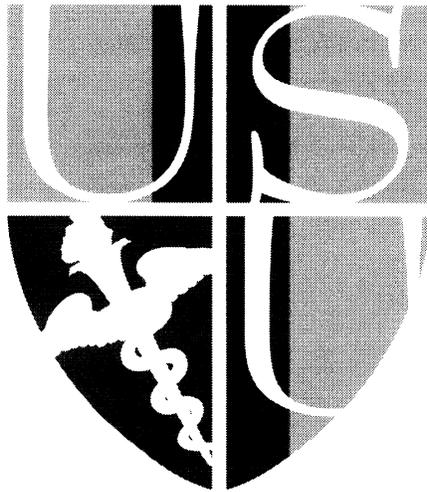


**USUHS
INSTRUCTION
6401**





UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES



SUBJECT: Biological Safety Manual Instruction 6401

(EHS)

SEP 5 2006

ABSTRACT

This Biosafety Manual was developed by the Center for Environmental Health and Occupational Safety in close cooperation with the Institutional Biosafety Committee (IBC) at the Uniformed Services University of the Health Sciences (USUHS). The manual is part of the USUHS Biosafety Program instituted to accomplish the following goals:

- Protect personnel from exposure to infectious agents.
- Prevent environmental contamination.
- Maintain a safe work place that facilitates an environment for high quality research.
- Comply with applicable federal, state and local safety regulations.
- Institute a secure laboratory environment that prevents unauthorized access or use of biological agents.

This manual provides university-wide safety guidelines, policies and procedures for the use of biohazards. This Instruction complements USUHS Instruction 6408, "Center for Disease Control Select Biological Agents Management" and USUHS Instruction 6403, "Biohazard Suite Management". The three USUHS Instructions should be used together in the overall management of the USUHS Biosafety Program.

The Principal Investigator (PI) is primarily responsible for ensuring safe operating procedures are followed in the laboratory. His/her knowledge and judgment are critical in conducting risk assessments of biohazards and appropriately applying the recommendations in this manual. Safety is a shared responsibility among all of the laboratory staff. Many resources are available to assist the PI in their responsibilities, to include the Institutional Biosafety Committee (IBC), the Biosafety Level-3 (BSL-3) Suite Subcommittee, and the Center for Environmental Health and Occupational Safety (EHS). All researchers who are involved or working with biological agents are required to read and understand the contents of this manual, complete the required training, and seek additional advice when necessary. The IBC Chairperson and the Biosafety Officer (BSO) are available to assist researchers in this endeavor.

This Instruction is effective immediately.

Charles L. Rice, M.D.
President

USUHS Biological Safety Manual

Instruction 6401

SUMMARY OF CHANGES

The draft USUHS Instruction 6401 has been formally renamed as the “Biological Safety Manual” for the USUHS and is expected to supercede the older version dated 15 May 1987, titled “Biohazards and Dangerous Materials Guide”. This new draft revision is a complete rewrite of the older Instruction. The 1987 version covered a number of different topics including biohazards, controlled substances, and dangerous materials such as hazardous chemicals and non-ionizing radiation. Over the past several years the USUHS has implemented more specific USUHS Instructions that fully cover many of the topics found in the older Instruction 6401. Examples of these are:

Instruction 6403 - Biohazard Suite Management

Instruction 6404 - Management of Controlled Substances and Regulated Chemicals

Instruction 6407 - USUHS Chemical Hygiene Plan

Instruction 6408 - CDC Select Biological Agents Management, and the
USUHS Laser Safety Plan

Therefore, what was left at task was to provide a usable guide on biosafety practices for the USUHS and to include in the revision more recently published and up-to-date standards and procedures for reference to researchers who work with biological materials in a laboratory setting.

As you review this manual, please provide comments and suggested improvements relating to the focus of this Instruction, which is primarily Biosafety in content.

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IMPORTANT TELEPHONE NUMBERS

EMERGENCY TELEPHONE NUMBERS

Fire, Police, Rescue,
Emergency Medical Service (Ambulance) **777**
HAZMAT Team *1

(*1 HAZMAT Team for response to large spills
or hazardous material situations that cannot
be handled by the laboratory.)

Campus Security Desk **(301) 295-3033**

ASSISTANCE TELEPHONE NUMBERS

University Health Clinic (301) 295-3630

Center for Environmental Health and Occupational Safety (EHS) (301) 295-9443/3305

Biosafety Officer/EHS (301) 295-9443

Occupational Health Nurse (301) 295-9444

CDC Select Agent Responsible Official (301) 295-3390

Radiation Safety Officer (301) 295-3390

Safety Officer (301) 295-9441

POLICY STATEMENT

Purpose

This is a statement of the official Uniformed Services University of the Health Sciences (USUHS) policy to establish the process for compliance with the following documents:

- *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, current edition.
- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, current edition.
- *42 CFR, Parts 72 & 73, Possession, Use, and Transfer of Select Agents and Toxins*.

Policy

The USUHS is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing recombinant DNA (rDNA) is necessary in university research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines*, the recommendations in the *BMBL*, and the rules of *42 CFR, Parts 72 & 73*. Compliance with all other applicable federal, state, and local regulations is also required.

ROLES AND RESPONSIBILITIES

The President, USUHS

The President, USUHS is responsible for ensuring that hazardous biological agents used at the University are secured, handled and disposed of in accordance with federal law and their use in laboratories does not pose a health risk to workers or the community.

Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee is a University wide committee charged with reviewing policy and procedures related to the use of biological agents, including: human pathogens, oncogenic viruses, other infectious agents, and recombinant DNA. The committee membership is established by the National Institutes of Health (NIH) through the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. The Committee must:

- Review rDNA research conducted at or sponsored by the University for compliance with the *NIH Guidelines*, and approve those research projects that are found to conform with the *NIH Guidelines*.
- Review research involving infectious agents conducted at or sponsored by the University for compliance with the guidelines in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, and approve those research projects that are found to conform with the recommendations in *BMBL*.

- Notify the PI of the results of the IBC's review and approval or disapproval; seek clarifying information as necessary in making approval/disapproval decisions.
- Recommend bio-containment levels in accordance with NIH and Centers for Disease Control and Prevention (CDC) guidelines, and adopts emergency plans covering accidental spills and personnel contamination.
- Report any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illness to the appropriate Institutional official and to the NIH Office of Biotechnology DNA Activities (OBA) within 30 days.
- Follow the guidelines for membership defined by NIH.

Center for Environmental Health and Occupational Safety (EHS)

The Center for Environmental Health and Occupational Safety (EHS) is the operational arm of the IBC. EHS provides instruction and training on safe work practices, conducts routine inspections of laboratories and work areas, investigates accidents and recommends preventative measures and corrective actions, reviews research protocols involving hazardous materials, reviews construction design for safety features and responds to emergencies. EHS must:

- Prepare and distribute this Biosafety Manual, with revisions as necessary.
- Receive and dispose of Biological Waste (also referred to as Biological, Pathological and Medical Waste -BPMW) or Medical Regulated Waste.
- Coordinate and provide routine laboratory safety, hazard communication and blood borne pathogen training to effected workers. (Laboratory specific/agent specific training is provided by the Principal Investigator.)
- Provide assistance to investigators in performing work hazard risk assessments as necessary.
- Conduct biosafety inspections of all laboratories including BSL-2 and BSL-3 level laboratories at USUHS.
- Inspect laboratory-specific Biosafety Plans/documentation and review PI training records for currency of training during annual laboratory health and safety inspections as periodically as necessary.
- Provides medical surveillance services as required by the OSHA Bloodborne Pathogens Standard (CFR 1910.1030), and as recommended in the *BMBL* and *NIH Guidelines*.
- Provide necessary vaccinations (as may be required) to occupational workers.

Facilities Director

The Facilities Director will:

- Ensure facilities personnel are properly trained, vaccinated as necessary, or are able to wear appropriate personnel protective equipment (PPE) to enter laboratory spaces and perform required maintenance in general laboratories and in the BLS-2 and BSL-3 facilities. EHS offers laboratory safety and hazard communication training for ancillary personnel who in the course of their work have occasion to enter laboratory spaces.
- Provide locksmith services to assist the Security Division with securing biological agents and associated laboratories and storage areas.
- Coordinate with EHS all non-emergency maintenance work requests involving structural, electrical, or mechanical equipment, or plumbing alterations or movements that would effect or alter the effectiveness of the engineering controls in place that protect the integrity of Biosafety operations in the laboratories. This includes the connection and disconnection of safety equipment such as fume hoods, biosafety cabinets, or any other related equipment or service.

Security Office

The Security Office will:

- Immediately notify EHS of any breach of security, incident or reported unsafe condition that involves biological agents or other hazardous materials.
- Be responsible for the physical security of buildings containing hazardous biological agents.
- Advise PIs in matters pertaining to the physical security of hazardous biological agents, including procedures for key custodian duties.
- Conduct annual security audits and ensure that security training occurs annually for workers who are authorized access to CDC Select Agents.
- Provide written procedures for laboratory access and physical security of hazardous agents (see Appendix A).

Logistics Division

The Logistics Division will:

- In cooperation with the CDC Select Agent Responsible Official, establish written procedures (Appendix B) for the ordering, receiving, and delivering of hazardous biological agents, especially CDC-Select agents, within the University.
- In cooperation with the CDC Select Agent Responsible Official, monitor all incoming and outgoing shipments of biological agents for compliance with federal and state shipping regulations and that the shipments are properly received, stored and delivered to authorized personnel.

- Ensure applicable Logistics personnel receive the necessary training to conduct their work.
- Provide appropriate notification procedures so that chain-of-custody can be maintained during receipt and delivery of CDC select agents to the user/ requestor.
- Maintain constant security of hazardous biological agents until delivered to an authorized user/requestor.
- With the assistance of the CDC Select Agent Responsible Official, update procedures (Appendix B) for ordering, receiving, and shipping CDC select agents.
- Follow shipping and receiving procedures as identified in Appendix B.
- The Technical Service Branch (TSB) is responsible for ensuring manufacturer recommended routing maintenance procedures are scheduled and performed on all Biosafety equipment such as autoclaves and biosafety cabinets.
 - Records for the past 3 years of maintenance and repairs must be kept on file for review.
 - The Department of Environment Health and Safety (EHS) will assist TSB in the tracking and management of annual BSC certification testing.
 - For repair, the manufacturer warranty will be employed if possible. For autoclaves out of warranty, call TSB at 301-295-3612.

Biological Safety Officer (BSO)

The Biosafety Officer (BSO) is responsible for implementation and maintenance of the Biosafety Program. The BSO duties include, but are not necessarily limited to:

- Consultation with faculty, staff, and the IBC regarding development and implementation policies and procedures to reduce the risks of work with biohazardous materials with consideration given to having minimal interference with the conduct of research and teaching.
- Providing technical advice and training as necessary to the IBC and researchers on laboratory containment concerning biosafety and biosecurity procedures.
- Developing emergency plans for inclusion in this manual regarding the handling of spills and personnel contamination.
- Developing and reviewing the Biosafety Manual and other related documents.
- Reviewing infectious waste disposal policies and procedures to comply with state, federal, and DoD regulations.

- Investigating laboratory accidents/incidents and reporting details and violations (if found) to the IBC per NIH Guidelines.

CDC Select Agent Responsible Official (RO)

The USUHS CDC Select Agent Responsible Official will ensure that the requirements of 42 CFR, Parts 72 & 73 entitled, "Possession, Use, and Transfer of Select Agents and Toxins Rule" are complied with at USUHS Facilities. See USUHS Instruction 6408 for requirements involving the use of CDC select agents and the duties of the Responsible Official (RO).

Departmental Chairpersons

The Departmental Chairpersons are responsible for providing support to researchers which ensures that appropriate facilities are available to contain biohazardous materials and to enable the PI to comply with pertinent USUHS policies. The Chairpersons are responsible for assuring that the PI has the training commensurate with the proposed project and that the project design and monitoring methods meet institutional safety standards.

Principal Investigator (PI)

The PI is responsible for full compliance with approved research protocols, the NIH Recombinant DNA Guidelines, the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard (human-derived materials), this Biosafety Manual, and other local, state and federal regulations that apply to research. In addition the PI will:

- Submit a Hazardous Biological Agents Use Protocol for all research projects. This must be completed and **approved** by the IBC before any research can occur. Issues regarding use of Biosafety Level Three (BSL-3) agents may be referred to the BSL-3- Suite Subcommittee.
- Register the following experiments with the IBC, as required:
 - (a) Recombinant DNA activities.
 - (b) Work with infectious agents.
 - (c) Experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, primary human cell lines, and certain body fluids.
 - (d) Animal and plant pathogens.
- Assess the risks of experiments and investigate all safety aspects of planned experimental work.
- Inform all individuals participating in the experiment of all potential hazards associated with the work.
- Prepare and maintain a basic biosafety manual (see Appendix C) for laboratories that includes records of training (See Appendix D).
- Ensure personnel working under an approved protocol obtain any necessary immunizations, if required. (Refer to Occupational Medicine/ EHS.)

- Ensure the safe operation of laboratories, proper maintenance and care of equipment, safe handling of bio-hazardous material and waste disposal.
- Train laboratory personnel in safe work practices.

Employee

An employee is to:

- Follow the University's health and safety policies, follow laboratory specific procedures, and the instructions of the responsible PI and BSO.
- Attend all required laboratory safety and biosafety training.
- Immediately report any hazardous or potentially unsafe condition to his/her Supervisor and/or Safety Officer.

REGISTRATION OF POTENTIALLY INFECTIOUS AGENTS

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by federal and state regulations and prescribed guidelines. Many granting agencies require grant recipients to certify that they adhere to both the guidelines and the regulations.

Microorganisms

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) both publish guidelines for working with infectious microorganisms. Publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* recommends work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), Biosafety Level 2 (BSL-2), Biosafety Level 3 (BSL-3), and Biosafety Level 4 (BSL-4). Appendix B in the *NIH Guidelines* classify pathogenic agents into one of four risk groups according to specific criteria. It is the policy at the USUHS that all laboratories adhere to the NIH and CDC guidelines.

Investigators must register any project involving the use of a pathogenic agent with the IBC and obtain IBC approval before work is begun. If the laboratory has not been classified for a specific containment level it must be surveyed by the Biosafety Officer (BSO) to ascertain that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

Genetically Engineered Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. These guidelines also classify recombinant DNA experiments into four levels of containment (BSL-1, BSL-2, BSL-3, and BSL-4) based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the U.S. Department of Agriculture (USDA) requires a permit for field testing of genetically engineered plants. It is the policy at the USUHS that all laboratories follow these guidelines.

Registration Document

Each PI is responsible for registering all recombinant DNA experiments, including those exempt from *NIH Guidelines*. Online registration forms are available at the EHS web site <http://www.usuhs.mil/ehs/ehsforms.html>. An example of the registration form can be found in Appendix E. The BSO will conduct an audit of all laboratories where BSL-2 or BSL-3 containment is required. BSL-1 laboratories are audited on request of the PI.

Review and Approval of Experiments

The IBC, or at a minimum, the Chair of the IBC, will review the registration.

a. Experiments covered by the *NIH Guidelines*

Many experiments involving rDNA molecules require registration and approval by the IBC before work may be initiated. Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4, or Restricted Agents as host-vector systems.
- Cloning DNA from Risk Group 2, 3, 4, or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems.
- Infectious virus, or defective virus in the presence of a helper virus in tissue culture systems.
- Whole plants or animals.
- More than 10 liters of culture.

Experiments that must be registered at the time of initiation include those that involve:

- The formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture.
- Recombinant DNA-modified whole plants, and/or recombinant DNA modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E of the *NIH Guidelines*.
- The generation of transgenic rodents that require BSL-1 containment.

b. Experiments exempt from the *NIH Guidelines*

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. The Chair of the IBC will review the registration and confirm that the work is classified correctly according to the *NIH Guidelines*.

Exempt experiments are those that:

- Use rDNA molecules that are not in organisms or viruses.
- Consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
- Do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment.
- Contain less than one-half of any eukaryotic viral genome propagated in cell culture.
- Use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* hostvector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts.
- Involve the purchase or transfer of transgenic rodents for experiments that require BSL-1 containment.

Human Clinical Materials

Please refer to the *Bloodborne Pathogens Exposure Control Plan* (Appendix F) for detailed information on handling human material.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR, Part 1910.1030. Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using BSL-2 work practices. This concept is called Standard (Universal) Precautions. Investigators are responsible for notifying EHS of their use of human materials so training and immunization can be provided as required by OSHA.

Plant and Animal Pathogens

The USUHS requires investigators to register their campus use of animal and plant pathogens with Institutional Animal Care and Use Committee (IACUC). The registration form for animal pathogens is available at <http://www.usuhs.mil/iacucforms/iacforms.html>. Registration of plant pathogens may be completed by forwarding a copy of the USDA/APHIS permit to the Chair of the IBC.

CDC Select Agents

CDC Select Agents are microorganisms and/or toxins that have potential for use in a terrorist act. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restrict the possession and use of select agents, and further require the USUHS to document and maintain information on their location and use. Contact the Select Agent Responsible Official (EHS) immediately if you plan to acquire select agents (Appendix G). Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University.

Laboratories possessing, using, and/or transferring select agents and certain levels of select agent toxins must comply with the regulations established in the 42 CFR, Part 73 and the guidelines

found in USUHS Instruction 6408, CDC Select Biological Agents Management. These regulations contain detailed information pertaining to laboratory registration, personnel security risk assessments, safety plans, security plans, emergency response plans, training, record keeping, inspections, and notifications. It is the CDC Select Agent Responsible Official's responsibility to ensure that the requirements of the 42 CFR Part 73 regulations are met on behalf of the Institution. If you have questions, you may contact the USUHS Select Agent Responsible Official, the BSO, or visit the CDC's Select Agent website found at <http://www.cdc.gov/od/sap/index.htm>, which provides links to select agent program information.

RISK ASSESSMENT FOR INFECTIOUS MATERIAL

Classification of Infectious Agents on the Basis of Hazard

Worldwide there are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ from each other, they all are based on the notion that some microorganisms are more hazardous than others. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures and/or effective treatment are only some of the criteria taken into consideration when classifying infectious agents. In the United States, the most current classification is found in the *NIH Guidelines*. The human etiologic agents addressed in these guidelines are classified into four risk groups with **Risk Group 1 (RG-1)**, of low or no hazard, through **Risk Group 4 (RG-4)** representing highly infectious agents:

Basis for the Classification of Biohazardous Agents by Risk Group

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

A comprehensive list of biological agents in Risk Groups 1, 2, 3, and 4 can be found in Appendix B of the *NIH Guidelines*. Please note that these lists are not all-inclusive, and agents not listed in RG-2, RG-3, and RG-4 are not automatically assumed to be classified in RG-1. Any unlisted agent needs to be subjected to a risk assessment based on the known and potential properties of the agent and its relationship to other agents that are listed.

Risk Groups and Biosafety Levels

Determining the risk group of a biological agent is part of the biosafety risk assessment that assists in assigning the correct biosafety level for containment. In general, RG-2 agents are handled at BSL-2, and RG-3 agents at BSL-3 containment. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated in a BSL-2, under certain conditions. For more information, contact the BSO or the IBC Chairperson.

BIOHAZARDOUS MATERIALS

Biohazardous materials are defined as materials of biological origin that have the capacity to produce deleterious effects on humans or animals including:

- Recombinant DNA molecules.
- Micro-organisms containing recombinant DNA molecules.
- Micro-organisms classified as risk group 1 (RG-1), RG-2, RG-3, or RG-4.
- Biological products derived from RG-1, RG-2, RG-3, or RG-4 microorganisms.
- Diagnostic specimens known or reasonably expected to contain pathogens in RG-1, RG-2, RG-3, or RG-4.
- Clinical/medical waste derived from the medical treatment of humans or animals or from biomedical research.

All studies using biohazardous materials must undergo IBC review. Experiments using RG-2 or RG-3 agents must be reviewed and approved prior to the initiation of experiments. Experiments using RG-1 materials must also undergo IBC review. However, this review process is expedited and research may commence simultaneously with submission.

HUMAN GENE TRANSFER EXPERIMENTATION

Human gene transfer continues to raise many safety, ethical, and scientific issues in need of public discussion and analysis. *NIH Guidelines* Section III-C describes "Experiments that Require Institutional Biosafety Committee (IBC) and Institutional Review Board Approvals and Recombinant DNA Advisory Committee (RAC) Review Before Research Participant Enrollment". In this section is listed one subsection entitled, "Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants" (Section III-C-1). The general process to obtain approval for human gene transfer experimentation is to submit for review the following:

(1) RAC [adhere to the NIH Guidelines that are outlined in Appendix M, "Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Research Participants"], (2) IBC, and (3) IRB.

For human gene transfer experimentation, it is the responsibility of the IBC to ensure that:

1. A RAC review has been conducted.
2. All issues raised by the RAC in a summarization letter to the Principal Investigator and the sponsoring institution have been considered.
3. No participant is enrolled until a RAC review has been completed and IBC and IRB

approval have been obtained.

RULES, REGULATIONS, AND GUIDELINES

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules. Copies of these documents are available by access to the appropriate website.

1. *National Institute of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules*, April 2002 (http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_guidelines_apr_02.htm). These guidelines 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. Because 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 *NIH 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.
2. *Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition, 1999 (BMBL manual) (<http://www.cdc.gov/od/ohs/biosfty/bmBSL-4/bmBSL-4toc.htm>). This manual 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 number of infectious agents in various laboratory settings. This manual has been revised several times and is commonly seen as the standard for biosafety.
3. *Occupational Safety and Health Administration: Bloodborne Pathogens Standard* (http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051). 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.
4. Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:

International Air Transport Association (IATA)
Dangerous Goods Regulations
<http://www.iata.org>

U.S. Department of Transportation
49 CFR
<http://hazmat.dot.gov/regs/rules.htm>

U.S. Postal Service
39 CFR Part 111

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

5. Importation permits are required for 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.
6. *Select Agent Regulation* (42 CFR Part 73)
This regulation became a final rule on March 18, 2005. It implements provisions of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. A copy of the most current list of select agents and toxins covered under this rule is included in Appendix G. (<http://www.cdc.gov/od/sap/docs/salist.pdf>)
7. The Department of Commerce also regulates shipping certain agents.

BIOSAFETY CONTAINMENT LEVELS

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. Most microbiological work at the USUHS is conducted at BSL-1 or BSL-2 containment. There are no BSL-4 laboratories at the University. A comprehensive description of each of the BSL containment level can be found in the BMBL, which is free to download from the CDC web site at <http://www.cdc.gov/od/ohs/biosfty/bmBSL-4/bmBSL-4toc.htm>

Biosafety Level 1 (BSL-1)

BSL-1 is appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with well-characterized agents not known to cause disease in healthy adult humans. The laboratory is not necessarily separated from the general traffic patterns in the building. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. The Standard Microbiological Practices listed in the Administrative Controls paragraph of this manual apply to all Biosafety Levels.

Biosafety Level 2 (BSL-2)

BSL-2 is similar to BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. With good microbiological techniques, work at BSL-2 can be conducted safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with BSL-2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown.

Biosafety Level 3 (BSL-3)

BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. Primary hazards to personnel working at BSL-3 relate to autoinoculation, ingestion, and exposure to infectious aerosols. A comprehensive description of BSL-3 requirements can be found in USUHS Instruction 6403, Biohazard Suite Management.

Biosafety Level 4 (BSL-4)

The USUHS does not conduct work requiring BSL-4 containment. BSL-4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. The facility is usually in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted. Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The BSL-4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

Biosafety Level 1 (BSL-1)	
Agents:	Not known to cause disease in healthy adult humans.
Practices:	Standard microbiological practices.
Safety Equipment: (Primary barriers)	None required.
Facilities: (Secondary barriers)	Open bench top with sink available.
Biosafety Level 2 (BSL-2)	
Agents:	Moderate risk agents that are present in the community and associated with human disease of mild to moderate severity.
Practices:	BSL-1 practice plus limited access, biohazard warning signs, "SHARPS" precautions, and an SOP defining any needed waste decontamination or medical surveillance policies.
Safety Equipment: (Primary barriers)	Primary barriers include a Class I or II Biological Safety Cabinet (BSC) or other physical containment devices used for the manipulations of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPEs) including laboratory coats, gloves, face and eye protection as needed.
Facilities: (Secondary barriers)	BSL-1 plus the availability of an autoclave for decontamination.
Biosafety Level 3 (BSL-3)	
Agents:	Indigenous or exotic agents with a potential for aerosol transmission; and which may cause serious or potentially lethal infection.
Practices:	BSL-2 practice plus controlled access, decontamination of all waste, and decontamination of lab clothing before laundering.
Safety Equipment: (Primary barriers)	Primary barriers include a Class II BSC or other physical containment device used for the manipulations of agents, PPEs to include protective lab clothing, gloves, face and eye protection, and respiratory protection as needed.
Facilities: (Secondary barriers)	BSL-2 plus physical separation from access corridors, self-closing and double door access, exhausted air not recirculated with negative airflow into laboratory

Animal Facilities

Four biosafety levels are also described for activities involving infectious disease work with experimental mammals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, (ABSL-1, ABSL-2, ABSL-3, ABSL-4), and provide increasing levels of protection to personnel and the environment.

Clinical Laboratories

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV.

GENERAL BIOSAFETY PRACTICES

Routes of Infection

Working in a biological research environment, it is reasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth by:
 - Eating, drinking and smoking in the laboratory.
 - Mouth pipetting.
 - Transfer of microorganisms to the mouth by contaminated fingers or articles.
2. Through the skin by:
 - Accidental inoculation with a hypodermic needle, another SHARPS instrument, or glass.
 - Cuts or scratches.
3. Through the eyes by:
 - Splashes of infectious material into the eyes.
 - Transfer of microorganisms to the eyes by contaminated fingers.
4. Through the lungs by:
 - Inhalation of airborne microorganisms.

The general laboratory procedures outlined in this manual provide for guidance in handling infectious or potentially infectious materials.

ADMINISTRATIVE CONTROLS

Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or RG-3 agents are handled or stored, or where BSL-2 or BSL-3 procedures are required. The appropriate place for

posting the label is at the main entrance door(s) to laboratories and animal rooms, and on equipment such as refrigerators, incubators, transport containers, and/or lab benches. Requests for standardized biohazard warning signs can be submitted to EHS, Room A2020.

Training

Good microbiological and laboratory practices are essential for a safe work environment. Training and education on these practices and procedures needs to start at the undergraduate level. All personnel working with RG-1, RG-2 or RG-3 agents are required to receive laboratory specific training from the Principal Investigator (PI) or laboratory supervisor in addition to the required basic training provided by the Institution. Training should include at a minimum:

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

This Manual and the USUHS Exposure Control Plan (Appendix F) describe the training requirements for personnel whose research involves recombinant DNA or biohazards. The BSO and EHS staff conducts basic biosafety training in accordance with applicable local and federal requirements. PIs are responsible for biohazard specific training in their laboratories.

Laboratory Audits

As part of the EHS audit program, the Environmental staff participates in routine laboratory inspections on an annual basis. The staff audits newly approved BSL-2 labs and also audits BSL-3 labs semi-annually. Significant deficiencies are reviewed with the BSO and the Director, EHS.

Recordkeeping

The IBC project approval records are maintained by the Chair, IBC in accordance with federal standards. Laboratory inspection results and training attendance records are maintained by EHS in accordance with applicable federal regulations. PIs are responsible for updating IBC approved projects with EHS, and providing current listings of personnel involved in IBC approved projects.

Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. All procedures are performed carefully to minimize creation of splashes or aerosols.
6. Work surfaces are decontaminated at least daily and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and federal regulations before removal from the facility.
8. An insect and rodent control program is in effect.

Bloodborne Pathogen (BBP) Program

In accordance with OSHA requirements, USUHS has established an Exposure Control Plan (see Appendix F) covering the potential exposure to bloodborne pathogens (e.g., HIV, Hepatitis B virus) found in human blood, serum and tissue, as well as in other potentially infectious materials. BBP training is required on an annual basis and available through a University sponsored CD-ROM-based training program.

Institutional Biosafety Committee (IBC)

The IBC gives oversight on all projects involving biohazardous agents (RG-1, RG-2, and RG-3) and certain toxins at the campuses. Membership is governed by the NIH Guidelines and includes the USUHS Biosafety Officer and/or CDC Select Agent Responsible Official.

ENGINEERING CONTROLS

Biological Safety Cabinets (BSCs)

BSCs are designated to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three types of BSCs, designated as Class I, II and III, have been developed to meet various research and clinical needs. The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases. The biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually. EHS administers a program for annual certification of all BSCs at the University. Contact EHS at (301) 295-9443 to confirm that your cabinet is included in this program.

Laboratory personnel must be trained in the correct use and maintenance of BSCs to ensure that personnel and product protection (where applicable) are maintained. Before selecting any biosafety cabinet for purchase, contact the EHS Industrial Hygiene Officer (301) 295-9442 for a work-specific assessment and selection criterion.

1. Class I BSC

Protects personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust. The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection (e.g., sterility).

2. Class II BSC

(Types A1, A2, B1, B2, and B3) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

3. Class III BSC (sometimes called Class III glove boxes)

This type of cabinet provides the highest level of product, environmental, and personnel protection, and is designed for work with infectious agents that require BSL-4 containment. The cabinet is gas-tight, maintained under negative air pressure, with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors.

Horizontal laminar flow "clean air benches" are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

OPERATING INSTRUCTIONS FOR CLASS II BSCs

- 1) Turn on cabinet fan 15 minutes before beginning work.
- 2) Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- 3) Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.) Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids. Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a pilot light and place it to the rear of the work space. Flames disrupt the airflow and contribute to the heat load inside

the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs. Do not work in a BSC while a warning light or alarm is signaling.

- 4) Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling.
- 5) Always wear gloves when there is potential for skin contact with infectious material.
- 6) Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
- 7) When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow the cabinet to run for 15 minutes.
- 8) Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.
- 9) The UV lamp should never be on while an operator is working in the cabinet.
- 10) Minimize traffic around the BSC and avoid drafts from doors and air conditioning.

Negative Pressure Rooms

All laboratories having a ducted exhaust air ventilation system where the directional airflow draws air into the laboratory (i.e. negative pressure), must incorporate a method to monitor the direction of airflow to ensure that it is properly working. It is recommended that a visual monitoring device be used to confirm directional inward airflow at the lab entry.

In special containment laboratory areas (BSL-3 labs), an electronic quantitative monitoring of the air flow should be continually maintained and tested at least annually.

Exhaust systems with HEPA filters require a mechanism to monitor proper functioning of the filter to determine when replacement is needed.

Protection of Vacuum Lines

All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BSL-2 containment and higher, a hydrophobic vacuum line filter should be used.

Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BSL-2 and BSL-3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies (Fisher Scientific, catalog #09-730-211, and VWR, catalog #55095-006).

Other Safety Equipment

Safety Showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are available from Facilities Maintenance.

Eyewash Stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials. Eyewash stations need to be checked and flushed periodically to ensure their operability. A log should be kept to track periodic inspections.

Ventilation Controls

Ventilation controls are intended to minimize employee exposure to infectious substances by removing air contaminants from the work site. The two types of ventilation controls are:

- a. General (Dilution) Exhaust - Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for RG-3 agents or BSL-3 work.
- b. Local (Removal) Exhaust - Local exhaust systems capture or contain contaminants at their source before they escape into the workroom environment. Typical systems consist of one or more hoods, ducts, an air cleaner, if needed, and a fan.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

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 must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The following PPE is recommended for regular use:

Face Protection

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

Laboratory Clothing

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

Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. Powered latex gloves should not be used at the USUHS.

Respirators

For certain protocols and projects, additional PPE like respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Fit testing for HEPA-filtered masks is available through EHS. All personnel utilizing a Biosafety Level 3 containment facility must have a passing "Fit Test Report" on file.

RECOMMENDED WORK PRACTICES

Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids inside a BSC if possible. If pipetting is done on the open bench,

use absorbent pads or paper on the bench. The following precautions should be followed:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
- Do not discharge biohazardous material from a pipette at a height. Whenever possible, allow the discharge to run down the container wall.
- Place contaminated reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size SHARPS container.
- When work is performed inside a biosafety cabinet, all pans or SHARPS containers for contaminated glassware should be placed inside the cabinet while in use.

Syringes and Needles

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

- Work in a BSC 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 a disinfectant.

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

Cryostats

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

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.

- Handle microtone knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained in operating instructions and safety precautions should be prominently posted on the unit. Aerosols are created by practices such as filling centrifuge tubes, removing supernatant and resuspending sediment pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, follow these procedures:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture in centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in case of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when resuspending sediment material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers

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

Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. If blender containers are not leak-proof, they should be tested with

sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Lyophilizer and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

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

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

Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended. Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence that disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hamper (labeled or color coded) or in biohazard bags. Contact EHS at (301) 295-9443 for additional information concerning the handling of contaminated clothing.

Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected. Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes. Old and unused chemicals should be disposed of promptly and properly.
- Providing a workplace that is free of physical hazards. Aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment and avoidance of the creation of electrical hazards in wet areas.

All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

USE OF ANIMALS IN RESEARCH AND TEACHING

The use of animals in research and teaching is subject to state and federal laws and guidelines.

Policy specifies that:

- All animals under the sponsorship of the Institution will be treated humanely.
- Prior to their inception, all animal projects receive approval by the Institutional Animal Care and Use Committee (IACUC).
- Researchers will comply with state and federal regulations regarding the use and care of animals.

The IACUC should be contacted for questions regarding the use of animals for teaching and research. Principal Investigators planning to use animals for any USUHS activity must submit an application to the IACUC for review prior to the start of the project, regardless of the source of funding for the project. A copy of the application can be obtained from the IACUC ((301) 295-3753) at http://usuhs.mil/usuhs_only/iacuc/. The completed form will include descriptions of experimental protocols, plans for animal care, available facilities, and information on the use of hazardous materials including infectious agents. All animal protocols involving the use of rDNA and infectious or transmissible agents must be submitted to the IBC for review prior to final approval by the IACUC.

EMERGENCY PROCEDURES

Biological Spills

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment for the kit contains: concentrated disinfectant (such as chlorine bleach), a package of paper towels, household rubber gloves, autoclave bags, a SHARPS container, and forceps to pick up broken glass.

General Spill Cleanup Guidelines

- Wear gloves and a lab coat.
- Use forceps to pick up broken glass and discard into a SHARPS container.
- Cover spilled material with paper towels.
- Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.
- Dispose of towels in biohazard waste container.
- Wipe spill area with diluted disinfectant.
- Wash hands with soap and water when finished.

Specific Spill Cleanup Guidelines

Spill inside of BSL-1

- Wearing gloves and a lab coat, pick up broken glass with forceps and place in a SHARPS container.
- Absorb the spill with paper towels or other absorbent material.
- Discard these contaminated materials into a biohazard waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism.
- Autoclave all towels, gloves, and other materials worn or used to clean up the spill.
- Wash hands with soap and water.

Spill of Human Blood

- Wear gloves and a lab coat to clean up a spill.
- If broken glass is present, use forceps to pick up and place in a SHARPS container.
- Absorb blood with paper towels and discard them in a biohazard waste container.
- Using a detergent solution, clean the spill site of all visible blood.
- Wipe the spill site with paper towels soaked in a disinfectant such as bleach diluted 1:10 (vol/vol).
- Discard all contaminated materials into a biohazard waste container.
- Wash hands with soap and water.

Spill inside of BSL-2

- Keep other workers out of the area to prevent spreading spilled material. Post warning sign, if needed.
- Remove contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin and inform the PI of the spill. Call the BSO at (301) 295-3058 for assistance, if necessary.
- Put on protective clothing (lab coat, gloves, and if needed, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, SHARPS container, and paper towels).
- Pick up broken glass with forceps and dispose it into a SHARPS container.
- Cover the spill with paper towels and add appropriately diluted disinfectant.
- After at least 20 minutes of contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.

- Collect all contaminated materials into a biohazard waste container and autoclave.
- Wash hands with soap and water.

Spill inside of BSL-3

- Stop work immediately.
- Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close the door, and post a warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- Notify the PI. Call the BSO at (301) 295-3058 (after hours and weekends call the Security Desk at (301) 295-3038 or 911) for assistance, if necessary.
- Allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- Put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, SHARPS container, and paper towels).
- Contain the spill with absorbent paper towels or disposable pads. Carefully add 10% chlorine bleach to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the bleach to inactivate the material.
- Pick up broken glass with forceps and discard in a SHARPS container.
- Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.
- Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean up.
- Wash hands thoroughly with soap and water.

Spill inside a Biological Safety Cabinet

- Leave the cabinet fan running.
- Wearing gloves and a lab coat, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as 70% ethanol. If necessary, flood work surface, as well as drain pans and catch basins below the work surface, with disinfectant. Allow at least 20 minutes contact time.
- Soak up the disinfectant and spill with paper towels, and drain catch basin into a container. Lift front exhaust grille and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into area below the grille.
- Surface disinfect all items that may have been spattered before removing them from the cabinet.
- Discard all clean-up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water.
- The BSO should be notified if the spill overflows into the interior of the cabinet.
- It may be necessary to do a more extensive decontamination of the cabinet.

Spill of Combined Radioactive & Biological Material

A spill involving combined radioactive and biological materials requires emergency response procedures that are different from the procedures used for either material alone. As a general rule, disinfect the microorganism using a chemical disinfectant, then dispose of all clean-up materials in a separate bag/container labeled to indicate that a radioisotope is mixed with a chemically disinfectant microorganism. Be sure to use procedures to protect yourself from the radionuclide while disinfecting the biological material. Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact the Radiation Safety Officer (RSO) at (301) 295-3390/1906 for specific radioisotope clean-up procedures.

Preparation for Clean-up

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- Wash all exposed skin with soap or hand washing antiseptic, followed by a three minute water rinse.
- Inform the PI and the RSO at (301) 295-3390/1906 of the spill, and monitor all exposed personnel for radiation.
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (diluted disinfectant, autoclavable containers, forceps, paper towels, SHARPS container).
- Confirm with the RSO that it is safe to re-enter the lab.

Clean-up of a Biological Spill Containing Radioactive Material

- Put on protective clothing (lab coat, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask. In setting up your spill plan, contact EHS for advice since the use of many types of respirators requires prior training, fit-testing, and medical approval.
- Pick up any sharp objects with forceps and put in a SHARPS container labeled according to Radiation Safety guidelines.
- Cover the area with paper towels, and carefully pour diluted disinfectant around and into the spill. Avoid enlarging the contaminated area. Use additional disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time. **Do not use bleach solutions on iodinated materials: radioiodine gas may be released. Instead, use an alternative disinfectant such as an iodophor.**
- Wipe surrounding areas where the spill may have splashed with disinfectant.
- Absorb the disinfectant and spill materials with additional paper towels, and place into an approved radioactive waste container. Keep separate from other radioactive waste. **Do not autoclave contaminated waste without the approval of the RSO.**
- Disinfect contaminated protective clothing prior to disposal as radioactive waste.
 - i. With the assistance of Radiation Safety Division personnel, place contaminated item(s) on absorbent paper and scan for radioactivity. **If no radiation is detected as cleared by the RSO, dispose of these items as biohazard waste.**
 - ii. If radioactive contamination is detected, spray with disinfectant and allow a 20 minute contact time.

- iii. Wrap the item(s) inside the absorbent paper and dispose of as radioactive waste.
- Wash hands and exposed skin areas with soap and water, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the RSO. If spill area has residual activity, the RSO will determine if it is fixed or removable and handle it accordingly.

Injury Involving Biological Materials

Severe Injuries

- Call “777” for assistance and transportation to the nearest emergency room.
- Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
- Report the accident to the PI and EHS.

Splash to the Eye

- Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Use an emergency eyewash if one is accessible.
- Contact the University Health Clinic at (301) 295-3630 to obtain care. If UHC is closed, go to the emergency room at NNMC or to the most convenient local emergency room.
- Report the accident to the PI and EHS, and seek additional medical assistance if necessary.

Contamination to the Body

- Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes.
- Contact the UHC at (301) 295-3630 to obtain care. If UHC is closed, go to the emergency room at NNMC or to the most convenient local emergency room.
- Report the injury to the PI and to EHS, and seek additional medical assistance if necessary.

Fires Involving Biological Materials

- **Without placing yourself in danger**, put biological materials in a secure location, such as an incubator or freezer.
- Activate the building’s fire alarm.
- Leave the building at once.
- Call the fire department from a safe location.
- Meet the fire department outside and direct them to the fire.

DECONTAMINATION AND DISPOSAL

Sterilization, disinfection, and antisepsis are all forms of decontamination. **Sterilization** implies the killing of all living organisms. **Disinfection** refers to the use of antimicrobial agents on

inanimate objects; its purpose is to destroy all non-spore forming organisms. **Antisepsis** is the application of a liquid antimicrobial chemical to living tissue.

Chemical Disinfectants

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

- 1) Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
- 2) Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
- 3) Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. cholerae* and *P. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
- 4) Antiseptic – an agent formulated to be used on skin or tissue – it is not a disinfectant.

Disinfectants Commonly Used in the Laboratory

Iodophors

- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- Stable in storage if kept cool and tightly covered.
- Built-in color indicator; if solution is brown or yellow, it is still active.
- Relatively harmless to humans.

Hypochlorites (bleach)

- Working dilution is 1:10 to 1:100 household bleach in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made daily.

Alcohols (ethanol, isopropanol)

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

Important Characteristics of Disinfectants

	Hypochlorites "Bleach"	Iodoform "Wescodyne"	Ethyl Alcohol
Shelf-life > 1 week		X	X
Corrosive	X	X	
Residue	X	X	
	Hypochlorites "Bleach"	Iodoform "Wescodyne"	Ethyl Alcohol
Inactivation by Organic Matter	X	X	
Skin Irritant	X	X	
Respiratory Irritant	X		
Eye Irritant	X	X	X
Toxic	X	X	X

Dilution Of Disinfectants

1. Chlorine compounds (Household Bleach)

Dilution in Water	% Available Chlorine	Available Chlorine mg/l or ppm
Not diluted	5.25	50,000
1/10	0.5	5,000
1/100	0.05	500

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 1%.

2. Iodophor

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces per 5 gallons water.

3. Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant.

Autoclaving Procedures

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

Container Selection

- **Polypropylene bags.** Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or may burst during autoclaving. Therefore, **place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving.** Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.
- Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.
- **Polypropylene containers and pans.** Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers,
 - ✓ Remove the lid (if applicable).
 - ✓ Turn the container on its side when possible.
 - ✓ Select a container with the lowest sides and widest diameter possible for the autoclave.
- **Stainless steel containers and pans.** Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

Preparation and Loading of Materials

- ✓ Fill liquid containers only 50%.
- ✓ Loosen caps, or use vented closures.
- ✓ Always put bags of biological waste into autoclavable pans to catch spills.
- ✓ Position biohazard bags on their sides, with the bag neck taped loosely.
- ✓ Leave space between items to allow steam circulation.
- ✓ Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

Cycle Selection

- ✓ Use liquid cycle (slow exhaust) when autoclaving liquids to prevent contents from boiling over.
- ✓ Select fast exhaust cycle for glassware.
- ✓ Use fast exhaust and dry cycle for wrapped items.

Time Selection

- ✓ Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- ✓ Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- ✓ Bags of biological waste should be autoclaved for 50 minutes to assure decontamination.

Removing the Load

- ✓ Check that the chamber pressure is zero.
- ✓ Wear a lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- ✓ Stand behind door when opening it.
- ✓ Slowly open door only a crack. Beware of a possible rush of steam.
- ✓ After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.

Monitoring

Autoclaves shall be tested periodically to ensure effectiveness. Testing parameters include biological indicators (described below) which are used to monitor the sterilization process. Chemical indicators (autoclave tape) are used in conjunction with biological indicators and physical parameters (i.e., pressure and temperature readings). They provide instantaneous feedback to confirm that the load has been sterilized; however, they must not be used as the sole indicator of sterility. The results of biological indicator testing must be entered in the *Equipment Log Sheet (see attached example)* in the *Autoclave Log Book*.

Chemical Indicators

Periodicity:

- One strip is dated and included in each load of the autoclave.

Method:

- Tape indicates that time, temperature, and the presence of steam have been adequate to ensure sterilization. The strip must completely change color (colors vary by manufacturer) or reveal the word “autoclaved” to ensure effective operation.
- If the indicator has not fully turned color, the operation of the autoclave should be reviewed, an entry is made on the *Equipment Log Sheet* in the *Autoclave Log Book*, and the load ran again. If the strip fails to indicate adequate exposure after the second attempt, notify the USUHS Technical Services Branch for assistance and possible corrective action.

Biological Indicators

Periodicity:

- Every 40 hours of use (required for autoclaves that are used to deactivate human or non-human primate blood, tissues, clinical samples, or human pathogens), OR
- Every 6 months (required for autoclaves used to deactivate other material).

Method

- A commercially available test indicator kit that uses bacterial spores such as *Bacillus stearothermophilus* that can be rendered unviable at 250 degrees F or 121 degrees C. For this type of test, ampoules of *B. stearothermophilus* are autoclaved along with a load of waste. Upon completion of the cycle, the ampoules need to be incubated for 48 hours and then observed for any sign of growth, which would indicate that the autoclave is not sterilizing properly.

If for any reason the integrity of the sterilization process is in question, the load should be considered contaminated and should be reprocessed.

When results of autoclave monitoring are unacceptable, an entry will be made on the ***Equipment Log Sheet*** stating the problem, the corrective action taken, and how it was resolved. This record is maintained in the ***Autoclave Log Book***.

When autoclave equipment fails to operate properly, an entry is made on the ***Equipment Log Sheet*** stating the problem, the corrective action taken, and how it was resolved. This record is maintained in the ***Autoclave Log Book***.

When autoclaves with the capability to generate printouts or temperature charts are utilized, these records of autoclave function should be reviewed from each load to assure that sterilization has occurred. The autoclave operator should make sure paper is in the printer at all times and replaced as needed.

Autoclave Record Keeping

The following records regarding autoclave operations must be maintained on site in the ***Equipment Log Sheet*** and kept in the ***Autoclave Log Book***:

1. Dates when maintenance and/or repairs are performed on the unit.
2. Operations log (each load of deactivated material shall be logged as follows):
 - Date, time, and operator's name.
 - Type and approximate amount of waste (lbs.).
 - Confirmation of sterilization.

Record the temperature, pressure, and length of time the load is sterilized.
Note that temperature sensitive autoclave tape is not sufficient to indicate

that the load reached sterilization conditions because the tape will change color at lower temperatures, OR

Save the autoclave printout if the autoclave has a working printer.

Autoclave Training and Operation

Principal investigators and/or supervisors must train and qualify their staff for operation of autoclaves. Qualified autoclave users should understand the time, temperature, pressure relationships required for proper materials decontamination. Additional training on handling materials to be decontaminated should also be provided. Supervisors should maintain a permanent record of training provided to their staff.

Autoclave Maintenance

Follow manufacturer recommended routine maintenance procedures. For repair, use manufacturer warranty if possible. For autoclaves out of warranty, call Technical Services Branch at (301) 295-3612.

AUTOCLAVE SAFETY

Caution - Autoclaves May Cause Serious Burns

To Prevent Injury:

- **Loosen screw caps on bottles and tubes of liquids before autoclaving.**
- **Check that chamber pressure has returned to zero before opening door.**
- **Wear eye and face protection.**
- **Stand behind door when opening it.**
- **Slowly open door only a crack. Beware rush of steam.**
- **Keep face away from door as it opens. Escaping steam may burn.**
- **Wait 5 minutes after opening door before removing liquids.**
- **Liquids removed too soon may boil up and out of container, burning operator.**

Use and Disposal of SHARPS

To prevent needle stick injuries:

- ✓ Avoid using needles whenever possible.
- ✓ Do not bend, break, or otherwise manipulate needles by hand.
- ✓ Do not recap needles by hand. Do not remove needles from syringes by hand.
- ✓ Immediately after use, discard needle and syringe (whether contaminated or not) into puncture-resistant SHARPS containers.
- ✓ Never discard SHARPS into regular trash.
- ✓ Never discard SHARPS into bags of biological waste.
- ✓ Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- ✓ Do not overfill SHARPS containers. Close completely when they are 3/4 full and request pickup from EHS at (301) 295-9443.
- ✓ Locate SHARPS containers in areas in which needles are commonly used. Make containers easily accessible.
- ✓ SHARPS containers must be purchased from laboratory supply distributors such as VWR and Fisher Scientific.

In the event of a needle stick injury:

- ✓ Wash thoroughly with soap and water. Notify supervisor and go immediately to the Clinic at the UHC. If the UHC is closed, go to the emergency room at the NNMC or to the most convenient local emergency room.

Biological Waste Disposal Procedures

Biohazardous Waste

- All biohazard bags must be conspicuously labeled “biohazardous waste” or with the international biohazard symbol and the word “biohazard.”
- All bags must be tied to prevent leakage or expulsion of contents during future storage, handling, or transport. (Recommendation: **Bags should not be more than 2/3 full and use autoclave tape to seal bag.**)
- Bags must be placed for storage, handling or transport in a rigid secondary container, which may be disposable, reusable, or recyclable. Containers must be leak resistant, have tight fitting covers, and be kept clean and in good repair. The secondary containers may be any color and labeled with the words “biohazardous waste” or the international biohazard symbol and the word “biohazard” on the lid and on the sides so as to be visible from any lateral direction.
- Full biohazard bags must not be stored **above 0°C (32°F)** for more than **seven days** or **below 0°C (32 °F)** for **more than 90 days** before treatment.

- Biohazardous waste must be separated from other waste at the point of origin in the producing facility. The color of the biohazard bag to be used is based on the biosafety level of the lab.

- ✓ White/Opaque Bag

EHS has approved the use of white/opaque autoclave bags for BSL-1 labs that generate non-sharp Risk Group 1 agent research waste. You may autoclave the white/opaque bags in any autoclave.

This new option allows BSL-1 labs to accumulate non-sharp items in white/opaque autoclave bags. These shall be autoclaved, and then placed in the regular trash for custodial pick-up.

Spill containment is still important both before and after autoclaving; do not set any waste bags directly on floors or counters at any point. White/opaque autoclave bags are available from the Campus Storehouse. Biohazardous bags must be placed in secondary containment.

- ✓ Red Bag

BSL-2 and BSL-3 labs shall accumulate non-sharp biological research waste in double red biohazard bags, and all SHARPS from all labs regardless of agent's Risk Group must to be accumulated in SHARPS containers. These items must be placed in the medical waste barrels for destruction off-site or steam-sterilized by a "certified" autoclave. EHS utilizes the CDC/NIH categorization system to determine an agent's Risk Group.

Red bags can be disposed of as non-regulated waste (regular trash) IF it has been sterilized in a "certified" medical waste autoclave and has a visible indication of decontamination (i.e. autoclave tape).

Red bags can also be picked up by an **approved vendor** and treated outside the campus (contact EHS at (301) 295-9442 for vendor information).

Human Surgery Specimens or Tissue Waste

Biohazardous waste comprised of human surgery specimens or tissues that have been fixed with formaldehyde or other fixatives shall be segregated for storage, and then disposed of by incineration at an off-site facility.

Multi-hazard or Mixed Waste

Avoid generating mixed waste if possible. Keep volume to a minimum. Do not autoclave mixed waste. When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, and then dispose as radioactive waste. Seek advice from the RSO at (301) (301) 295-3390 before beginning inactivation procedures. When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, and

then dispose as chemical waste. Seek advice before beginning inactivation procedures. Contact EHS at (301) 295-9443 for instructions.

Disposal of Animal Tissues, Carcasses and Bedding

Disposal of animal carcasses/tissues is coordinated through the Laboratory Animal Medicine (LAM). Place animal carcasses/tissues into a plastic bag. Double-bag when carcass contains a zoonotic agent (transmissible from animals to humans). Place bag in freezer until pickup. Call LAM at (301) 295-3753 for pickup. Disposal of animal carcasses/tissues that are contaminated with radioactive materials or hazardous chemicals is performed through EHS. Disposal instructions are available by phoning (301) 295-9443.

Radioactive Biohazardous Waste

All radioactive biohazardous waste must be chemically disinfected and then disposed of through EHS as radioactive waste. See the EHS website and fill out the "Radioactive Waste Collection Form" or the "Chemical Waste Collection Form." EHS will pick up your waste within 1-3 days.

Pharmaceutical Waste

- Non-Controlled substances - Dispose of by placing the material in a cardboard box (no larger than 12in. x 12in. x 12in.). The box must be securely taped shut and labeled "Pharmaceutical Waste." Arrangements can be made to bring it to the Pharmacy. Contact EHS at (301) 295-3668
- Controlled substance - Must be returned to the Pharmacy with a copy of the yellow sheet. The Pharmacy Officer and Controlled Substances Officer will arrange for controlled substance waste to be picked up and disposed of by an outside vendor. Controlled Substance Custodians must transport controlled substances.

Liquid or Semi-Liquid Biohazardous Waste

Waste such as blood or culture solutions that have been treated by chemical disinfection with bleach may be discharged into the public sewage system. No other disinfectants may be discharged into the sanitary sewers. Other disinfectants must be collected and disposed of by EHS. An example of proper decontamination of liquid waste is adding bleach solution and allowing at least 30 minutes of contact time. Make sure that suction flasks always have the appropriate disinfectant inside the flask before suctioning off the media. The National Institutes of Health, the CDC, or the American Biological Safety Association must recognize the disinfecting method used.

SHARPS Containers

Full SHARPS containers must be tightly sealed or taped to ensure that contents will not spill. **Do not overfill SHARPS containers!** SHARPS containers should be closed and disposed when $\frac{3}{4}$ full in order to minimize risk of puncture.

- SHARPS Contaminated with Infectious Materials - Contaminated needles, syringes, scalpels, blades, broken glass, etc. must be placed in rigid, puncture- and leak-resistant containers, which are labeled with the words "SHARPS Waste" and with the international biohazard symbol or the word "Biohazard". (Appropriate SHARPS containers can be ordered). The containers are picked up by a vendor for off-campus incineration.

- Broken glass NOT Contaminated with Infectious Materials - Broken glass can be placed in rigid, puncture- and leak- resistant containers and taped shut before disposal in the regular trash. Broken glass can be placed in a broken glass container or rigid box, taped shut, and disposed as regular trash. Syringes and razor blades should be disposed in a biohazard SHARPS container with the biohazard symbol defaced.
- SHARPS Contaminated with Radioactive Materials - SHARPS can be contained in rigid, puncture-resistant, non-biohazardous containers, and then disposed through EHS as dry radioactive waste. Label containers “Radioactive Waste.” The composition would be dry SHARPS.
- SHARPS Contaminated with Hazardous Materials - SHARPS must be contained in rigid, puncture resistant non-biohazardous containers, then disposed of through EHS. Label containers as “Hazardous Waste.” The composition would be solid SHARPS.

TRANSPORT OF BIOLOGICAL MATERIALS

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

On-Campus Transport between Laboratories or Buildings

When moving infectious substances between labs or buildings on campus, the following minimum procedures must be followed:

- Sample must be in sealed primary container. Utilize plastic containers whenever possible.
- Place primary container in sealed secondary container, with absorbent (paper towels) between primary and secondary container suitable for the volume transported.
- If dry ice is needed, the secondary container should be placed in an outer container, with the dry ice placed between the secondary and tertiary container (never place dry ice in a sealed container).
- Place biohazard label with agent name, lab address, and phone number on outer container.

SHIPMENT OF BIOLOGICAL MATERIALS

General Information

Shipment of infectious agents, biological products, and diagnostic specimens is regulated by many agencies, and requirements are not always uniform. In addition, regulations are continually modified and new ones are added. A summary of current requirements is presented here, but it is recommended that the investigator check with the USUHS BSO, the CDC Responsible Official, or EHS and the various agencies before shipping any material that may be regulated. In general, **first** determine whether the material you wish to ship requires a permit and documented training before you begin the application process. **Second**, decide on a carrier, and learn the packaging

and labeling requirements of that carrier. **Third**, contact the BSO to determine if a certifying official's signature is needed for the shipment.

Permits

- Permits are required from the Centers for Disease Control and Prevention (CDC) to **import or transport**:
 1. Any microorganism that causes disease in humans.
 2. Biological materials, such as blood and tissues, when known or suspected to contain an infectious agent.
 3. Live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans.
 4. Any animal known or suspected of being infected with any disease transmissible to humans.

Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC Select Agent Responsible Official at (301) 295-3390, or through the CDC web site at <http://www.cdc.gov/od/ohs/biosfty/impptper.htm>.

- Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for **importation or transport** infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS web site at <http://www.aphis.usda.gov/forms/index.html>.
- Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests**. Application should be made at least 120 days in advance of the anticipated release or shipment date. Information and application forms may be obtained by calling the USDA/APHIS at (877) 770-5990, or through the APHIS web site at <http://www.aphis.usda.gov/ppq/permits/>.
- Facility registration and completion of the APHIS/CDC Form 2 are required by the CDC prior to transfer of **select agents and toxins** (42 CFR Part 73). Select agents are listed in Appendix G, and a copy of the regulation is available at http://www.cdc.gov/od/sap/final_rule.htm. Please contact the CDC Responsible Official at (301) 295-3390 if your work might include any of the agents listed in Appendix G.

- A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins to all countries outside of the United States, except Canada. Most agents in this category are included in the List of Select Agents found in Appendix G. As a general rule, the USUHS will not export biohazardous agents or material. Questions concerning this may be directed to the CDC Select Agent Responsible Official in EHS ((301) 295-9443).

Packaging

Various carriers (FedEx, UPS, Postal Service or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies such as the International Air Transport Association (IATA), and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances. Therefore, it is important to check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and/or labeling.

- Infectious Substance (Category A): Substances known, or reasonably expected, to contain pathogens such as microorganisms, or its toxin and are transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals. (DOT requires shippers of infectious substances to attend training every 2 years.)
- Infectious Substance (Category B): An infectious substance which does not meet the criteria for inclusion in Category A.
- Biological Product: Products derived from living organisms such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen.
- A secondary container that contains the primary container and packaging capable of absorbing the specimen.
- An outer rigid shipping container that contains the secondary container and other material.

Genetically Modified Microorganisms

The *NIH Guidelines for Experiments Involving Recombinant DNA Molecules* (April 2002) states that:

- Host organisms should be shipped as etiologic agents, regardless of whether they contain recombinant DNA (rDNA), if they are regulated as human pathogens, animal pathogens, or plant pests.
- Host organisms should be shipped as etiologic agents if they contain: (1) rDNA that includes the complete genome of an organism that is a human or animal pathogen or plant pest; (2) rDNA that codes for a toxin involved in eliciting human, animal, or plant

disease, and that is carried on an expression vector or within the host chromosome; or (3) rDNA from an organism regulated as a human or animal pathogen or a plant pest that has not been adequately characterized.

Human Clinical Materials

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Various carriers may have additional requirements.

BIOSECURITY

Biosecurity is defined as **protection of high-consequence microbial agents and toxins, or critical relevant information against theft or diversion by those who intend to pursue intentional misuse.** The following biosecurity issues should be considered by all laboratories handling biohazardous agents:

- Risk and threat assessment.
- Facility security plans.
- Physical security.
- Data and electronic technology systems.
- Security policies for personnel.
- Policies regarding accessing the laboratory and animal areas.
- Specimen accountability.
- Receipt of agents into the laboratory.
- Transfer or shipping of biohazardous agents from the laboratory.
- Emergency response plans.
- Reporting of incidents, unintentional injuries, and security breaches.

As part of a biosecurity program and to comply with Federal legislation, special procedures are required for the possession and transfer of specific biohazardous agents called CDC Select Agents (see Appendix G for a listing of these agents).

As part of the Institutional biosecurity program, the PI should address the following issues in the conduction of research activities: (1) personnel suitability and reliability (including student access), (2) pathogen accountability (both on-site and through the transfer process), and (3) response to biosecurity incidences. Refer to USUHS Instruction 6408 and USUHS Instruction 6403 for more information concerning biosecurity.

USEFUL WEB SITES

NIH Guidelines for Research Involving Recombinant DNA Molecules:

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

Biosafety in Microbiological and Biomedical Laboratories:

<http://www.cdc.gov/od/ohs/biosfty/bmBSL-4/bmBSL-4toc.htm>

NIH Office of Biotechnology Activities:

<http://www4.od.nih.gov/oba>

CDC Select Agents Program:

<http://www.cdc.gov/od/sap/sitemap.htm>

USDA/APHIS Select Agents Program:

http://www.aphis.usda.gov/programs/ag_selectagent/index.html

CDC Permit to Import or Transport Etiologic Agents:

<http://www.cdc.gov/od/ohs/biosfty/impptper.htm>

Selection, Installation, and Use of Biological Safety Cabinets

<http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm>

USUHS Environmental Health and Occupational Safety

<http://www.usuhs.mil/ehs/>

Health Canada's MSDSs for microorganisms

<http://www.phac-aspc.gc.ca/msds-ftss/index.html>

ADDITIONAL BIOSAFETY REFERENCES

USUHS Instructions:

- 1020** Proper wearing of Laboratory Coats and Name Tags by USUHS Personnel.
- 4120** Infectious Waste Decontamination Quality Control Program (cancelled 2/14/94)
- 6002** USUHS Occupational Health & Safety Program
- 6402-M** Radiation Safety Guide
- 6403** Biohazard Suite Management
- 6404** Management of Controlled Substances and Regulated Chemicals
- 6407** USUHS Chemical Hygiene Plan
- 6408** CDC Select Biological Agents Program

SECURITY DEPARTMENT- KEY AND ACCESS POLICES AND PROCEDURES

The Microbiology Department has the internal responsibility for the issuance and accountability of keys, and the Safety and Security of biohazardous agents within the Department.

1. The Chair, Microbiology shall:

a. Appoint a key custodian and alternate in writing. A copy of the appointment letter will be submitted to the Security Division.

b. Establish department/activity key control and access procedures in accordance with this Instruction.

c. Authorize, in writing, individuals to use the BSL-3 Suite and BSL-2 Laboratories.

d. Ensure that authorizations are issued to the absolute minimum, consistent with operational requirements. (Convenience or the position or status of individuals is not sufficient criteria for issuance of a key.)

e. Obtain prior approval from the CDC Select Agent Responsible Official before granting any researcher access to CDC select agents or entry into the BSL-3 suite.

2. The Key Custodian shall:

a. Issue keys only to individuals authorized by the Chair, Microbiology and, if CDC Select Agent access is desired, only to individuals who have been granted approval by the CDC Select Agent Responsible Official..

b. When issuing keys, fill out USUHS Form 5211, which list the key number, room number, the date the specific key was issued and a signature of the individual being assigned a key.

c. Request additional keys by submitting USUHS Form 5335, through the Chair, Microbiology, to the Facilities Division. Facilities will review each request, assign identification number, and forward the form 5335 to the Security Division for approval.

d. Ensure individuals return keys immediately upon termination or transfer of employment or duties. Record the date and time the keys were returned; and have the individual returning the key to sign the USUHS Form 5211.

e. Update and maintain key issuance/control records for yearly auditing by the Security Division.

f. Report immediately to the Security Division the loss or suspected loss of keys or other compromises of keys/locks, which would possibly allow unauthorized access to the BSL-3 Suite or BSL-2 Laboratories.

3. Principal Investigators shall:

a. Ensure the BSL-3 suite is locked at all times. BSL-2 laboratories are required to be lock when unoccupied.

b. Verify that Facilities or contractor personnel requesting access to the BLS-3 Suite and BS1-2 Laboratories have been authorized by the Chair, Microbiology.

c. Ensure that Individuals requesting access to the BSL-3 Suite or BSL-2 laboratories have a medical clearance from EHS. This documentation must include the individual's name, date, reason for needing access, and medical clearance.

d. Record all entries (including entries by visitors, maintenance workers, repairman and others needing one time or occasional entry) into the BSL-3 Suite by signature into a logbook. The logbook should include the date, time, and names of individuals entering the BSL-3 Suite.

e. Refrain from passing keys from one person to another. Keys assigned to PIs on a continuing basis shall not leave the personal custody of those PIs. PIs assigned keys are ultimately responsible and accountable for their keys.

f. No longer use combination locks to secure freezers, refrigerators, cabinets, and other containers containing select agents.

g. Label containers holding select agents with the PIs name, date and type of select agent.

h. Lock freezers, refrigerators, cabinets, and other containers where select agents are kept when not being used.

i. Immediately notify the Chair, Microbiology, EHS and the Security Division of any incidents indicating the possible loss or compromise of select agents.

**LOGISTICS AND CONTRACTING DEPARTMENTS
PROCEDURES FOR ORDERING AND RECEIVING CDC SELECT AGENTS**

Requesting CDC Select Agents

- Instructions for requesting CDC Select Agents may be found in USUHS Instruction 6408.
- All requests for CDC select agents may be entered in the CUFS financial system (see USUHS Instruction 6408 for the list of select agents).
- Purchasing select agents through the Henry M. Jackson Foundation or with a departmental government purchase card (GPC) is strictly prohibited.

When entering the requisition in the CUFS for select agents, the following commodity code must be used:

Commodity Code	Description
6505-CDC Agents	CDC Select Agent

- When entering requisitions the following text must be included in the RXD: “THIS ITEM IS A CDC SELECT AGENT AND SPECIAL HANDLING IS REQUIRED! THIS ITEM CAN BE RECEIVED ONLY BY AN INDIVIDUAL WHO IS TRAINED AND APPROVED TO RECEIVE CDC SELECT AGENTS. THE FIRM MUST NOTIFY USUHS AT 301-295-0799 OR 301-295-9443 OF THE CARRIER AND THE ESTIMATED TIME OF DELIVERY AT LEAST 24 HOURS IN ADVANCE.”
- Upon entry of the RXD, the requestor must send an e-mail to the mail group “dl-CDC Select Agents” which provides notice that a CDC Select Agent has been requested, the RXD#, and contact information for at least two department personnel.

Ordering CDC Select Agents - CONTRACTING

- Instructions for the ordering of CDC Select Agents may be found in USUHS Instruction 6408.
- When the order is placed by contracting, the special handling instructions must be unequivocally conveyed to the vendor. The person placing the order must also send an e-mail to the mail group “dl-CDC Select Agents” with notice that a CDC Select Agent has been ordered, the order #, the estimated delivery date, and a copy of the actual order or other pertinent information.

Receiving CDC Select Agents - LOGISTICS

- All received select agents will be immediately processed and delivered to the user/requestor.
- The delivery ticket notifies the receiving area of CDC select agents, other pertinent information relating to the items and to contact EHS.

- If received select agents cannot be immediately delivered, the Receiving Section will contact the CDC Select Agent Responsible Official to coordinate pick-up and delivery to an approved storage location listed on the CDC Select Agent Registration.
- The CDC Responsible Official (CDC RO) will be immediately notified of any CDC select agents that cannot be delivered to the user/requestor within 3 hours of receipt.
- Logistics Receiving section will immediately notify the CDC RO by E-mail once the select agent has been delivered to the user/requestor.

Transfer of CDC Select Agents.

Instructions for the Transfer of CDC Select Agents can be found in USUHS Instruction 6408.

Requirement for Protocol in the Use of CDC Select Agents

Accidents occurring in the presence of biological agents may result in infection. An exposure may result from subtle causes, such as the production of **aerosols** during a routine laboratory procedure. When working with biological agents or materials whose epidemiology and etiology are unknown or incompletely understood, it should always be assumed that the work presents a hazard. Certain biological agents infect both humans and laboratory animals. Safety procedures should be directed toward the prevention of infection in both groups. Therefore, the USUHS requires that **any investigator** who needs to work with a hazardous biological agent develop a written experimental protocol describing the methods used for **handling, containment and decontamination** of the agent, exposed area and objects. The protocol should describe the pathogenicity and public health aspects of the agent used. The Hazardous Biological Agents Use Protocol must then be submitted to the Institutional Biosafety Committee for review and **approval** or **disapproval**, prior to performing any experimentation.

**UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS)
SAMPLE
LABORATORY BIOSAFETY MANUAL**

This Biosafety Manual is written to comply with the requirements of the OSHA Bloodborne Pathogen Standard, 29 CFR 1910.1030 and the USUHS Safety Manual. This laboratory manual shall be reviewed annually, and updated as necessary.

AREA ACCESS

During times when work with human blood, body fluid, tissue or pathogenic organisms is in progress, each laboratory shall have access restricted to only those individuals who are authorized. Any individual may be “authorized” to enter, if they have met the following criteria:

- They have trained, and shown proficiency with the microbiological practices used in the laboratory.
- They have been trained in the procedures used in the laboratory.
- They have been advised of the potential hazards.

While work with potentially infected substances is in progress, each laboratory must have its doors closed and labeled. The label must be red or orange in color, contain the universal biohazard symbol, a listing of the pathogen being used, and the name and telephone number of the individual in charge of the laboratory.

Work Practices

All work areas will be properly equipped for hand washing, waste disposal and SHARPS disposal facilities. While working with potentially infected materials, employees will not be allowed to eat, drink, (needless to say) smoke, apply lip balm or cosmetics, or handle contact lenses. Mouth pipetting is not allowed at any time.

Containment

All refrigerators, freezers, biological safety cabinets, animal cages or other storage containers that contain potentially infected samples or tissues must be labeled with the biohazard symbol and pathogen. Food may not be stored in these refrigerators or freezers. All potentially infectious specimens will be kept in leak proof containers during transport and storage. For transport between work areas, samples should be in nested, sealed containers (e.g., tubes in a sealed pouch, pouch in a latched cooler).

BIOLOGICAL SAFETY CABINETS (BSC)

Class II BSC must be used when working with potentially infectious substances that are likely to produce significant aerosols, and for any work with materials known to contain Class II

pathogens, as defined in Federal Register 51, #88, pp. 16967-68, 1986. No work with Class III or Class IV pathogens is presently permitted in the USUHS.

Vacuum Lines

Vacuum lines used to aspirate potentially infectious fluids shall be protected by a liquid disinfectant trap and a HEPA filter in line between the collection flask and the control valve. These will be checked periodically and replaced when necessary.

NEEDLES & SHARPS

Accidental needle sticks or other SHARPS injuries are a major health risk for lab personnel who work with body fluids or tissues, and every effort must be made to prevent needless SHARPS exposures. Proper disposal of syringes requires that the needles are not removed, recapped, bent or sheared, but that the syringe and attached needle is promptly and carefully deposited in a puncture-proof, leak-proof, lockable SHARPS container which is labeled with the universal biohazard symbol. Over-filled containers are hazardous and cause unnecessary needle sticks. Never force the syringe into the SHARPS container; replace the container. Full containers should be incinerated. In case of a needle stick or other SHARPS injury, immediately notify your supervisor and contact the Occupational Health Nurse in EHS/ Occupational Medicine.

ACCIDENTS & SPILLS

Spills or other accidents which result in overt exposure to potentially infectious substances will be reported immediately to the laboratory director who will report to EHS. Medical evaluation, surveillance and treatment will be provided as appropriate and written records maintained.

CONTAMINATED WASTE

All contaminated items must be placed in red or biohazard labeled bags which are contained in leak proof and labeled receptacles. This material will be autoclaved and/or incinerated for disposal.

CENTRIFUGES

Centrifugation of potentially contaminated samples will be done only in sealed tubes and rotors or with approved biohazard safety cups.

PERSONAL PROTECTIVE EQUIPMENT

Universal Precautions will be employed by all individuals with potential exposure to any human bloodborne pathogen.

All contaminated personal protective equipment must be handled as biohazardous. Disposables (gloves and gowns) will be disposed through incineration. Contaminated clothing will be kept isolated until the contracting company will pick up for laundering. All linen service contractors handle all laundry with Universal Precautions.

Gloves must be worn whenever potentially infected substances are in use.

Gloves will be suitable for the task, changed if torn or contaminated and should not be worn outside the laboratory. Hands will be washed when gloves are removed.

Clothing

Lab coats and/or repellent gowns will be worn whenever potentially infected substances are in use. These must be changed if contaminated and should not be worn outside the laboratory.

Eye Protection

Glasses with side shields, goggles and/or face shields must be worn whenever potentially infected substances are in use.

Masks

Water repellent masks will be worn when there is potential of spray, splash, splatter or the generation of aerosols.

TRAINING REQUIREMENTS

All employees working with potentially infected substances must participate in the basic required training for all employees offered by the EHS and extended training offered by their laboratory supervisor.

BIOSAFETY TRAINING LINKS

1. http://www.lbl.gov/ehs/biosafety/Biosafety_Training/biosafety_training.shtml = Excellent web based and comprehensive basic biosafety training. The training makes reference to some facts unique to Berkeley Laboratory, but this is only a minor limitation to the program. You can have your workers take the exams provided or not.
2. <http://www.vcu.edu/oehs/chemical/training/Biosafety-Training-CDCNIH--Module.html> = Excellent voice- over lecture using Power Point slides. BMBL based training. May be more appropriate for lab supervisors and PIs.
3. <http://www.cdc.gov/od/ohs/safety/S2.pdf> = The CDC's Biosafety Training manual .

FOR COMMITTEE USE ONLY IBC# _____
Application is Exempt Status [] yes [] no
Approved by full IBC [] yes [] no
Approved with Conditions _____
Not Approved _____
Approved IBC Chair/BSO _____
Date Review _____
Completed _____

Uniformed Services University of The Health Sciences

Institutional Biosafety Committee
Biological Agent/Toxin Registration Form

Complete this application to register and obtain approval for use of a biological agent/toxin in research or teaching projects. This information can be submitted at any time and approvals are granted for a 3 year period. Application must be resubmitted when there is a change made to the proposed work. Please do not hesitate to contact the Biosafety Officer or members of the IBC concerning any policy or procedure.

New Submission Teaching Purposes Grant Renewal Previous IBC # _____

Principal Investigator _____ Phone _____

Department _____ Fax _____ E-mail _____

Laboratory/rooms where work will be performed _____

Project Title _____

Funding Agency _____

Dates of Project: From _____ To _____

1. Attach a concise scientific summary and rationale of the proposed study. The abstract from the grant application may be used.

2. Name(s) of biological agent/toxin that will be used in this project:

3. Name of strains or isolates:

4. Where will the agent/toxin(s) be stored? (Bldg., Room) _____

5. Is the agent pathogenic to humans? Yes No

6. Will the project utilize human blood, body fluids or tissue? Yes No

7. Describe the sources of human tissue, blood or body fluids to be used in your project:

8. Human Risk Group _____ (*Laboratories using Risk Group 3 agents must submit a Laboratory Safety and Procedures manual and have it approved by the IBC prior to work with that agent.*)

9. Is the agent pathogenic to animals? Yes No

10. Is the agent pathogenic to plants? Yes No

11. Is the agent antibiotic resistant? Yes No

12. Does the project involve, or does the microorganism synthesize, a toxic molecule lethal for vertebrates? Yes No Not Known If yes, what toxin? _____

13. Is a USDA or CDC permit required for use of this agent? Yes No

14. Does the project involve the infection of animals? Yes No Species _____

If yes, can the infected animal(s) release this microorganism into the environment? Yes No

15. Does the project generate > 10 liters of culture? Yes No

16. Specify amount/concentration of agent generated during this project.

17. What Biosafety Level (BSL-1, BSL-2 or BSL-3) will be used during this project?

18. Is there a vaccine available and recommended for persons handling this agent? Yes No

19. **Provide laboratory protocols specific to this research.** Be sure to include the following information:

a. Identification of potential exposure hazards during sample preparation and experimental manipulations (e.g. aerosol generation when transferring, mixing or centrifuging; use of SHARPS; excretion by animals, etc.).

b. Safety procedures that will be used to minimize risk and prevent release of infectious agents (e.g. protective clothing, use of biological safety cabinet, SHARPS disposal procedures, waste disposal procedures, etc.).

c. Methods to inactivate/decontaminate agent.

d. Accidental spill/exposure procedures.

20. Identify personnel conducting the experiments (including students and temporary staff). Specify applicable training and experience including duration (e.g. 2 years), and project responsibilities. * New PIs must attach CV.

NAME	TRAINING/EXPERIENCE	PROJECT RESPONSIBILITIES

By signing below you are agreeing that all work on this project will be conducted using biosafety practices described in the CDC/NIH Publication entitled *Biosafety in Medical and Biomedical Laboratories (BMBL)* and following FAU policy and procedures. Work must not be conducted before IBC approval is granted.

As Principal Investigator, I hereby certify that prior to initiation of this project, all laboratory staff will be given the protocols that describe potential biohazards and precautions to be taken while working with this material. Laboratory staff involved in this project will be trained in laboratory practices and techniques to ensure safety of personnel and the environment. Personnel will be informed of procedures involving accidents and if medical surveillance is necessary. All laboratory staff will attend compliance training in applicable government rules and regulations.

Principal Investigator's Signature _____ Date _____

As Department Chair, I hereby certify that I have had the opportunity to review the proposal information and have granted departmental approval.

Department Chair's Signature _____ Date _____

After completing the form, sign and obtain departmental signature, submit the signed form and a copy of the proposal to the Biosafety Officer located at EHS Room A2020, or fax (301) (301) 295-3320. Please save a copy for your records.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS)

BLOODBORNE PATHOGENS - EXPOSURE CONTROL PLAN

I. STATEMENT OF POLICY

It is the policy of USUHS to limit or prevent occupational exposure to blood or other potentially infectious materials by strict adherence to the Universal Precautions, by providing suitable personal protective equipment, training, and where appropriate, Hepatitis B immunization. This Exposure Control Plan describes the procedures necessary to comply with the Occupational Safety and Health Administration's (OSHA) Blood borne Pathogen Standard (29CFR1910.1030) and PA Act 148.

II. SCOPE

This policy applies to all University employees, Henry M. Jackson Foundation employees and visiting scientists and researchers who could reasonably anticipate occupational exposure to blood or other potentially infectious materials in the performance of their duties.

III. DEFINITIONS - as per 29CFR1910.1030(b)

BLOODBORNE PATHOGENS - Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, Hepatitis B Virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency Virus (HIV).

OCCUPATIONAL EXPOSURE - Reasonably anticipated skin, eye, mucous membrane, or other parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

OTHER POTENTIALLY INFECTIOUS MATERIALS -

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, 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 dead).
3. HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

UNIVERSAL PRECAUTIONS - "Universal Precautions for the Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus and Other Blood borne Pathogens in Health-

Care Settings" published by the Centers for Disease Control in *Morbidity and Mortality Weekly Report*, 1988; 27(24):377

IV. EXPOSURE DETERMINATION

EHS is responsible for identifying University employees who are or may be exposed to blood borne pathogens, as well as their job classifications/titles and tasks as follows:

1. A list of job classifications and job titles in which ALL employees have an occupational exposure to blood borne pathogens (see below).
2. A list of job classifications and job titles in which SOME employees have an occupational exposure to blood borne pathogens.
3. For job classifications and job titles where SOME employees have an occupational exposure to blood borne pathogens, a list of the tasks and procedures where exposure occurs (see Appendix B for a complete list).

Principal Investigators and/or their designee(s) are responsible for informing Environmental Health and Safety (EHS) of new employees who are hired and are in "at-risk" situations. Determination of risk shall be made without regard to the use of Personal Protective Equipment.

V. METHODS OF COMPLIANCE

- A. Universal Precautions - Strict adherence to the Universal Precautions is required for this exposure control program to be effective. The cornerstone of the Universal Precautions is that all blood, regardless of the source, be treated as if it is infectious. Appropriate personal protective equipment and work practices must be observed to reduce the possibility of skin and/or mucous membrane exposure to blood and other potentially infectious materials.
- B. Personal Protective Equipment - Personal protective equipment including (but not limited to) gloves, masks and eye protection shall be available and worn by all persons who can reasonably anticipate exposure to blood and other potentially infectious materials during the course of their duties.
 1. At no cost to the employee, such personal protective equipment is provided, cleaned and/or replaced as required by law by the University unit where the exposed employee works.
 2. Employees are required to use appropriate personal protective equipment whenever contact with blood or other potentially infectious material is anticipated.

3. Personal protective equipment is considered to be appropriate only if it prevents contact of blood and/or other potentially infectious materials from coming into contact with skin/mucous membranes.
- C. Needlestick Prevention - Devices that are capable of reducing or eliminating the potential for needlestick and other sharp instrument injuries are now available. Examples of such technology include needle-less delivery systems, self-sheathing needles and catheters, retractable hypodermic needles, and needle guards and shields. It is vitally important that the use of these devices becomes a standard practice in clinical and research laboratories. They should be used wherever and whenever possible. Those employees who use these devices the most (i.e., nurses and phlebotomists) should be consulted for input in the type of needlestick prevention equipment purchased.

VI. HEPATITIS B VACCINATION

- A. Each employee whose duties may reasonably be anticipated to involve exposure to blood or other potentially infectious materials will be offered Hepatitis B vaccine by the University at no cost to the employee.
- B. Information about the vaccine, its efficacy, safety, method of administration, and the benefits of being vaccinated will be provided to the employee during a blood borne pathogen training program. The vaccine is provided in accordance with current CDC recommendations and EHS protocols.
 1. An employee may choose to take the vaccine or decline. If the employee declines the vaccine, a waiver stating that fact must be signed by the employee.
 2. If at any time, a potentially exposed employee who initially declined to receive the vaccine wishes to receive the vaccine, the University will provide the vaccine at no cost to the employee.
 3. The Occupational Medicine branch of EHS is responsible for maintaining vaccination records.

VII. POST EXPOSURE EVALUATION AND FOLLOW-UP

- A. All exposures to blood or other potentially infectious materials are to be reported to Occupational Medicine. Following the report of an exposure incident, confidential medical evaluation, treatment and follow-up shall be made available within 72 hours to the employee who experienced such exposure. Such services shall be provided at no cost to the employee.
- B. Occupational Medicine will determine the required follow-up or treatment to be taken based on the exposure, applicable CDC guidelines, and EHS policies.

1. Occupational Medicine is responsible for documenting all exposures and medical actions taken.
 2. Occupational Medicine is responsible for maintaining and retaining medical records of such evaluations, treatment and follow-up. These records are maintained in accordance with PA Act 148.
 3. EHS is responsible for evaluating the circumstances surrounding an exposure incident, and shall recommend appropriate safety equipment and/or changes in procedure to prevent further exposures of this type.
- C. At off campus locations, exposures to blood or other potentially infectious materials should be evaluated as soon as possible by the nearest appropriate Military or civilian health care facility (i.e., local hospital or medical clinic). This evaluation and subsequent medical follow-up should be done as soon as possible after the exposure occurs. This medical evaluation and follow-up shall be provided at no cost to the employee.
- D. Medical Evaluation - following the report of an exposure incident, the University shall make immediately available to an exposed employee a confidential medical evaluation and follow-up, which shall include the following elements:
1. Documentation of the route(s) of exposure, and the circumstances under which the exposure occurred.
 2. Identification and documentation of the source individual, unless the employer can establish that identification is infeasible or prohibited by state or local law.
 3. The source individual's blood shall be tested as soon as it is feasible to, and after consent is obtained, in order to determine HBV, HCV and HIV infectivity. If consent is not obtained, the employer shall establish that legally required consent cannot be obtained. When the source individual's consent is not required by law, the source individual's blood, if available, shall be tested and the results documented.
 4. When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated.
 5. Results of the source individual's testing shall be made available to the exposed employee, and the employee shall be informed of applicable laws

and regulations concerning disclosure of the identity and infectious status of the source individual within the confines of PA Act 148.

6. Collection and testing of the exposed individual's blood for HBV and HIV status.
 7. The exposed employee's blood shall be collected as soon as it is feasible to, and tested after consent is obtained.
 8. If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as it is feasible to do so.
 9. Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service.
 10. Counseling - prior to collection of blood.
 11. Evaluation of reported illnesses.
- E. Information Provided to the Healthcare Provider - the University shall ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided with a copy of this regulation.
1. The University shall ensure that the healthcare professional evaluating an employee after and exposure incident is provided with the following information:
 - a. A description of the exposed employee's duties as they relate to the exposure incident.
 - b. Documentation of the route(s) of exposure and circumstances under which exposure occurred.
 - c. Results of the source individual's blood testing, if available.
 - d. Medical records relevant to the appropriate treatment of the employee including vaccination status, which are the University's responsibility to maintain.
- F. Healthcare Professional's Written Opinion - The employer shall obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

1. The healthcare professional's written opinion for Hepatitis B vaccination shall be limited to whether Hepatitis B vaccination is indicated for the employee, and if the employee has received such vaccination.
2. The healthcare professional's written opinion for post-exposure evaluation and follow-up shall be limited to the following information:
 - a. That the employee has been informed of the results of the evaluation
 - b. That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment.
3. All other findings or diagnoses shall remain confidential and shall not be included in the written report.

VIII. COMMUNICATION OF HAZARDS

The universal biohazard symbol shall be used throughout the University to indicate the presence of blood or other potentially infectious materials and shall be affixed to containers of infectious waste, refrigerators and freezers containing these materials, containers used to transport these materials, contaminated equipment and at the entrances of areas where these materials are used or stored.

IX. INFORMATION AND TRAINING

- A. All employees who may have occupational exposure to blood borne pathogens shall attend a training program which explains the hazards of working with blood and other potentially infectious materials and the methods of compliance used by the University to minimize this exposure. It shall be the responsibility of Department Heads to ensure that their at-risk employees attend the training.
- B. Initial training shall be provided to all at-risk employees. Retraining is provided annually or, in the event of employee reassignment, training on new tasks or procedures shall be provided at the time of such reassignment.
- C. The training shall include, but not be limited to the following:
 1. A copy of the OSHA Blood borne Pathogen standard and an explanation of its contents.
 2. A general explanation of the epidemiology and symptoms of blood borne diseases.
 3. An explanation of the modes of transmission of blood borne pathogens.

4. An explanation of the University's exposure control plan and the means by which an employee can obtain a copy of the plan.
 5. An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials.
 6. An explanation of the use and limitations of the method that will prevent or reduce exposure, including appropriate work practices and personal protective equipment.
 7. Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment.
 8. Information on the basis for selection of personal protective equipment.
 9. Information on the Hepatitis B vaccine as described in section VI of this document.
 10. Information on the appropriate actions to be taken and persons to contact in an emergency involving blood or other potentially infectious materials.
 11. An explanation of the procedures to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available.
 12. An explanation of pertinent signs and warning labels in use at the University.
 13. An opportunity for questions and answers.
- C. Training records shall be maintained by EHS, as appropriate. Such records shall be retained for a minimum of three years.

X. ANNUAL REVIEW

This Exposure Control Plan is reviewed on an annual basis and updated as needed to reflect changes in University policies and procedures.

XI. JOB CLASSIFICATION

A - Job classifications and job titles in which ALL employees have an occupational exposure to blood borne pathogens.

University Health Clinic

Physician
Physician's Assistant
Medical Assistant
Medical Lab Technician
Clinical Assistant
Emergency Medical Technician
Medical Corpsman
Nurse
Nurse Practitioner
Nurse's Assistant

B - Job classifications and job titles in which SOME employees have an occupational exposure to blood borne pathogens; and tasks and procedures where exposure occurs.

Job Classification

University Academic and Administrative Departments

Tasks

Provide instruction in patient care; performing venipuncture; starting IVs; training students in the cleaning of dressings; drawing blood from test subjects; analysis of blood and blood components; culturing of blood cells.

Professor
Associate Professor
Assistant Professor
Associate Director
Clinical Instructor
Instructor
Nurse
Research Nurse
Graduate Student
Research Assistant
Nurse/Medical Assistant
Post-Doctoral Fellow
Technician
Research Aide
Staff Scientist
Graduate Assistant
Senior Research Aide
Research Associate
Visiting Scientist

EHS

Providing first aid care/CPR; collection and packaging of infectious waste; responding to accidents and incidents involving blood

Director

Biosafety Officer

CDC Select Agent RO and ARO

Manager

Fire Protection Engineer

Industrial Hygiene Specialist

Radiation Safety Officer

EHS Specialist

Special Waste Technician

Nurse - Providing basic health care services, including: dressing wounds; physical examinations; handling infectious waste; providing medical treatment to athletes; First Aid and CPR; handling infectious waste.

LIST OF SELECT AGENTS AND TOXINS

HHS NON-OVERLAP SELECT AGENTS AND TOXINS

Crimean-Congo haemorrhagic fever virus
Coccidioides posadasii
Ebola viruses
Cercopithecine herpesvirus 1 (Herpes B virus)
Lassa fever virus
Marburg virus
Monkeypox virus
Rickettsia prowazekii
Rickettsia rickettsii
Reconstructed 1918 Influenza virus

South American haemorrhagic fever viruses

Junin
Machupo
Sabia
Flexal
Guanarito

Tick-borne encephalitis complex (flavi) viruses

Central European tick-borne encephalitis
Far Eastern tick-borne encephalitis
Russian spring and summer encephalitis
Kyasanur forest disease
Omsk hemorrhagic fever

Variola major virus (Smallpox virus)

Variola minor virus (Alastrim)

Yersinia pestis

Abrin

Conotoxins

Diacetoxyscirpenol

Ricin

Saxitoxin

Shiga-like ribosome inactivating proteins

Tetrodotoxin

HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS/ SELECT AGENTS
(OVERLAP AGENTS)

Bacillus anthracis

Brucella abortus

Brucella melitensis

Brucella suis

Burkholderia mallei (formerly *Pseudomonas mallei*)
Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*)
Botulinum neurotoxin producing species of *Clostridium*
Coccidioides immitis
Coxiella burnetii
Eastern equine encephalitis virus
Hendra virus
Francisella tularensis
Nipah Virus
Rift Valley fever virus
Venezuelan equine encephalitis virus
Botulinum neurotoxin
Clostridium perfringens epsilon toxin
Shigatoxin
Staphylococcal enterotoxin
T-2 toxin

USDA HIGH CONSEQUENCE LIVESTOCK PATHOGENS AND TOXINS (NON-OVERLAP AGENTS AND TOXINS)

Akabane virus
African swine fever virus
African horse sickness virus
Avian influenza virus (highly pathogenic)
Blue tongue virus (Exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Cowdria ruminantium (Heartwater)
Foot and mouth disease virus
Goat pox virus
Lumpy skin disease virus
Japanese encephalitis virus
Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1)
Menangle virus
Mycoplasma capricolum/ M.F38/*M. mycoides Capri*
(contagious caprine pleuropneumonia)
Mycoplasma mycoides mycoides (contagious bovine pleuropneumonia)
Newcastle disease virus (velogenic)
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Vesicular stomatitis virus (Exotic)

LISTED PLANT PATHOGENS

Candidatus Liberobacter africanus

Candidatus Liberobacter asiaticus

Peronosclerospora philippinensis

Phakopsora pachyrhizi

Ralstonia solanacearum race 3, biovar 2

Schlerophthora rayssiae var *zeae*

Synchytrium endobioticum

Xanthomonas oryzae pv. *oryzicola*

Xylella fastidiosa (citrus variegated chlorosis strain)

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES (USUHS)

Biosafety Laboratory Annual Audit Checklist

Date: _____

Principal Investigator		Department	
Lab Phone #		Lab Room #	
EHS Inspector			
Name & Title of Person providing information			

I. RECOMBINANT DNA/ BIOLOGICAL AGENT INFORMATION

Y N	1. Bacteria	
	a. Specific name of agent (strain, serotype)	
Y N	2. Virus	
	a. Specific name of agent (strain, serotype)	
	b. Is the agent replication deficient?	Molecular basis: e.g., E1 or E1/E3 deleted
Y N	3. Parasite	
	a. Specific name of agent	
Y N	4. Biological toxins (e.g., tetrodotoxin, conotoxin)	
Y N	5. Is viable material stored in lab? Where?	Location (Room # with biohazard sign)
Y N	6. Are personnel experienced/trained to work with agents?	By whom?
Y N	Is training updated as needed and documented?	
Y N	7. Are potential health effects of agent known to staff?	
Y N	8. Is PI familiar with NIH Guidelines for rDNA research?	www4.od.nih.gov/oba/rac/guidelines/guidelines.html
Y N	9. Is medical surveillance recommended?	

II. Does research involve human blood, tissue and/or tissue culture? Specify

--	--

III. Does research involve Non-human Primate tissue or tissue culture ? Specify

IV. BLOODBORNE PATHOGEN INFORMATION

Y N	1. Is a department-specific Exposure Control Plan available? Reviewed annually and updated?	Name of Exposure Control Officer:

Y N	2. Annual Bloodborne Pathogen Training is required if employees are potentially exposed to human blood or body fluids. Is everyone's training current?	Conducted by:

Y N	3. Do you know where the training records are kept?	Location:

Y N	4. Is the Bloodborne Pathogen poster clearly displayed?	

Y N	5. Are universal precautions practiced?	

Y N	6. Have all at risk personnel either been vaccinated for Hepatitis B virus or signed a waiver?	

V. BIOHAZARD SIGNS DISPLAYED

Y N	1. Are doors labeled?	

Y N	2. Are labels displayed on refrigerators, freezers and incubators containing bioagents?	

Y N	3. Is the Hazardous Material Spill Response Card displayed?	

Y N	4. Do you have a spill kit?	

Y N	5. Do you have tools for picking up broken glass?	Describe (e.g., autoclavable dust pan, cardboard)

VI. WORK PRACTICES

Y N	1. Are food and beverages stored or consumed in the lab?	

Y N	2. What disinfectant is used to decontaminate daily or after a spill?	Specify:

Y N	3. Are all containers properly labeled including the disinfectant?	

Y N	4. Do you decontaminate broth cultures with bleach or by autoclaving prior to disposal?	
Y N	5. Do you transport biohazardous material outside of your lab? If yes, what? _____	
Y N	6. Are all injuries or accidents reported to the PI?	
Y N	7. Are all glass pasteur pipettes, needles, syringes, razor blades & scalpels placed in a SHARPS disposal container?	

VII. EQUIPMENT-PRIMARY BARRIERS

Y N	1. Is a biosafety cabinet (Tissue Culture Hood) used whenever there is potential for splashes or creation of aerosols?	Last date of certification

VIII. PERSONAL PROTECTIVE EQUIPMENT (PPE)

- Y N 1. Are Lab coats/protective clothing worn while working? Specify PPEs available in lab:
- Y N 2. Are gloves worn and hands washed after removing gloves? (Disposable gloves are not reusable.)

IX. LABORATORY FACILITIES

- Y N 1. Are emergency shower & eyewash areas easily accessible? (Access not blocked.) Eye Wash flushed routinely:
Location:

Comments:

Resources:

www.nih.gov

www.osha.gov

www.absa.org (American Biological Safety Association)

www.cdc.gov/od/ohs/biosfty/bmBSL-4/bmBSL-4toc.htm

www4.od.nih.gov/oba/rac/guidelines/guidelines.html

www.absa.org/resguides.html (Laboratory Biosafety Guidelines)

Staff completing audit: _____

Auditor signature: _____

Date of Audit: _____

Safety Awareness Roster

The following employees in _____ lab have reviewed this lab audit checklist.

Employee Name	Employee Signature	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____