still apply to such research. For details on which experiments are considered exempt, see the NIH *Guidelines*, Section III-F.

Table 7. Level of Review for rDNA Experiments

Level of Review	Example of rDNA Experiment	NIH Guideline Section
IBC Approval RAC Review NIH Director Approval	Experiments that compromise the control of disease agents in medicine through deliberate transfer of a drug resistance trait to a microorganism not known to acquire the trait naturally.	Section III-A
IBC Approval NIH Review	Cloning of toxin molecules with an LD50 of <100 ng/kg.	Section III-B
IBC Approval IRB Approval NIH Review	Introduction of rDNA into human subjects (human gene transfer) or vaccines.	Section III-C
IBC Approval prior to initiation	Wide range (rDNA experiments involving pathogens, defective vectors, animals, plants, or large-scale cultures).	Section III-D
IBC Notice at initiation	Creating transgenic rodents that require BL-1 containment, low risk rDNA plant experiments.	Section III-E
Exempt—Requires Exemption Form	Those that do not represent a significant risk to health or the environment.	Section III-F

8.4 rDNA Accident, Spill, and Disposal Procedures

A spill contingency plan must be described and implemented as part of the laboratory specific safety manual. This plan must provide for the containment as well as the safe clean up and decontamination of any spilled recombinant or synthetic nucleic acid molecule material. Disposal methods must be documented.

See Appendix C for the Recombinant or Synthetic Nucleic Acid Molecules Emergency, Spill, and Exposure Response Procedures and Reporting Requirements.

Section 9: Nanotechnology

Currently, there is limited occupational safety information on nanoparticles and nanomaterials in the university research environment. The purpose of this section is to proactively address the safety issues in the emerging field of nanotechnology and ensure that UAMS employees performing nanotechnology research are aware of the potential hazards and risks involved and the control measures that should be utilized to limit exposures. Research involving the use of nanotechnology must have a biosafety protocol submitted to the IBC for review and approval. The protocol must be approved by the IBC before research is initiated.

9.1 Nanoparticles and Nanomaterials

Nanotechnology involves the manipulation of matter at nanometer scales to produce new materials, structures, and devices. Nanoparticles are materials that have been engineered to have a size with at least one dimension (length, width, or depth) between 1 and 100 nm and may exhibit unique physical and chemical properties not seen in larger molecules of the same composition. Nanoparticles may be suspended in a gas or liquid or embedded in a matrix. When in these states they are commonly referred to as nanomaterials. Substantial private and public investments are flowing into the exploration and development of products that can take advantage of the unique properties of nanomaterials.

Currently, there are no federal regulations that specifically address the environmental, health and/or safety issues of nanotechnology. At UAMS, several research groups are researching nanomaterials for use in various applications. Researchers must consider the potential health and environmental risks that might result during this research and development. This section will provide guidance to UAMS researchers working with nanotechnology and will evolve as nanotechnology regulations increase.

9.2 Working Safely with Nanomaterial

Though there are currently no federal regulations surrounding nanotechnology, the following are recommended control methods for research involving nanoparticles or nanomaterials.

9.2.1 Inhalation Exposure Control Methods

Nanoparticles will follow airstreams making them easily collected and retained in standard ventilated enclosures such as fume hoods and biosafety cabinets with HEPA filters. Synthesis of nanomaterials in enclosed reactors or glove boxes will prevent airborne exposures. Inhalation exposures can occur when processing materials are removed from reactors. To minimize the risk of inhalation exposure, the following practices should be followed:

- Work with nanomaterials should be performed in a fume hood or, where practicable, a biosafety cabinet with a HEPA filter.
- Maintenance on reactor parts that might cause the release of residual particles should be performed in a fume hood or, where practicable, a biosafety cabinet with a HEPA filter.
- Any work with nanomaterials in solution form should also be performed in a fume hood or biosafety cabinet with HEPA filter.
- Research techniques should stress slow and careful handling of the material to prevent aerosolization.

9.2.2 Dermal Exposure Control Measures

The ability of nanoparticles to penetrate skin is unknown at this time, therefore gloves should be worn when handling particulates or particles in solution. In addition, the following practices should be followed:

- For liquids, gloves should have good chemical resistance to the solute.
- For dry particulates, a sturdy glove such as nitrile laboratory gloves with good integrity should be used.
- Disposable nitrile laboratory gloves should provide good protection for most procedures that don't involve extensive skin contact. If contact is extensive, double-gloving should be performed.
- There should be no exposed skin around the hands and wrists.
- Check gloves regularly for holes, tears, etc.
- Wash hands immediately after removing gloves.

9.2.3 Ingestion Control Measures

When working with nanoparticles or nanomaterials, normal hygienic principles should be followed:

- Scrupulously avoid hand-to-mouth contact.
- Wear gloves at all times where there is potential for exposure to nanoparticles.
- Wash hands immediately after removing gloves.
- No eating, drinking, smoking, applying cosmetics, etc. in the laboratory or before hands are washed.

It is advised that the Standard Microbiological Practices found in Section 5, Subsection 5.1 in this manual are also followed.

9.2.4 Injection Control Measures

When working with nanoparticles or nanomaterials, follow normal laboratory sharps work procedures. Sharps procedures can be found in Section 5, Subsection 5.3.4 (Sharps, Needles, and Syringes) in this manual.

9.2.5 Clean-Up of Nanomaterials

In the event of a spill the following procedure should be followed:

- Use dampened cloths to wipe up powders. Apply absorbent materials suitable for the solute to large liquid spills.
- Dry sweeping or using compressed air is NEVER an appropriate clean-up method.
- Use a HEPA filtered respirator and double gloves when cleaning up large spills.

9.2.6 Disposal of Nanomaterials

Recommended disposal of nanoparticles or nanomaterials are as follows:

- Never dispose of nanoparticle waste in regular trash or down the drain.
- When disposing of dry nanoparticle waste, use a sealable container that remains closed.

- Dispose of all nanoparticle waste, including contaminated debris, as you would the base material (e.g., carbon nanotubes should be disposed of as carbon, metallic particles consistent with base metal).
- If the nanoparticles are in solution, they should be managed as a solution of the solvent and the parent nanomaterial (e.g., flammable solvents are handled as flammable waste materials).
- All nanoparticle waste must be labeled with the base metal or solute and identified as containing nanomaterial.
- Proper disposal of nanoparticle waste will be based on the type of material and will be coordinated through the UAMS Biohazard Waste Pickup Team.
- Please call OH&S at 501-686-5536 for further details pertaining to disposal.

9.3 Nanotechnology Safety Resources

Laboratory personnel should be made aware of and trained in the hazards and uncertainties associated with nanomaterials by utilizing nanotechnology safety resources. The following resources offer recommendations for safe practices when working with nanotechnology:

- OSHA Fact Sheet: Working Safely with Nanomaterials
  https://www.osha.gov/Publications/OSHA_FS-3634.html

- CDC and NIOSH "Safe Nanotechnology in the Workplace"
  https://www.cdc.gov/niosh/docs/2008-112/

- NIH Environmental Health & Toxicology
  "Nanotechnology Health Information Resources"
  https://envirotoxinfo.nlm.nih.gov/nanotechnology-and-human-health.html

- Safe Nano "Current Guidance for Safe Handling and Control of Nanomaterials"
  https://www.safenano.org/knowledgebase/guidance/safehandling/

Section 10: Biohazardous Spill Response

It is important to be prepared for spills of biohazardous materials prior to a spill incident. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. In addition, a spill procedure should be included in the laboratory specific safety manual; the procedure should be appropriate for the biohazard material being used in the laboratory. The purpose of this section is to serve as a guide for biohazardous spill clean-up; the information contained in this section will need to be modified for specific situations.

In case of emergency, stay calm, call 911 if necessary, and proceed with common sense. If further assistance is required, call OH&S at 501-686-5536. If the spill outgrows the resources in the laboratory, notify OH&S and the Biological Safety Officer (501-686-5536).

For spills involving rDNA, refer to Section 8.4 of this manual. A more detailed rDNA spill procedure can be found in Appendix C.

10.1 Responsibility

Each PI is responsible for developing spill clean-up procedures appropriate for the materials used in the laboratory, as well as assuring that a spill kit or spill clean-up materials are stored in an easily accessible location within the laboratory.

Furthermore, anyone working with biological materials must receive training in spill clean-up appropriate for the biological agents routinely used. It is the responsibility of the PI to ensure all laboratory workers complete this training. Laboratory Biosafety Spill Kit/Station and Emergency Notification signage must be current, available, and maintained.

For spills involving rDNA, refer to Section 8.4 of this manual. A more detailed rDNA spill procedure can be found in Appendix C.

10.2 Biohazardous Spill Kit

In most instances, a basic spill kit can be assembled with materials already used in the laboratory. All labs operating at BL-2 or higher must have an assembled spill kit available in the laboratory. In BL-1 labs, although it is preferable to have the contents of the spill kit in one location, as long as the materials are easily accessible to everyone in the laboratory, prior assembly may not be necessary.

Biohazardous spill kits can be packed in a 5-gallon plastic bucket or container. The bucket/container should be clearly labeled to indicate that it is a biohazardous spill kit. Spill kits can be assembled to fit specific laboratory needs. The following table lists basic biohazardous spill kit components.

Table 8. Biohazardous Spill Kit Components

An appropriate chemical disinfectant	• A freshly prepared 1:10 dilution of household bleach, or • Other disinfectant appropriate for agent in use
Absorbent Materials	• Paper towels, • Absorbent pads, or • Any other special materials designed to absorb large volumes of liquid
Personal Protective Equipment (PPE)	• Nitrile or heavy duty gloves, • Long-sleeved lab coat or gown, • Safety glasses or goggles, • Facial protection for large spills, and • Any additional PPE required for agent in use
Mechanical tools to pick up broken glass	• Tongs, forceps, scoops, sponges, • Autoclavable dust pan, or • Any other method that prevents direct contact with broken glass
Waste container for treatment and disposal	• Biohazard bags for clean-up waste, • Sharps container for broken glass, and • Plastic bucket or other secondary container for transport
Printed spill clean-up instructions	• Laboratory specific spill clean-up procedures should be available and accessible for laboratory staff.

10.2.1 Biohazardous Spill Kit Maintenance

Your biological spill kit should be restocked after each use. It should also be checked for completeness on an annual basis. The following maintenance activities should be done regularly:

- Check expiration on disinfectant and replace as needed (e.g., bleach should be replaced every 6 months once opened and after 24 hours when diluted);
- Replace gloves;
- Replace absorbent material if old or disintegrating;
- Check waste container for cracks or other damage;
- Ensure PPE is present and free of damage.

10.3 Biohazardous Spill Procedures—Spills Inside the Laboratory

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. All spills or breaks involving biohazardous materials should be cleaned up using appropriate biosafety procedures. An appropriate decontaminant for the material that is to be cleaned up must be identified. If there is any doubt about what to do, call the PI or the UAMS Biological Safety Officer (501-686-5536).

If a spill occurs inside the laboratory, clear out personnel from the area. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing. Don a disposable gown or lab coat, safety goggles, and gloves and have a complete biohazardous spill kit ready to go before you start the clean-up.

10.3.1 Small Spills with NO Broken Glass/Sharps

1. Remove spill supplies from container and line the container with a biohazard bag.
2. Don appropriate PPE.
3. Prepare the disinfectant solution, following the laboratory specific safety manual procedure.
4. Cover the spill area with absorbent material.
5. If using an absorbent powder, use a broom and dustpan to remove the powder and discard it in the biohazard bag. If using absorbent material such as paper towels, place them in the biohazard bag for disposal.
6. Spray the contaminated area with the appropriate disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
7. Repeat step 6 to allow for sufficient disinfection of contaminated surfaces.
8. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazardous waste.
9. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
10. Wash your hands thoroughly after removing gloves.
11. Restock spill kit for next use.
12. Reopen the area to general use only after spill clean-up and decontamination is complete.

10.3.2 Small Spills Involving Broken Glass/Sharps

1. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps.
2. Don appropriate PPE.
3. Prepare the disinfectant solution, following the laboratory specific safety manual procedure.
4. Using tongs or forceps, place broken glass/sharps in sharps container. Do not pick up any contaminated sharp object with your hands.
5. Cover the spill with absorbent material.
6. If using an absorbent powder, use a broom and dustpan to remove the powder and discard it in the biohazard bag. If using absorbent material such as paper towels, place them in the biohazard bag for disposal.
7. Spray the contaminated area with the appropriate disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
8. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
9. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazardous waste.
10. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
11. Wash your hands thoroughly after removing gloves.
12. Restock spill kit for next use.
13. Reopen the area to general use only after spill clean-up and decontamination is complete.

10.3.3 Large Spills Inside the Laboratory

DO NOT attempt to clean up a major spill if you do not have the proper training, resources, or confidence. For assistance, contact the PI, laboratory supervisor, or OH&S (501-686-5536).

1. Hold your breath and leave the room immediately. Ask other laboratory occupants to leave the room. Close all doors.
2. Warn others not to enter the contaminated area and post a sign on all doors.
3. Remove any contaminated PPE and place in a biohazard bag for autoclaving.
4. Thoroughly wash your hands and any exposed areas of the body.
5. Wait 30 minutes for aerosols to dissipate.
6. Assemble spill clean-up materials.
7. Don appropriate PPE before re-entering the room (e.g., long-sleeve gown, goggles, and nitrile or heavy-duty gloves).
8. Remove any contaminated sharp items from the spill using tongs or forceps and place in sharps container. Do not pick up any contaminated sharp object with your hands.
9. Slowly pour an appropriate disinfectant solution around the spill and allow the solution to flow into the spill. Paper towels soaked with the disinfectant may also be used to cover the area. Wait the appropriate contact time.
10. Remove excess disinfectant by wiping with a sponge or several paper towels. Place contaminated clean-up materials in a biohazard bag.
11. Repeat steps 9-10 to allow for sufficient disinfection of contaminated surfaces.
12. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazard waste.
13. Wash hands thoroughly after removing gloves.
14. Restock spill supplies for next use.

10.3.4 Spills Inside a Biological Safety Cabinet that are Confined to the Work Surface

1. Do not turn off the BSC during spill clean-up. Do not place your head inside the cabinet or under the sash at any time.
2. Don appropriate PPE.
3. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps if necessary.
4. Remove any contaminated sharp items from the spill using tongs or forceps and place in sharps container. Do not pick up any contaminated sharp object with your hands.
5. Cover spill with absorbent material.
6. Slowly pour an appropriate disinfectant around the spill and allow to flow into the spill. Wait the appropriate contact time.
7. Wipe up spill, work surfaces, walls, and any equipment in the BSC with paper towels dampened with disinfectant.
8. Place contaminated paper towels and other clean-up materials in the biohazard bag.
9. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
10. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazardous waste.
11. Wash hands thoroughly after removing gloves.
12. Restock spill kit for next use.

10.3.5 Spills Inside a Biological Safety Cabinet that Flow into Front or Rear Grills

1. Do not turn off the BSC during spill clean-up. Do not place your head inside the cabinet or under the sash at any time.
2. Don appropriate PPE.

3. Close the drain valve under the BSC if open.
4. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps if necessary.
5. Remove any contaminated sharp items from the spill using tongs or forceps and place in sharps container. Do not pick up any contaminated sharp object with your hands.
6. Flood the top work surface tray and drain pans and catch basin (if a Class II BSC) below the work surface with an appropriate disinfectant. Wait the appropriate contact time.
7. Remove excess disinfectant from the work surface by wiping with a sponge or cloth. For Class II BSCs, drain the tray into the catch basin below the work surface, lift out the tray and removable front intake grill, and wipe top and bottom surfaces with sponge or cloth soaked in disinfectant. Then replace and drain disinfectant from base into appropriate container, and dispose via the sewer.
8. Place contaminated paper towels and other clean-up materials in a biohazard bag.
9. Repeat steps 6-8 to allow for sufficient disinfection of contaminated surfaces.
10. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazardous waste.
11. Wash hands thoroughly after removing gloves.
12. Restock spill kit for next use.

10.3.6 Spills Inside a Centrifuge

1. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps if necessary.
2. Don appropriate PPE (e.g., lab coat, gloves, safety glasses or goggles) prior to opening the centrifuge.
3. Carefully open the centrifuge to assess the damage. If the spill is contained within a closed cup, bucket, or rotor, spray the exterior with an appropriate disinfectant and wait the appropriate contact time.
4. Remove the carrier to the nearest biosafety cabinet.
5. Open the centrifuge rotor or bucket inside of the BSC. Remove any contaminated sharp items using tongs or forceps and place in sharps container. Do not pick up any contaminated sharp object with your hands.
6. Wipe carrier/bucket with disinfectant. After disinfection, wash the carrier, bucker, or rotor with a mild soap and water.
7. Spray the interior of the centrifuge chamber with disinfectant and wait the appropriate contact time. Wipe the interior of the centrifuge and dispose of all clean-up materials in a biohazard bag.
8. Remove any contaminated PPE in a manner to avoid cross-contamination. Dispose of as biohazardous waste.
9. Wash hands thoroughly after removing gloves.
10. Restock spill kit for next use.

If you are concerned that the spill is not contained within the rotor or bucket:

1. Ensure that other people in the area are notified that a spill has occurred, and the room should be evacuated. Post a sign on all doors and notify the PI or laboratory supervisor.
2. If you need assistance with the spill clean-up, contact OH&S (501-686-5536).
3. Wait 60 minutes before re-entering the room to allow aerosols to settle.

4. Proceed with clean-up as described above.

10.4 Biohazardous Spill Procedures—Spills Outside the Laboratory

The following procedures are provided as a guideline to biohazardous spill clean-up outside the laboratory in public spaces. All spills or breaks involving biohazardous materials should be cleaned up using appropriate biosafety procedures. An appropriate disinfectant for the material that is to be cleaned up must be identified. If there is any doubt about what to do, call the PI, laboratory supervisor, or the UAMS Biological Safety Officer (501-686-5536).

10.4.1 Spills During Transport

All biohazardous materials must be transported in a secondary, leak-proof container to minimize the potential for spills. Use a cart if necessary. If a spill does occur in a common hallway or public space, cordon off the area, restrict access, and disinfect the spill with appropriate disinfectant. Contact OH&S for assistance (501-686-5536).

10.4.2 Radioactive Biohazardous Spills

Anyone working with both radioactive and biohazardous materials should develop a spill clean-up plan appropriate for all materials used. The following general principles should apply:

- Contain the spill
- Prevent spreading the contamination
- Choose methods for decontamination that do not create "mixed waste"
    - NOTE: The use of bleach may be incompatible with some radioactive materials, especially those containing radioiodine

Contact the Radiation Safety Officer and Biological Safety Officer in OH&S (501-686-5536) for additional information concerning these materials and creating an appropriate clean-up plan.

10.5 Spill Exposure Response Procedures

The following procedures are provided as a guideline to biohazardous spill exposures and will need to be modified for specific situations. If there is any doubt about what to do, call the PI, laboratory supervisor, or the UAMS Biological Safety Officer (501-686-5536). In the event of an exposure incident an Injury & Incident Report must be completed. This form can be found here: http://www.uams.edu/campusop/depts/ohs/forms/accident.aspx.

10.5.1 Exposure to Skin or Clothing

1. Stop work. Remove any contaminated clothing and wash affected area thoroughly with soap and water, careful not to abrade the skin.
2. If necessary, use the emergency shower in the laboratory or exit the laboratory and immediately take a shower, washing thoroughly with soap and water. Be careful not to abrade the skin.
3. Notify the PI or laboratory supervisor of the exposure.
4. If exposed to BL-2/RG-2 (or above) agent, notify the UAMS Biological Safety Officer (501-686-5536).

10.5.2 Exposure to Penetrating Wound

1. Stop work and wash the affected area immediately with soap and water.
2. Notify the PI or laboratory supervisor, who must notify the Biological Safety Officer.

3. Proceed to Employee Health/Student Preventative Services M-F 8:00-4:00 or the UAMS Emergency Department if after hours.

10.5.3 Exposure to Eyes or Mucous Membranes

1. Stop work. Immediately flush eyes or mucous membranes with water for 10-15 minutes.
2. Notify the PI or laboratory supervisor, who must notify the Biological Safety Officer.
3. Proceed to Employee Health/Student Preventative Services M-F 8:00-4:00 or the UAMS Emergency Department if after hours.

10.5.4 Emergency Spills: Environmental Risk

1. Stop work. Ensure that any other people in the vicinity are notified that a spill has occurred and that the room should be evacuated. Post a sign on all doors and notify the PI or laboratory supervisor.
2. Call OH&S for assistance (501-686-5536).
3. Take precautions to limit exposure or spread of spill to other areas.

Section 11: Decontamination

Decontamination is defined as the process of removing or inactivating microorganisms to render surfaces, areas, devices, or equipment reasonably free from a risk of transmission of infectious agents or materials to laboratory personnel, the public, and the environment. In addition, it reduces or eliminates cross-contamination within the laboratory.

This section describes basic strategies for decontamination in laboratories to minimize the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment. Methods for sterilization and disinfection and the levels of antimicrobial activity associated with liquid chemical germicides are reviewed. The following are considered general approaches, not detailed protocols and methods. The PI is responsible for including laboratory specific decontamination procedures in the laboratory specific safety manual.

11.1 Decontamination Terms

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

- Antimicrobial—An agent that kills microorganisms or suppresses their growth and multiplication.
- Antiseptic—A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- Biocide—A general term for any agent that kills organisms.
- Chemical germicide—A chemical or mixture of chemicals used to kill microorganisms.
- Disinfectant—A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- Microbicide—A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide," "chemical germicide" or "antimicrobial."
- Sporicidal—A chemical or mixture of chemicals used to kill microorganisms and spores.

11.2 Decontamination Methods

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

When choosing a method of decontamination, it is important to consider the following aspects:

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

Ways to Decontaminate

Physical and chemical means of decontamination fall into four main categories:

- Heat

- Liquid Chemicals
- Vapors and Gases
- Radiation

Disinfection if normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (e.g., steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time.

11.2.1 Heat

The application of heat, either wet or dry heat, is recommended as the most effective method of sterilization. The advantage of wet heat is a better transfer of heat to and into the cell, resulting in overall shorter exposure time and lower temperature. Steam at 121°C under pressure in the autoclave is the most convenient method of rapidly achieving sterility under ordinary circumstances. This type of heat kills all microbial cells including spores, which are normally heat resistant. Dry heat at 160°C to 170°C for periods of two to four hours is required for destruction of viable agents on an impermeable non-organic material such as glass but is not reliable in even shallow layers of organic or inorganic material that can act as insulation. Incineration is another use of heat for decontamination and serves as an efficient means of disposal for human and animal pathological wastes.

The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure could be a source of scalding jets if the equipment is misused. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too soon after treatment. This may cause a sudden and violent boiling of contents from the containers that can splash scalding liquids onto personnel handling the containers. Always follow the manufacturer's guidelines for use when operating equipment.

11.2.2 Liquid Decontaminants

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. In general, liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes.

When choosing a liquid decontaminant, consideration must be given to such factors as temperature, contact time, pH, the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Number of microorganisms present, and resistance of microorganisms should also be considered. Small variations in these factors may make large differences in the effectiveness of decontaminants. See Table 9 below for microorganism resistance to chemical disinfectants.

Table 9. Descending Order of Microorganism Resistance to Germicidal Chemicals

BACTERIAL SPORES *Bacillus subtilis, Clostridium sporogenes* ↓
MYCOBACTERIA *Mycobacterium tuberculosis* var. *bovis,* non-tuberculous mycobacteria ↓
NONLIPID OR SMALL VIRUSES Poliovirus, Coxsackievirus, Rhinovirus ↓
FUNGI *Trichophyton* spp., *Cryptococcus* spp., *Candida* spp. ↓
VEGETATIVE BACTERIA *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella choleraesuis,* Enterococci ↓
LIPID OR MEDIUM-SIZE VIRUSES Herpes simplex virus, cytomegalovirus, respiratory syncytial virus, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), Hantavirus, Ebola virus

Adapted from CDC BMBL, 5th Edition, Appendix B.

Note: There are exceptions to this list. *Pseudomonas* spp. are sensitive to high-level disinfectants, but if they grow in water and form biofilms on surfaces, the protected cells can approach the resistance of bacterial spores to the same disinfectant. The same is true for resistance to glutaraldehyde by some non-tuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Cheatomium globosum*, and the pink-pigmented *Methylobacteria*. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this section.

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids and alkalies, heavy metal salts, quaternary ammonium compounds, phenols, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics such as corrosivity. None is equally useful or effective under all conditions for all infectious agents.

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned to the task of making up use-concentrations from stock solutions must be informed of the potential hazards and trained in the safety procedures to follow and appropriate personal protective equipment to use, as well as the toxicity associated with ocular, skin and respiratory exposure.

The more common liquid disinfectants are discussed below:

Alcohols
Ethyl or isopropyl (rubbing) alcohol in a concentration of 70% to 90% in water are good general-use disinfectants. However, they evaporate quickly 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 than 70% to 90% concentrations.

Formalin
Formalin is 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. A concentration of 8% formaldehyde exhibits good activity against vegetative bacteria, spores, and viruses. Formaldehyde is a human carcinogen and creates respiratory problems at low levels of concentration.

Glutaraldehyde
Glutaraldehyde may be used for cold sterilization of equipment (e.g., medical equipment) that cannot be steam sterilized, but sterilization often requires many hours of exposure. Two percent solutions exhibit good activity against vegetative bacteria, spores, and viruses. Its use, however, must be limited and controlled due to its toxic properties and ability to damage the eyes. Vapors of glutaraldehyde can also irritate nasal passages and the upper respiratory tract. Always use appropriate PPE when using glutaraldehyde.

Phenol and Phenol Derivatives (Phenolics)
Phenols and phenol derivatives come in various concentrations ranging mostly from 5% to 10% phenol-based compounds. These derivatives including phenol have an odor, which can be somewhat unpleasant. Phenol itself is toxic and appropriate PPE is necessary during use. The phenolic disinfectants are used frequently to disinfect contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacteria tuberculosis*, fungi, and lipid-containing viruses. They are not active against spores or non-lipid viruses. Phenols and phenolics should not be used on food contact surfaces.

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. They are less active against Gram-negative bacteria and are not active against non-lipid viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If quaternary ammonium compounds 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 (NaOCl) 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 clean-up

purposes. Chlorine-containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate PPE when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered cold sterilizers since they inactivate bacterial 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.

11.2.3 Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde gas and ethylene oxide. Applied in closed systems under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating biological safety cabinets and associated air-handling systems and air filters; bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may be damaged by other decontamination methods; and rooms, buildings and associated air-handling systems.

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide; toxic and hypersensitivity effects are well-documented for formaldehyde.

In the event a BL-3 laboratory is closed, must undergo major renovations, or has significant changes in laboratory usage, an outside contractor will be employed. A process known as vaporized hydrogen peroxide will be used to decontaminate the laboratory.

For more information regarding the use of formaldehyde and ethylene oxide contact OH&S (501-686-5536).

11.2.4 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. UV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.

Long-term exposure to UV light may be used for disinfecting surfaces and air; however, UV light is not recommended or necessary for use inside biosafety cabinets. This is because UV light is limited by many factors, such as penetrating power, relative humidity, temperature and air movement, lamp cleanliness, and lamp age. It is also harmful to human tissue. Therefore, other means of disinfection (e.g., chemical) are recommended for use inside BSCs.

11.3 Decontaminants for Research using rDNA

Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Oxidizing agents such as bleach will destroy the nucleic acid. However, the chosen decontaminant's ability to destroy the nucleic acid should be confirmed in the laboratory.

Section 12: Transport of Biohazardous Materials

This section outlines the proper procedures for transporting biohazardous agents and laboratory specimens within buildings and between buildings, as well as shipping biohazardous materials. For procedures on transporting biohazardous waste, including rDNA waste, refer to Section 13 of this manual. Biohazardous agents must be packaged so that PPE in not needed during transport. PPE should not be worn in public corridors. 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.

It is the responsibility of the PI to ensure all laboratory personnel responsible for packaging, transporting, or shipping biohazardous materials are trained on proper procedures and requirements.

12.1 Transporting Biohazardous Materials Within Buildings

When packing biohazardous agents for transportation within the same building but through public areas, the following guidelines apply:

1. Biohazardous agents including specimens of blood or other potentially infectious materials must be placed in a primary container that prevents leakage during transportation. A test tube, for example, is a primary container.
2. The primary container must be closed prior to being transported. The test tube, for example, must have a tight-fitting cap or the cap must be taped in place or otherwise secured.
3. Label the container with the name of the PI and the room number.
4. The primary container must then be placed in a leak-proof secondary container. A sealable plastic bag, for example, is a secondary container.

12.2 Transporting Biohazardous Materials Between Buildings

When biohazardous agents are transported between buildings, the following guidelines apply:

1. Biohazardous agents, including specimens of blood or other potentially infectious materials, must be placed in a primary container that prevents leakage during transportation. A test tube, for example, is a primary container.
2. The primary container must be closed prior to being transported. The test tube, for example, must have a tight-fitting cap or the cap must be taped in place or otherwise secured.
3. Label the container with the name of the PI and the room number.
4. The primary container is placed within a leak-proof secondary container, such as a sealable plastic bag. It is good practice to place absorbent material between the bag and tube to cushion the tube and absorb leakage from improperly sealed tubes. It is mandatory that the absorbent material be used for items transported in a motor vehicle.
5. The packages are then placed in an outer transport container labeled with the biohazard symbol. This container can be a cardboard box with a Styrofoam liner, a cooler, or other sturdy transport container.
6. If the material is not transported by the original packager, the outer transport container must show the following information in addition to the biohazard label:

- Identification of the material being transported (e.g., human blood, animal blood, cultures, etc.)
- The name, department, building, box number, and phone number of the receiving party
- The name and phone number of the sender
- The date sent

Anyone involved in packaging or transporting biohazardous materials between buildings, including infectious substances, must have task specific training prior to beginning the assignment and annually thereafter.

12.3 Shipping Biohazardous Materials

The shipment of biohazardous materials, including diagnostic and clinical specimens, biological products, infectious agents, and rDNA molecules is regulated by national and international transportation rules. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation and labeling, and permits. Anyone involved in packaging, shipping, or preparing paperwork for the shipment of biohazardous materials, including infectious substances, must complete the Department of Transportation shipping training before packaging and shipping any biohazardous material. For more information about specific packaging requirements and training, contact OH&S (501-686-5536).

12.4 Resources for Packaging, Shipment and Transportation of Biohazardous Materials

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
  https://www.unece.org/?id=3598

- International Civil Aviation Organization (ICAO)
  Technical Instructions for the Safe Transport of Dangerous Goods by Air
  https://www.icao.int/safety/dangerousgoods/pages/technical-instructions.aspx

- International Air Transport Association (IATA)
  Dangerous Goods Regulations
  https://www.iata.org/publications/dgr/Pages/index.aspx

- U.S. Department of Transportation
  49 CFR Parts 171-178

- U.S. Postal Service
  39 CFR Part 111

- U.S. Department of Labor, OSHA

29 CFR 1910.1030

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

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

Section 13: Biohazardous Waste Management

Some waste associated with biological materials must be disposed of in special ways because they may have been contaminated with infectious organisms or agents. These potential infectious or biohazardous materials are defined by Arkansas regulations as Regulated Medical Waste. This section includes responsibility, definitions, and procedures for biohazardous waste. The subsections will address how to identify, package, transport, and decontaminate different types of biohazardous waste.

13.1 Responsibility for Biohazardous Waste Management

PIs are responsible for developing laboratory specific protocols for properly identifying, packaging, and decontaminating biohazardous waste, including rDNA waste, prior to disposal. These procedures are based on state and federal law, requirements from the Occupational Safety and Health Administration (OSHA), Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH), and good laboratory practice. Failure to manage biological waste properly could result in personal injury, disruption to research, fines, or criminal prosecution. For assistance contact OH&S (501-686-5536).

13.2 Biohazardous Waste Terms

Common terms related to biohazardous wastes are defined in this section.

13.2.1 OSHA Definition of Medical Waste

Regulated waste means liquid or semi-liquid blood or other potentially infectious materials (OPIM); contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

Other Potentially Infectious Waste (OPIM) is defined as 1) the following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, and any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; 2) any unfixed tissue or organ (other than intact skin) from a human (living or deceased); and 3) human immunodeficiency virus (HIV)-containing cell or tissue cultures, organ cultures, and HIV- or hepatitis B virus (HBV)-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

13.2.2 Arkansas Department of Health Definition of Medical Waste

A waste from a generator or a health care related facility as outlined in Section IV of the Arkansas Department of Health Regulations, which if improperly treated, handled, or disposed of may serve to transmit an infectious disease and which includes the following:

- Pathological Waste: All human unfixed tissues, organs and anatomical parts, other than intact skin, which emanate from surgeries, obstetrical procedures, dental procedures,

autopsies and laboratories. Such waste shall be exclusive of bulk formaldehyde and other preservative agents.

- Liquid or Semi-Liquid Blood: This includes human blood, human blood components and/or products made from human blood (e.g., serum, plasma) and other potentially infectious materials, to include regulated human body fluids such as semen, vaginal secretions, cerebrospinal fluid, pleural fluid, pericardial fluid, synovial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, and body fluid that is visibly contaminated with blood and all body fluids where it is difficult or impossible to differentiate between body fluids, not to include urine or feces, which cannot be discharged into the collection system of a publicly owned treatment works (POTW) within the generating facility.
- Contaminated Items: This includes dressings, bandages, packing, gauze, sponges, wipes, personal protective equipment, cotton rolls and cotton balls, etc., which cannot be laundered or disinfected and from which blood, blood components, or regulated body fluids drip freely, or that would release blood or regulated body fluids in a liquid or semi-liquid state if compressed or are caked with dried blood or regulated body fluids and are capable of releasing these materials during handling.
- Microbiological Waste: This includes, but is not limited to, cells and tissue cultures, culture medium or other solutions and stocks of infectious agents, organ cultures, culture dishes, devices used to transfer, inoculate and mix cultures, paper and cloth which has come into contact with specimens or cultures, and discarded live vaccines.
- Contaminated Sharps: This includes, but is not limited to, any contaminated object that can penetrate the skin, such as hypodermic needles, intravenous tubing with needles attached, syringes with attached needles, razor blades used in surgery, scalpel blades, Pasteur pipettes, capillary tubes, broken glass from laboratories, and dental wires. Potentially breakable containers of blood, regulated body fluids, microbiological wastes, or infectious material must be treated as contaminated sharps when disposed of.

13.2.3 Biohazardous Agent

This term refers to an agent that is biological in nature, capable of self-replication, and has the capacity to produce deleterious effects upon biological organisms. Biohazardous agents include, but are not limited to; bacteria, fungi, viruses, rickettsiae, chlamydia, prions, parasites, recombinant products, allergens, cultured human and animal cells and the potentially biohazardous agents these cells may contain, infected clinical specimens, tissue from experimental animals, plant viruses, bacteria and fungi, toxins, and other biohazardous agents as defined by State and Federal regulations.

13.2.4 Biological Waste

Biological waste is any material that contains or has been contaminated by a biohazardous agent. Biological waste includes, but is not limited to; Petri dishes, surgical wraps, culture tubes, syringes, needles, blood vials, absorbent material, personal protective equipment and pipette tips.

13.2.5 Sharps

Sharps are items that are capable of puncturing, cutting or abrading the skin. Sharps include but are not limited to; glass and plastic pipettes, broken glass, test tubes, razor blades, syringes, and needles.

13.3 Identifying Biohazardous Waste

The following materials are defined as biohazardous (biomedical) waste and should be treated as such:

- Sharps waste is regulated by state law. Sharps waste must not be disposed of in the regular waste stream. The term "sharps" is a regulatory waste classification that refers to items used to puncture, cut, or scrape body parts and that, in a waste container, can cause punctures or cuts to solid waste handlers or the public. Therefore, all sharps waste must be placed in appropriate sharps containers and decontaminated prior to disposal. Sharps include the following:
    - Needles, including syringes with needles and IV tubing with needles attached
    - Syringes without needles when removed from their original sterile containers
    - Lancets
    - Scalpel blades
    - Other sharps not defined above only if contaminated with biohazardous material, including rDNA (e.g., broken glass, razor blades, fragile glass tubes, vials, or ampoules, etc.)
- Human and non-human primate blood, blood products, body fluids, tissues, and cells: includes human and non-human primate blood, blood components, and materials containing free-flowing blood and blood products. Both human and non-human primate cells lines, regardless of origin, are also defined as biohazardous waste.
- Cultures and stocks of etiologic agents and associated biologicals: include, but are not limited to specimen cultures, discarded live and attenuated vaccines, cultures and stocks of etiologic agents, and wastes from the production of biologicals and serums.
- rDNA: includes but is not limited to waste products from laboratory research procedures involving rDNA in plasmids, viral vectors, *E. coli*, yeast, cell cultures, as well as naked DNA from polymerase chain reaction (PCR) and sequencing reactions. This also includes tissue and cells harvested from animals containing rDNA (e.g., transgenic animals).
- Laboratory waste that has come in contact with a biohazard as listed above: includes but is not limited to culture dishes, blood specimen tubes, devices used to transfer, inoculate, and mix cultures, and other materials that have come into contact with biohazards (including disposable PPE and clothing).
- Animal waste, animal carcasses, and body parts exposed to pathogens or rDNA: includes animal bedding and other waste from such animals and all non-human primate tissue and carcasses.
- Human pathological waste: includes human source biopsy materials, tissues, and anatomical parts. This does not include teeth, human corpses, remains, and anatomical parts that are intended for interment or cremation.

13.4 Packaging, Handling, and Disposal of Biohazardous Waste

The following are guidelines for packaging and handling of biohazardous waste and may need to be modified for specific situations.

13.4.1 Sharps Waste

All sharps must be placed into properly labeled sharps containers or other rigid, puncture-proof containers. Sharps containers must not be filled more than 2/3 full. Make sure the container is sealed, labeled, and intact. When filled, request pickup of sharps containers by contacting the UAMS Biohazard Waste Pickup Team (501-526-0000) or requesting a pickup online http://www.uams.edu/campusop/depts/ohs/forms/biowaste.aspx.

13.4.2 Solid Biohazardous Waste

Solid waste includes cloth, plastic, and paper items that have been exposed to infectious agents hazardous to humans, animals, or plants, and solidified agarose gels. These contaminated items should be collected in appropriate biohazard waste autoclave bags. When ¾ full, loosely tie the bag closed and secure the lid on the container. Contact the UAMS Biohazard Waste Pickup Team (5001-526-0000) to request pickup or request a pickup online http://www.uams.edu/campusop/depts/ohs/forms/biowaste.aspx.

13.4.3 Liquid Biohazardous Waste

Liquid biological waste should be collected in leak proof, rigid, durable containers for autoclaving or chemical disinfection. Autoclaved or chemically disinfected liquid wastes can be disposed of via the laboratory sink. Do not pour melted agarose down the drain. Allow it to cool and solidify, then dispose of it as solid waste in biohazard waste bags.

13.4.4 Biosafety Level 3 Waste Procedures

All biohazardous waste including RG-2 and RG-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. Exceptions might include animal carcasses that need to be incinerated. Contact the UAMS Biohazard Waste Pickup Team (501-526-0000) to request pickup of waste after autoclaving.

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Acknowledgements

This document was developed from earlier University of Arkansas for Medical Sciences Biosafety and Laboratory Safety Manuals and Standard Operating Procedures (SOP) documents, as well as Safety Manuals developed at other Universities, including Michigan State University, University of Missouri, University of Southern California, University of Utah, University of Washington, and Yale University.

Appendix A
Required Training

New and current employees are required to complete training during the onboarding process and annually thereafter. Ultimately, it is the Principal Investigator's responsibility to ensure that all laboratory workers complete these and laboratory specific trainings. Below are guidelines for new employee and current employee trainings, as well as additional trainings that may be required by the UAMS Department of Occupational Health and Safety. These are only guidelines and may need to be modified in some cases. For questions, contact OH&S at 501-686-5536.

New Employee	
Course	**Format**
Basics of Biosafety Training	Online via My Compass
Chemical/Laboratory Safety	Classroom; register on My Compass
Bloodborne Pathogens	Completed in New Employee Orientation

Current Employee	
Course	**Format**
Basics of Biosafety Training	Online via My Compass
Chemical/Laboratory Safety Refresher	Online via My Compass
Bloodborne Pathogens	Online via My Compass

Additional Courses		
Course	**When Required**	**Format**
NIH Guidelines for Recombinant DNA Research	Required annually for anyone working with rDNA.	Online via My Compass
DOT-IATA Shipping Infectious Substances	Required annually for anyone shipping infectious substances.	Online via My Compass
Zoonoses Training	Required annually for anyone working with animals.	Online via My Compass
Respirator Safety—CO N95 Respirator Safety Training	Required annually for anyone using N95 respirators. Additional information: http://www.uams.edu/campusop/depts/ohs/forms/n95approval.aspx	1. N95 Respirator Medical Evaluation Questionnaire 2. Online via My Compass 3. N95 fit test (within 90 days of completing online training)
Respirator Safety—Air Mate (PAPR) Powered Air Purifying Respirator Training	Required annually for anyone using Air Mate PAPR. Additional information: http://www.uams.edu/campusop/depts/ohs/forms/papr_approval.aspx	1. Respirator Medical Evaluation Questionnaire 2. Online via My Compass 3. Respirator fit test (within 90 days of completing online training)
Respirator Safety—Breathe Easy (PAPR) Powered Air Purifying Respirator Training	Required annually for anyone using Breathe Easy PAPR. Additional information: http://www.uams.edu/campusop/depts/ohs/forms/papr_approval.aspx	1. Respirator Medical Evaluation 2. Online via My Compass 3. PAPR fit test (within 90 days of completing online training)

Biological Safety Training

Links to commonly required Biological Safety Training courses can be found here: http://uams.edu/campusop/depts/traincenter.aspx?ListID=1#viewlist. These courses can be found on the UAMS My Compass website. Once signed in to My Compass, register for the course and complete the training. My Compass will keep a record of your training and serves as training documentation. No additional documentation is required.

Respirator Safety Training

Links to commonly required Respirator Safety Training courses can be found here: http://uams.edu/campusop/depts/traincenter.aspx?ListID=9#viewlist. These courses are also found on the UAMS My Compass website, where you can register for and complete the training after signing in to your account. Be aware that respirator safety training usually requires online training as well as a fit test for the type of respirator you are required to use. This fit test must be completed within 90 days of the online training. For questions, contact OH&S at 501-686-5536.

Additional Training Opportunities

Additional training opportunities can be found here: http://uams.edu/campusop/depts/traincenter.aspx.

Laboratory Specific Training

Laboratory specific training and documentation of training is the responsibility of the PI. Check with your laboratory's PI for required laboratory specific training.

Appendix B
Biohazard Warning Signs

Biohazard warning signs must be posted on doors where required and serve to restrict laboratory access during work with biohazardous material. These signs must communicate the agent(s) in use, specify entry and exit requirements, and list the Principal Investigator's contact information.

This Biohazard Warning Sign template below can be used to fulfill this requirement. To complete the Biohazard Warning Sign, fill in all information, print the sign in color, and post on all necessary doors. The biohazard symbol on the sign is required to have the orange background with the symbol and lettering in a contrasting color and must be printed in color. If a color printer is not available to you, contact OH&S at 501-686-5536 for a color copy.

CAUTION

Admittance to Authorized Personnel Only

Room No.:	Date:

BL-2

Biological Agent(s): _____

Special Procedures, PPE or Precautions For Entry/Exit: _____

Principal Investigator(s)		Emergency Contact	
Name	Phone	Name	Phone

Appendix C
Recombinant DNA Spill Procedure

Recombinant or Synthetic Nucleic Acid Molecules
Emergency, Spill, and Exposure Response Procedures
and Reporting Requirements

This procedure outlines response protocols and reporting requirements for emergencies, spills, and exposures involving recombinant or synthetic nucleic acid molecules at the University of Arkansas for Medical Sciences (UAMS).

The University of Arkansas for Medical Sciences is required to report incidents involving recombinant or synthetic nucleic acid molecules (rDNA) to the National Institutes of Health (NIH) Office of Science Policy (OSP) and other regulatory agencies. This procedure includes necessary information to determine the nature and extent of the incident, appropriate spill and exposure response, and reporting requirements according to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).* *https://osp.od.nih.gov/wp-content/uploads/2013/06/NIH_Guidelines.pdf*

RESPONSIBILITIES

Principal Investigator is responsible for:

- Making available to all laboratory personnel (staff and students) the protocols that describe the potential biohazards and the precautions to be taken.
- Instructing and training laboratory personnel on the emergency, spill, and exposure response procedures outlined here and ensuring that spills are addressed in a prompt manner.
- Utilizing these procedures to tailor emergency, spill, and exposure response plans specific to agents used in their laboratory. The laboratory specific spill procedure must be posted in the lab and include emergency contact name(s) and phone numbers.
- Immediately reporting incidents to the UAMS Biological Safety Officer in the Department of Occupational Health and Safety (501-686-5536) if the incident involves rDNA.

Laboratory Personnel (Staff, Students, Visiting Scientists) are responsible for:

- Adhering to laboratory specific emergency, spill, and exposure response plans.
- Attending all appropriate laboratory safety training.
- Immediately notifying the laboratory supervisor of any incidents, spills, injuries, or possible exposures involving rDNA.

SPILL PROCEDURES

Disinfectants suitable to specific agents/rDNA being used in the laboratory shall be used for spill response procedures and shall be made available to laboratory personnel. The Department of Occupational Health and Safety is available to provide consultation on spill response and will

assist with decontamination and clean-up of large spills. They can be contacted at 501-686-5536.

This document outlines procedures for the following:

- Large spills outside containment
- Small spills outside containment
- Spills inside containment
- Spills inside a centrifuge
- Skin, mucous membrane, or injury exposure
- Reporting requirements for rDNA spills and exposures

1. **Large Spills Outside Containment (Biosafety Cabinet)**
   Spills larger than 200 mL are considered large spills. In the event of a large spill:
   - Evacuate the room, account for all personnel, close all doors, and prevent others from entering. Remove any contaminated clothing and place in autoclave bag, and wash any exposed skin.
   - Notify the laboratory supervisor or PI immediately.
   - Contact OH&S (501-686-5536 during work hours) or the UAMS Call Center (526-0000 after hours) for assistance if necessary.
   - If you are able to clean up the spill yourself, leave all doors closed for at least 30 minutes to allow aerosols to settle before re-entering the room.
   - Prior to re-entering the room for clean-up, don the appropriate personal protective clothing and equipment, which can include, but is not limited to:
     - Lab coat and optional waterproof apron
     - Shoe covers
     - Rubber or nitrile gloves
     - Eye protection (such as goggles or a face shield)
   - Upon re-entry to the room, place paper towels or other absorbent material over the rDNA spill area.
   - Pour an appropriate disinfectant over paper towels starting at the edges of the spill and working towards the center. Avoid splashing or generating aerosols. Disinfect any equipment or surfaces which may have been splashed.
   - Allow disinfectant to remain in contact with spilled rDNA for a minimum of 20 minutes.
   - Use a squeegee and dust pan to recover absorbent materials and put in biohazard bag. Clean area once more with fresh paper towels soaked in disinfectant; place towels in biohazard bag.
   - Any sharps or broken glass must be picked up with forceps and placed in a hard-sided sharps container.
   - Remove protective clothing and dispose of in biohazard bag or segregate for cleaning.
   - Wash hands thoroughly with a disinfectant hand soap before leaving the area.

2. **Small Spills Outside Containment (Biosafety Cabinet)**
   Spills less than 200 mL are considered small spills. The spill response for small spills depends on the size of the spill and hazard of the material.
   - Minimally hazardous materials spilled without generating considerable aerosols can be cleaned with a paper towel soaked in an appropriate disinfectant.
   - Spills of small to moderate size containing low risk agents should follow the same procedures as large spills outside containment.

3. **Spills Inside Containment (Biosafety Cabinet)**
   If a spill occurs in a biosafety cabinet, leave the cabinet turned on during clean-up to contain aerosols and allow exhaust air to be HEPA-filtered.
   - Immediately notify the laboratory supervisor or Principal Investigator of the spill. Notify others in the area.
   - Don appropriate personal protective equipment if not already wearing them, such as gloves, lab coat, and eye protection.
   - While cabinet remains on, cover spilled rDNA material with paper towels or other absorbent material.
   - Pour an appropriate disinfectant over paper towels starting at the edges of the spill and working towards the center, careful not to splash or generate aerosols.
   - Close the sash and allows aerosols to settle for a minimum of 15 minutes. Then wipe up the spill and decontaminate any equipment or surfaces that may have been splashed.
   - If personnel are exposed, remove potentially contaminated clothing at the biosafety cabinet (BSC) and place in autoclave bag. Wash hands and any potentially exposed skin with soap and warm water. Don fresh PPE and continue with the clean-up procedure.
   - If a bleach solution is used as the decontaminant on metal surfaces, rinse the area with water or 70% ethanol after decontamination is complete.
   - If the spill overflows into the interior of the biosafety cabinet, more extensive decontamination may be required. Contact OH&S (501-686-5536) for further instructions.

4. **Spills Inside a Centrifuge**
   Spills inside a centrifuge have the potential for multiple exposures, as aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. Opening centrifuges suspected of having a spill or breakage should occur slowly. Notify the laboratory supervisor or PI of the spill immediately.
   - **Spills Inside a Centrifuge with Unsealed Buckets**
     - If breakage or a spill is suspected while the centrifuge is running, turn the motor off and allow the machine to rest for a minimum of 30 minutes before opening to allow aerosols to settle. If breakage or a spill is discovered after the centrifuge has stopped, re-close the lid immediately and allow the machine to rest for a minimum of 30 minutes before opening.
     - Before beginning the clean-up procedure, unplug the centrifuge.

- Don thick rubber gloves and other appropriate PPE before beginning the clean-up.
- Slowly open the centrifuge lid after a minimum resting period of 30 minutes. Place paper towels or other absorbent material over the entire spill area, then cover the spill with an appropriate disinfectant. Allow a minimum of 20 minutes of contact time.
- Use forceps or other mechanical means to remove and broken tubes or glass fragments, and place them in a sharps container to be decontaminated and disposed of as infectious waste.
- Remove centrifuge buckets, trunnions, and rotor and place in disinfectant for a minimum of 20 minutes or autoclave.
- Unbroken, capped tubes may be placed in disinfectant and recovered after 20 minutes of contact time or autoclaved.
- Use forceps or other mechanical means to remove disinfectant soaked materials from centrifuge bowl and discard in biohazard bag.
- Disinfect the centrifuge bowl once more, wipe down, then wash with water and dry. Discard disinfectant materials in biohazard bag.
- Remove protective clothing used during clean-up and dispose of in biohazard bag or segregate for cleaning.
- Wash hands thoroughly with a disinfectant hand soap before leaving the area.

- **Spills Inside a Centrifuge with Sealed Buckets (Safety Cups)**
  - If breakage or a spill is suspected, remove the sealed bucket and place in biosafety cabinet before opening.
  - If breakage occurred, replace the cap on the sealed bucket loosely and autoclave.

5. Skin, Mucous Membrane, or Injury Exposure

All exposures to recombinant or synthetic nucleic acid molecules or recombinant pathogens must be reported to the laboratory supervisor or PI immediately, as well as the Biological Safety Officer (501-686-5536). If the exposure results in an emergency, call the UAMS Call Center at 501-526-0000 or 911 to request help. Otherwise, the following steps should be taken:

- If there is an agent-specific protocol for exposure, such as those for HIV or Hepatitis B, follow those protocols.
- For skin contact exposure or injury with a contaminated instrument:
  - Thoroughly wash the area with soap and water. Do not squeeze the wound to induce bleeding.
  - Avoid using abrasive chemical soaps or disinfectant washes, as they can cause skin abrasions and a possible additional route of entry for the agent.
  - Cover the wound with sterile dressing.
  - For mucous membranes (e.g., eyes, mouth), flush with water for a minimum of 15 minutes.
- Contact UAMS Student and Employee Health as soon as possible after exposure
  - Exposed personnel must complete the Employee/Student Injury and Incident Report (I&I) Form

    - Employees of UAMS must call the Company Nurse Injury Hotline at 1-855-339-1893 as soon as possible after injury or exposure.
    - During normal business hours, exposed individuals should contact the Preventive Occupational Environmental Medicine Clinic at 501-686-6565 (open 8 a.m. to 4:30 p.m.)
    - After hours, exposed individuals should contact the UAMS Emergency Department at 501-686-2085 for evaluation.
- Any exposures to recombinant DNA must be reported to the following:
    - Principal Investigator or Laboratory Director—Immediately
    - Biological Safety Officer (501-686-5536)—Immediately
    - Institutional Biosafety Committee (IBC)—May be reported through Biological Safety Officer
    - NIH/OBA—Report will be coordinated by the IBC

REPORTING INCIDENTS

Laboratory incidents, illness, exposure, or injury involving recombinant or synthetic nucleic acid molecules or recombinant pathogens or non-compliance with NIH Guidelines may be brought forward by any person and should promptly be reported to the UAMS Biological Safety Officer in the Department of Occupational Health & Safety (501-686-5536) for investigation and reporting to the NIH Office of Science Policy (NIH/OSP).

The University of Arkansas for Medical Sciences is required to report any significant problems or violations of the NIH Guidelines as well as any substantial research-related accidents or illnesses to the appropriate institutional official and the NIH/OSP within 30 days.

Other incidents that are reported to NIH OSP within 30 days include:

- Failure to follow approved containment conditions
- Failure to obtain Institutional Biosafety Committee approval
- Incomplete activation
- Loss of containment
- Loss of a transgenic animal
- Personnel exposure

The Biological Safety Officer will work with the Principal Investigator and the IBC to complete the NIH OSP Template for Reporting Incidents Subject to the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.
The incident report must be sent to NIH OSP within 30 days.

The Biological Safety Officer will submit the final incident report on behalf of the university to NIH OSP via email to NIHGuidelines@od.nig.gov within 30 days.

Template for Reporting Incidents: https://osp.od.nih.gov/wp-content/uploads/Incident-Reporting-Template-2016_2.docx