UNIVERSITY OF SOUTH FLORIDA

Institutional Biosafety Manual

Division of Research Integrity & Compliance

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Emergency Contacts

Institutional Contact List

Emergency Notification:
For all emergencies – contact the University Campus Police at the following numbers:

Tampa, Main Campus – 911-from a campus phone. State the location and description of the emergency. Based upon the description, the proper emergency responder and the appropriate USF safety personnel will be notified.

St. Petersburg Campus – 3-4140-from campus phone or 727-553-4140 for off-campus.
If 911 is dialed, call is forwarded from the city/county emergency system to the USF Campus Police. Please state location and description of the emergency.

University Contacts:

Bloodborne Pathogen Exposure,
Immediately Notify: Diana Doughty Office: (813)974-3163 Pager: (813)216-0153
After regular business hours, contact: (813)974-2201
The Infectious Disease Physician (answering service - ID on call)

Biohazardous Materials Spill Notification:
Notify the Principal Investigator, the Lab Director/supervisor
Notify - Farah Moulvi Office: (813)974-0954 Cell: (813)469-1625
If emergency/after regular business hours, contact University Police 911

Biomedical Waste Spill Notification Environmental Health & Safety
Notify the Principal Investigator
Notify Environmental Health and Safety Office: (813)974-4036
If emergency/after regular business hours, contact University Police 911

Notification of Occupational Laboratory Exposures:
Immediately Notify: Diana Doughty Office: (813)974-3163 Pager: (813)216-0153

Work Related Injury/exposure
If a work related injury Workman's Compensation (AmeriSys 1-800-455-2079):
Meica Elridge: (Workers' Compensation Specialist) Office: (813)974-5775
If emergency/after regular business hours, contact University Police 911

In the event of an emergency, if medical care is needed, proceed to the nearest Emergency Room.

Foreword

This biosafety manual has been developed by Research Integrity & Compliance at the University of South Florida (USF). The purpose of the manual is to institute sound biosafety practices and procedures that when followed will reduce the risk of disease to all those involved who face potential occupational exposure to infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules. In addition, when the biosafety practices and procedures are followed, it will reduce the risk of accidental release of an organism into the environment, provide an environment for high quality research while maintaining a safe work place, and comply with applicable federal, state, and local requirements.

These guidelines are applicable to all faculty, staff, students, volunteers, and visitors at USF and its affiliates using/working with infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.

The USF Biosafety Program achieves its goals through the use of:

- Administrative controls
- Engineering controls (e.g., containment)
- Personal Protective Equipment (PPE)
- University Biosafety policies, procedures, practices, and guidelines

The importance of personal responsibility for safe laboratory activities is stressed throughout the manual. A safe laboratory environment is the product of individuals who are well trained and technically proficient in safe practices, and share responsibility for their own safety and for the safety of their colleagues, their communities, and the environment. Personal responsibility also involves the practice of assessing risks prior to the conduct of activities that involve infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.

USF's Biosafety Program depends on investigators, staff, students, and volunteers who are committed to maintaining a safe working environment and who are knowledgeable about laboratory safety. It is the Principal Investigator's (PI) responsibility to become thoroughly familiar with the contents of this manual, and to make sure that his or her workers become equally familiar with it, and to ensure that all work with infectious agents, biological toxins, Select Agents, recombinant or synthetic nucleic acid molecules is in accordance with USF's policies, procedures, practices, and guidelines.

The USF Biosafety Manual provides institution-wide safety guidelines, and procedures for the use and manipulation of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules. The successful implementation of this manual depends largely on the combined efforts of administrators, safety staff, Principal Investigators, laboratory supervisors, and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules are used.

Researchers and students must follow the recommendations in the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL), 5th edition* and *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) as the minimum containment level required for their work. Containment requirements may be subject to modification by the Institutional Biosafety Committee (IBC).

This manual was drafted in accordance with the CDC's *BMBL 5th edition, NIH Guidelines* and the *World Health Organization, Laboratory Biosafety Manual*.

Section 1 Program Administration

Section 1.1 Institutional Official-Vice President for Research

The Vice President for Research is the Institutional Official responsible for the Biosafety Program regarding infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules. The Vice President for Research has appointed an Institutional Biosafety Committee and alternate members, through Research Integrity & Compliance, who are responsible for oversight of the Biosafety Program (regarding infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules), to ensure compliance with the regulations and guidelines, and establish and implement policies that govern the safe conduct of research and teaching involving biohazardous agents and to advise the Vice President for Research accordingly in matters involving the use of biohazardous agents.

Section 1.2 Institutional Biosafety Committee (IBC)

The IBC is responsible for:

- Reviewing and approving prior to initiation of the research all use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.
- Advising the Vice President for Research on all matters pertaining to the safe use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules in research at USF.
- Establishing guidelines for faculty, staff, students, volunteers, and visitors at USF and its affiliates conducting research or academic programs involving infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.
- Establishes policies for review of all projects involving the use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules to assure compliance with the most current federal, state, and local regulations and guidelines

- Guides and supports the work of the researchers toward these goals.
- Promotes awareness of personal and environmental safety in the research community.
- Establish, monitor, and enforce policies and procedures which meet or exceed applicable norms or regulations for the use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.
- Maintaining a diverse membership representing the community and a variety of University interests.

Section 1.3 Principal Investigator (PI)

A. Principal Investigator shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the requirements set forth in this manual.
2. Select appropriate microbiological practices and laboratory techniques to be used for the research.
3. Complete and submit the appropriate registration application to the IBC for review and approval. Current applications can be downloaded at the following website http://www.research.usf.edu/dric/biosafety/forms.asp
4. Prior to initiation submit all changes (Change in Biological Agent, Change in Protocol Title, Change in Protocol Sponsor, Change in Lab Location, Change in Lab Equipment, Change in Procedure, Change in Personnel, Other) in a given project to the IBC for review and approval using a Modification Request form accessed at http://www.research.usf.edu/dric/biosafety/docs/modification.doc.

B. Prior to Initiating Research the Principal Investigator shall:

1. Make available to all laboratory staff and involved facilities staff the protocols that describe the potential biohazards and the precautions to be taken.
2. Instruct and train all research personnel in:
   a. The practices and techniques required to ensure safety;
   b. The procedures for dealing with accidents and spills.
   c. The reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations).
3. Ensure that transportation of any biohazardous material comply with all applicable packaging and shipping requirements.

C. During the Conduct of the Research the Principal Investigator shall:

1. Supervise the safety performance of the laboratory staff to ensure that the required safety practices are employed.
2. Report any significant problems pertaining to the operation and implementation of containment practices and procedures to the IBC.
3. Immediately notify the Institutional Biosafety Officer (IBO) at (813) 974-0954 (during normal working hours) or the University Police using 911 (after hours) of any biological spills, accidents, near misses, containment failure, or violations of biosafety practice which result in the release of biohazardous material and/or the

exposure of laboratory personnel (or the public) to infectious agents and/or recombinant or synthetic nucleic acid molecules.

4. Restrict access to the laboratory when experiments are in progress to those who are aware of the risks associated with use of biohazardous agents and are trained to safely handle the biohazardous agents.
5. Accept responsibility for full compliance with the policy, practices, and procedures set forth in this manual and as described in the approved IBC registration application.

As part of the general responsibilities, the Principal Investigator will:

a. Complete the Biosafety core requirements and annual refresher training.
b. Develop and implement written laboratory-specific biosafety procedures and containment practices (for BSL-2 and higher) that are consistent with the nature of current and planned research activities. In addition, make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken. The PI shall ensure that all laboratory personnel, including other faculty members, understand and comply with these laboratory-specific biosafety procedures.
c. Ensure that all laboratory personnel, maintenance personnel, and visitors who may be exposed to any biohazard are informed in advance of their potential risk and of the behavior required to minimize that risk.
d. Ensure that all maintenance work in, on, or around contaminated equipment is conducted only after that equipment is thoroughly decontaminated by the laboratory staff or PI.
e. Ensure that research materials are properly decontaminated before disposal and that all employees are familiar with the different methods of waste disposal.
f. Report any significant problems, violations of the policies, practices, and procedures set forth in this manual, or any significant research-related accidents and illnesses, to the Biosafety Office (813) 974-0954.
g. Notify the USF Health Medical Health Administration immediately (813) 974-3163, Pager: (813) 216-0153, E-mail to:llennert@health.usf.edu: for all blood borne pathogen exposures.
h. Be trained in standard microbiological techniques.
i. Create and foster an environment in the laboratory which encourages open discussion of biosafety issues, problems, and violations of procedure. The PI will not discipline or take any adverse action against any person for reporting problems or violations to the IBC.
j. Comply with shipping requirements (federal, state, local, USF) for infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.

Section 1.4 Laboratory Worker

Whoever works in the laboratory in a technical capacity is defined as a laboratory worker, whether the person is faculty, staff, student, intern, visiting scholar, or volunteer.

It is the laboratory staff's responsibility to:

a. Conscientiously follow lab-specific biosafety practices and procedures.
b. Inform the PI of any health condition that may require additional safety precautions so that they may be implemented.
c. Report to the PI or the lab supervisor all problems, violations in procedure, spills or near misses as soon as they occur.

Section 1.5 Institutional Biosafety Officer (IBO)

The responsibilities of the IBO include:

- Managing the Biosafety Program and implementation of Institutional Biosafety Committee (IBC) policies and procedures.
- Maintenance of records regarding, but not limited to, infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.
- Consulting with researchers regarding biosafety issues.
- Reporting to the institutional management on program status.
- Reporting to the IBC problems related to accidents and illnesses, operations, or other activities involved with proposed or approved protocols.
- Assisting laboratories in conforming to pertinent regulatory guidelines and IBC policies by providing training, facility inspection, and communication of program requirements.
- Conducting audits, site inspections, and enacting policies and procedures to ensure adherence to federal, state, local, regulations and University policies and procedures for the use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules.
- Monitoring national, state, and local regulatory trends and communicate any changes to the IBC, responsible institutional representatives, research faculty, and staff.
- Serving as a voting member of the IBC.
- Preparing and filing of annual IBC membership report to NIH/OSP.
- Acting as a liaison with the University, the Institutional Review Boards (IRBs), Institutional Animal Care and Use Committee (IACUC), Infection Control Units, and the Environmental Health and Safety (EHS) office to preserve a safe working environment.

Section 2 General Principles

Section 2.1 Containment

The term "containment" is used in describing safe methods for managing biohazardous materials in the laboratory environment.

The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

The three elements of containment include **engineering controls, administrative controls, and practices and procedures**. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements to implement. It can be accomplished through the following means:

1. **Primary Containment**: Protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.
2. **Secondary Containment**: Protection of the environment external to the laboratory from exposure to infectious materials and/or recombinant or synthetic nucleic acid molecules through a combination of facility design and operational practices.

Section 3 Biohazardous Agents

Section 3.1 Definitions

Biohazardous agents include infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules that are potentially hazardous to humans, animals, plants, and/or the environment. They include (but not limited to) known pathogens and infectious agents, which include bacteria and their plasmids and phages, viruses, fungi, mycoplasmas, parasites, cell lines, animal remains, and laboratory animals including insects that might harbor such infectious agents, and primate (human and non-human) body fluids. Also included are potentially biohazardous organisms used in procedures such as recombinant DNA (recombinant or synthetic nucleic acid molecules) and genetic manipulations.

An **infectious agent** is any microorganism (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; or deleterious alteration of the environment.

A **biological toxin** is the toxic material or product of plants, animals, or microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes:

- poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or

- poisonous isomer or biological product, homolog, or derivative of such a substance.

Select Agents and Toxins/High Consequence Livestock Pathogens and Toxins are any organism or substance that the United States Department of Health and Human Services (HHS) and/or the United States Department of Agriculture (USDA) has identified (certain bacteria, viruses, toxins, rickettsia, and fungi) as a potential threat to public health/animal health or welfare. The Select Agent Program currently requires registration of facilities including government agencies, universities, research institutions, and commercial entities that possess, use, or transfer biologic agents and toxins that pose a significant threat to public health.
Recombinant or Synthetic Nucleic acid molecules (as defined by the ***NIH Guidelines*****)** - any nucleic acid molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can be replicated in a living cell and/or DNA molecules that result from the replication of those molecules described above.

Section 3.2 Institutional Requirements

All research and teaching activities involving the use of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules must be registered with the IBC using the appropriate registration application forms prior to initiation of project or handling of the biohazardous agent.

The categories below represent the items that require IBC approval:

1. Infectious agents - any microorganism (including but not limited to bacteria, viruses, fungi, rickettsia, or protozoa).
2. Biological toxins - a toxic material or product of plants, animals, microorganisms (including but not limited to bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule (whatever the origin and method of production).
3. Recombinant or synthetic nucleic acid molecules (as defined by the *NIH Guidelines)*, or naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance.
4. Select Biological Agents and Toxins / High Consequence Livestock Pathogens and Restricted Plant Pathogens as identified by 42 CFR 73, 9 CFR 121, and 7 CFR 331. Note this also requires registration with the applicable federal agency (e.g., CDC and/or USDA).
5. All materials that may contain wild poliovirus.
6. Prions.

Note: The definition also includes projects that involve known biohazards that do not appear to fall into one of the above criteria. When it is unclear as to whether a material constitutes a potential biohazard, the Institutional Biosafety Officer (IBO) (813) 974-0954 should be consulted.

Exceptions: Research using any Risk Group 4 agents or any materials that require Biosafety Level 4 containment is restricted at any USF location or facility (dictated by the lack of qualifying facilities at USF).

Section 3.3 Risk Groups

Biohazards are classified according to the theoretical risk they present to a healthy individual. The risk classification determines the type of containment level.

Definitions of risk are described in the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. 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 and Risk Group 4 (RG-4) representing highly hazardous microorganism.

Throughout this manual, references are made to the relative hazards of infectious microorganisms by Risk Group (RG-1, -2, -3, and -4). This risk group classification is to be used for laboratory work only.

Risk Group 1 (no or very low individual and community risk)
Agents that are not associated with disease in healthy adult humans. -*NIH Guidelines*

Risk Group 2 (moderate individual risk, low community risk)
Agents that are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. -*NIH Guidelines*

Risk Group 3 (high individual risk, low community risk)
Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may or may not be available. -*NIH Guidelines*

Risk Group 4 (high individual and community risk)
Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. -*NIH Guidelines*

A list of RG-2, -3, and -4 agents can be found in Appendix B of the NIH *Guidelines*. It is important to realize however, that none of the lists are all inclusive. Any unlisted agent needs to be subjected to a risk assessment based on the known and potential properties of the agents and their relationship to agents that are listed.

Determining the RG of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level (BSL) for containment. A BSL may be adapted to compensate for the absence of certain recommended safeguards. In general, RG-2 agents are usually handled at BSL-2, and RG-3 agents at BSL-3. The RG and/or BSL containment level may vary depending upon the risk assessment. For more information refer to the section on risk assessment in this manual, the CDC/NIH BMBL, the NIH *Guidelines*, or contact the Institutional Biosafety Officer (IBO) at (813) 974-0954.

Section 4 Risk Assessment

"Risk" implies the probability that harm, injury, or disease will occur. In the context of the microbiological and biomedical laboratories, the assessment of risk focuses primarily on the prevention of laboratory-associated infections. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical step. It helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker's and the environment's risk of exposure to an agent and/or recombinant or synthetic nucleic acid molecules to an absolute minimum. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level.

There are many resources available to assist in the assessment of risk for a given procedure or experiment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The principal investigator (PI) is responsible for ensuring that adequate and timely risk assessments are performed prior to possessing and using biohazardous agents. The PI is encouraged to consult with the Institutional Biosafety Officer (IBO), biosafety staff, or the Institutional Biosafety Committee (IBC) regarding risk assessment. The IBC will make the final decision as to the level of risk and appropriate biological containment. **During the IBC registration application review, the committee can change the BSL based upon risk analysis**.

This manual will assist in the evaluation, containment, and control of infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules. However, research or teaching activities involving infectious agents, biological toxins, Select Agents, and/or recombinant or synthetic nucleic acid molecules cannot be initiated without prior approval by the University of South Florida’s (USF) Institutional Biosafety Committee (IBC). Research Integrity & Compliance and its biosafety staff are available to assist in this endeavor.

The IBC determines if appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be routinely reviewed and revised when necessary, taking into consideration new data or other relevant new information from the scientific literature.

Section 4.1 Components of Risk Assessment

Factors considered include:

- pathogenicity of the agent and infectious dose
- consideration of the outcome of exposure
- natural route of infection

- other routes of infection, resulting from laboratory manipulations (ingestion, absorption, inhalation, and parenteral)
- stability of the agent in the environment
- concentration of the agent and volume of concentrated material to be manipulated
- presence of a suitable host (human or animal)
- information available from animal studies and reports of laboratory-acquired infections or clinical reports
- laboratory procedures such as concentration, sonication, aerosolization, centrifugation, etc.
- any genetic manipulation of the organism that may extend the host range of the agent
- altering the agent's sensitivity to known, effective treatment
- local availability of effective prophylaxis or therapeutic interventions.

Some of the above mentioned factors of interest in a risk assessment are discussed in detail below:

- The ***pathogenicity*** of the infectious or suspected infectious agent, including disease incidence and severity (i.e., low morbidity versus high mortality, acute versus chronic disease). The more severe the potentially acquired disease, the higher the risk. For example, *Staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is relegated to BSL-2. Viruses such as Ebola, Marburg, and Lassa fever, which cause diseases with high mortality rates and for which there are no vaccines or treatment, are worked with at BSL-4. However, disease severity needs to be tempered by other factors. Work with human hepatitis B virus is also done at BSL-2, and although it can cause potentially lethal disease, it is not transmitted by the aerosol route. Also, there is an effective vaccine available for hepatitis B.

- ***Agent stability*** is a consideration that involves not only aerosol infectivity, but also the agent's ability to survive over time in the environment (e.g., from spore-forming bacteria). Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.

- The ***infectious dose*** of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The laboratory worker's ***immune status*** is directly related to his/her susceptibility to disease when working with an infectious agent.

- The ***route of transmission*** (e.g., parenteral, airborne, or by ingestion) of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. When planning work with a relatively uncharacterized agent with an uncertain mode of transmission consider the

potential for aerosol transmission. The greater the aerosol potential, the higher the risk.

- The ***concentration*** (number of infectious organisms per unit volume) will be important in determining the risk. Such a determination will include consideration of the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation). The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-titered microorganisms increases, since additional handling of the materials is often required.

- The ***origin*** of the potentially infectious material is also critical in doing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger livestock and poultry.

- The ***availability of data from animal studies***, in the absence of human data, may provide useful information in a risk assessment. Information about pathogenicity, infectivity, and route of transmission in animals may provide valuable clues. Caution must always be exercised, however, in translating infectivity data from one species of animal to another species.

- The established ***availability of an effective prophylaxis*** or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Risk assessment includes determining the availability of effective immunizations. In some instances, immunization may affect the biosafety level (e.g., the BSL-4 Junin virus can be worked on at BSL-3 by an immunized worker). Immunization may also be passive (e.g., the use of serum immunoglobulin in HBV exposures). However important, immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management.

- ***Immune Status of Individual***-Persons with altered immunocompetence may be at an increased risk when exposed to infectious agents. Immunodeficiency may be hereditary, congenital, or induced by a number of neoplastic or infectious diseases, by therapy, or by radiation. The risk of becoming infected or the consequence of infection may also be influenced by such factors as age, sex, race, pregnancy, surgery (e.g., splenectomy, gastrectomy), predisposing diseases (e.g., diabetes, lupus erythematosus), or altered physiological function. These and other variables must be considered in applying the generic risk assessments of the agent summary statements to specific activities of selected individuals.

Please be informed that certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These conditions can include: pregnancy, immunosuppression, and animal related allergies. If any of these conditions apply, inform your personal physician/health care professional of your work.

Section 4.2 Materials Containing Known Infectious Agents

The characteristics of most known infectious agents have been well identified. Information useful to risk assessment can be obtained from some of the sources below:

1. ***Biosafety in Microbiological and Biomedical Laboratories*** (BMBL): available from the CDC and at BMBL 5th Edition - Table of Contents
2. The Canadian Laboratory Centre for Disease Control (LCDC): The LCDC maintains Safety Data Sheets for microbial agents on its web site at http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php.
3. The listing of risk groups for microbiological agents in Appendix B of the *NIH Guidelines*.

Other sources include the American Public Health Association's manual, *Control of Communicable Diseases* (Benenson, Abram S., Editor. Control of Communicable Diseases Manual. 17th Edition, 2000. American Public Health Association, Washington, D.C. 2005)

Section 4.3 Materials Containing Unknown Infectious Agents

There are situations when the information is insufficient to perform an appropriate risk assessment, for example, with vaccine strains or attenuated strains of infectious agents. Some questions that may help in this risk assessment include:

1. Why is an infectious agent suspected?
2. What epidemiological data are available? What route of transmission is indicated?
3. What is the morbidity or mortality rate associated with the agent?
4. What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In these cases (i.e., absence of hard data), it is prudent to take a conservative approach in determining the risk of handling these agents. Consult with the Institutional Biosafety Officer (813) 974-0954 for questions regarding risk assessment.

Section 4.4 Materials containing recombinant or synthetic nucleic acid molecules

Recombinant or synthetic nucleic acid molecules as defined by the NIH Guidelines Nucleic acid molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can be replicated in a living cell and/or DNA

molecules that result from the replication of those molecules described above. It is the policy of the USF IBC that research involving the use of recombinant or synthetic nucleic acid molecules as defined by the *NIH Guidelines* must be documented by a registration and be reviewed and approved by the IBC.

- All research and teaching activities involving the use of recombinant or synthetic nucleic acid molecules material, whether Exempt or Non-Exempt from *NIH Guidelines*, must be registered with and reviewed by the IBC, using the appropriate registration forms.
- Investigators planning to administer recombinant DNA to animals should refer to Appendix Q and Section III-D-4 of the *NIH Guidelines* for information on biological and physical containment practices, and should visit the Institutional Animal Care and Use Committee (IACUC) Website for additional guidance and to obtain applications to work with animals.
- Work involving laboratory animals requires an additional application. For information visit the IACUC web page.
- PIs are responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories. This responsibility may not be shifted to inexperienced or untrained personnel.
- The use of recombinant or synthetic nucleic acid molecules in animal research requires approval by both the IBC and IACUC before initiation of study.

Recombinant DNA technology involves combining genetic information from different sources thereby creating genetically modified organisms (GMOs). This category of agents includes microorganisms that have been genetically modified through recombinant DNA technologies. Consult the "*Guidelines"*, published by the National Institutes of Health (NIH), as the reference for recombinant or synthetic nucleic acid molecules research. The NIH Guidelines may be found via this link: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

Biological Expression Systems

Biological expression systems are vectors and host cells that fulfill a number of criteria that makes them safe to use. A good example of a biological expression system is plasmid pUC18 (or derivatives thereof), which is frequently used as a cloning vector in combination with *Escherichia coli* K12 cells. The pUC18 plasmid and its derivatives have been entirely sequenced. More importantly, all genes required for efficient transfer to other bacteria have been deleted from the precursor plasmid pBR322 providing significant containment. *E. coli* K12 is a strain that lacks the genes known to render some *E. coli* strains pathogenic. Furthermore, *E. coli* K12 cannot permanently colonize the gut of healthy humans or animals. Thus, most routine genetic engineering experiments can be performed safely with *E. coli* K12/pUC18 at Biosafety Level 1 provided the inserted foreign DNA sequences do not require a higher biosafety level (see below).

Properties of the Donor Organism and Cloned DNA

Risk assessment must consider not only the vector/host system used but also the properties of the DNA to be cloned.

Viral Vectors for Gene Transfer

Among the recombinant viruses now routinely developed are adenoviruses, alphaviruses, retroviruses, vaccinia viruses, herpesviruses, and others designed to express heterologous gene products. However, the nature of the genetic modification and the quantity of virus must be carefully considered when selecting the appropriate biosafety level for work with a recombinant virus.

Among the points to consider in work with recombinant microorganisms are:

- Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
- Does the modification have the potential to alter the host range or cell tropism of the virus?
- Does the modification have the potential to increase the replication capacity of the virus?
- Does the inserted gene encode a known oncogene?
- Does the inserted gene have the potential for altering the cell cycle?
- Does the viral DNA integrate into the host genome?
- What is the probability of generating replication-competent viruses?

This list of questions is not meant to be all inclusive. Rather, it serves as an example of the information needed to judge whether a higher biosafety level is needed in work with genetically modified microorganisms. Although such vectors are usually replication-defective, they should be handled at the same biosafety level as the parent virus from which they are derived. In many cases the risk assessment will show that the recombinant organism can be handled at the same biosafety level as the wild-type recipient. In some instances, however, higher biosafety levels will be required. The reason for this is that the virus stocks may be contaminated with replication-competent viruses, which are generated by rare spontaneous recombination events in the complementing cell line.

Transgenic and "Knock-Out" Animals

Animals carrying foreign genetic information (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes ("knock-out" animals) do not generally present particular biological hazards.

Transgenic Plants

Transgenic plants expressing genes of animal or human origin should remain strictly contained within the facility. Such transgenic plants should be handled at biosafety levels appropriate to the characteristics of the products of the expressed genes.

PI's Responsibilities under NIH Guidelines

General Responsibility

Principal Investigators (PIs) are responsible for full compliance with the NIH Guidelines during the conduct of research involving recombinant or synthetic nucleic acid molecules. As part of this general responsibility, the PI should:

1. Be adequately trained in good microbiological techniques.
2. Provide laboratory research staff with protocols describing potential biohazards and necessary precautions.
3. Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents.
4. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
5. » Supervise laboratory staff to ensure that the required safety practices and techniques are employed.
6. Correct work errors and conditions that may result in the release of recombinant or synthetic nucleic acid materials.
7. Ensure the integrity of physical containment (e.g., biological safety cabinets) and biological containment (e.g., host-vector systems that preclude survival of the agent outside the laboratory).
8. Comply with permit and shipping requirements for recombinant or synthetic nucleic acid molecules.
9. Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination

Before initiating research subject to the NIH Guidelines, the PI must:

1. Determine whether the research is subject to Section III-A, III-B, III-C, III-D, or III-E of the NIH Guidelines.
2. Propose physical and biological containment levels in accordance with the NIH Guidelines when registering research with the IBC.
3. Propose appropriate microbiological practices and laboratory techniques to be used for the research.
4. Submit a research protocol to the IBC for review and approval.
5. Seek NIH OSP's determination regarding containment for experiments that require case-by-case review.
6. Petition NIH OSP, with notice to the IBC, for proposed exemptions from the NIH Guidelines.
7. Obtain IBC approval before initiating research subject to the NIH Guidelines.
8. Seek NIH approval, in addition to IBC approval, to conduct experiments specified in Sections III-A and III-B of the NIH Guidelines.

While conducting research subject to the NIH Guidelines, the PI must:

1. Determine the need for IBC review before modifying recombinant or synthetic nucleic acid research already approved by the IBC.
2. Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval.
3. Remain in communication with the IBC throughout the duration of the project.
4. Report any significant problems pertaining to the operation and implementation of containment practices and procedures, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the IBC, NIH OSP, and, as applicable, the Biological Safety Officer, Greenhouse or Animal Facility Director, and other appropriate authorities.

PIs conducting human gene transfer research subject to Section III-C of the NIH Guidelines must:

1. Ensure that all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OSP for review by the NIH Recombinant DNA Advisory Committee (RAC).
2. Provide a letter signed by the PI(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OSP complies with the requirements set forth in Appendix M.
3. Not enroll research participants in a human gene transfer clinical trial until the RAC review process has been completed; IBC approval (from the clinical trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained. » Comply with reporting requirements for human gene transfer experiments (see Appendix M-I-C of the NIH Guidelines).

Section 4.5 Materials Containing Biological Toxins

Biological toxins are toxic materials or products of biological origin. Laboratory use of biological toxins falls under the auspices of the Institutional Biosafety Committee.

Safety Data Sheets (SDSs): (must be maintained if available)

- SDSs for the specific toxin should be received from the vendor upon receipt of the toxin. MSDSs may also be available on the Internet through various vendor websites or through the biosafety office upon request.
- Toxicology textbooks such as *Casarett's and Doull's Toxicology* are also good sources of hazard information for toxins.

Section 4.6 Animal Studies

Laboratory studies involving infectious agents and animals may present different kinds of physical, environmental, and biological hazards than that in a laboratory environment. The specific hazards present in an animal model are unique, varying according to the species involved and the nature of the research activity. The risk assessment for the

biological hazard should particularly focus on the animal facility's potential for increased exposure, both to human pathogens and to zoonotic agents.

Laboratory animals may harbor microorganisms that can produce human diseases following bites, scratches, or exposure to excreted/secreted microorganisms. In the process of inoculating animals, an investigator can be exposed to infectious material by accidental self-inoculation or inhalation of infectious aerosols. During surgical procedures, necropsies and processing of tissues, aerosols can be produced unintentionally, or the operator can inflict self-injury with contaminated instruments.

Since animal excreta can also be a source of infectious microorganisms, investigators should take precautions to minimize aerosols and dust when changing bedding and cleaning cages. Animals can also harbor infectious organisms which are acquired naturally. Some infectious agents can give rise to a chronic carrier state, or an agent might be shed intermittently. If the possibility that such an agent may be excreted, secreted, exhaled, or shed by an animal during the course of an experiment cannot be excluded, then all those animals should be kept at the containment level appropriate to the risk.

The animal routes of transmission must also be considered in the risk assessment. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous. Animal handlers in research facilities working on infectious agents have a greater risk of exposure from the animals' aerosols, bites, and scratches.

Visit the USF IACUC Website for additional guidance and to obtain applications to work with animals. PIs are responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories. This responsibility may not be shifted to inexperienced or untrained personnel. Animal biosafety level criteria must be adhered to, as specified in the CDC BMBL, when using infectious agents in conjunction with animal protocols.

All work with animals involving the use of infectious agents, Select Agents, biological toxins, and recombinant or synthetic nucleic acid molecules must be submitted to the IBC for review and approval, in addition to approval from the IACUC.

Section 5 Biosafety Containment Levels (BSL)

The CDC/NIH BMBL 5th edition describes four biosafety levels (BSLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity.

The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Laboratories are designated as Biosafety Level 1 (BSL-1), Biosafety Level 2 (BSL-2), Biosafety Level 3 (BSL-3), and Biosafety Level 4 (BSL-4). A Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Level 4 (BSL-4) requires a special containment laboratory or facility, which is not available at USF. Since most of the research at USF is conducted at BSL-1 and BSL-2, with a few experiments at BSL-3, this manual will mainly focus on these three Biosafety Levels.

Note: There are no designated BSL-4 laboratories at USF. Therefore, no agents requiring BSL-4 containment may be used or possessed by USF faculty and staff.

Section 5.1 Biosafety Level One: BSL-1

Biosafety Level 1
Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

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

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice

controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

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

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.

    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

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

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

B. Special Practices

None

C. Safety Equipment (Primary Barriers)

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

D. Laboratory Facilities (Secondary Barriers)

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

Section 5.2 Biosafety Level Two: BSL-2

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures
- access to the laboratory is restricted when work is being conducted

- all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

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

A. Standard Microbiological Practices

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

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

B. Special Practices

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

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

C. Safety Equipment (Primary Barriers)

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

D. Laboratory Facilities (Secondary Barriers)

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

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

Section 5.3 Biosafety Level Three: BSL-3

Biosafety Level 3
Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.
The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

It is recognized, however, that some existing facilities may not have all the facility features recommended for BSL-3 (e.g., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a BSL-2 facility, **providing:**

1. the exhaust air from the laboratory room is discharged to the outdoors;
2. the ventilation to the laboratory is balanced to provide directional airflow into the room;
3. access to the laboratory is restricted when work is in progress; and
4. recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for BSL-3 are rigorously followed.

The decision to implement this modification of BSL-3 recommendations will be made only by the Institutional Biosafety Committee (IBC).

The following standard and special safety practices, equipment, and facilities apply to agents assigned to BSL-3:

A. Standard Microbiological Practices

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

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

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

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

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

C. Safety Equipment (Primary Barriers)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splash guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers:
    a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

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

    Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
    a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
    b. The laboratory exhaust air must not re-circulate to any other area of the building.
    c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

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

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

Health and Medical Surveillance for BSL3

The objectives of a health and medical surveillance program (initial medical evaluation will include medical history form preparation and baseline laboratory evaluation as applicable) for a BSL-3 laboratory are as follows:

1. Medical examination of all laboratory personnel who work in Biosafety Level 3 containment laboratories is recommended. This should include recording of a detailed medical history and a physical examination.
2. If applicable, a baseline serum sample should be obtained and stored for future reference.
3. Individuals who are immunocompromised should consult their physician prior to working in BSL-3 containment laboratories.
4. Special consideration should be given to the employment of pregnant women.

USF Blood Borne Pathogen Exposure/Health Surveillance

Diana Doughty (813) 974-3163
USF Health Medical Health Administration Pager: (813) 216-0153
After regular business hours, please contact: (813) 974-2201
The Infectious Disease Physician (USF Physician's Group answering service)

Additional Important Concepts for BSL-3 Labs

- Solid front/wrap-around gowns should be worn; front-buttoned standard laboratory coats are unsuitable.
- Access to the laboratory area must be designed to prevent entrance of arthropods and other vermin.
- Access doors must be self-closing.
- Windows should be closed and sealed.
- Effluents should be decontaminated before being discharged to the sanitary sewer.

Section 5.4 Selection of BSL Containment Level

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work and appropriate personal protective equipment selected. The biosafety level assigned to an agent is based on:

1. The growth and manipulation of the quantities (volumes) and concentrations of infectious agents required ("production quantities").
2. Activities with clinical materials may pose a lesser risk to personnel than those activities associated with manipulation of cultures.
3. Manipulations which are likely to produce aerosols or which are otherwise intrinsically hazardous.

"Production quantities":

1. Large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities.
2. Propagation and concentration of infectious agents, as occurs in large-scale fermentations, antigen, and vaccine production.

The IBC must make an assessment of the activities conducted and select practices, containment equipment, and facilities appropriate to the risk, irrespective of the volume or concentration of agent involved.

The IBC may require a biosafety level higher by the unique nature of the proposed activity (e.g., the need for special containment for experimentally generated aerosols for inhalation studies). Similarly, a biosafety level may be adapted to compensate for the absence of certain recommended safeguards. An acceptable safety level may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features provide the recommended containment level and standard microbiological practices, special practices, and safety equipment for the next higher biosafety level are rigorously followed (i.e., BSL-2 facility features with BSL-3 practices).

Section 6 Biosafety Levels for Laboratory Animal Facilities

General Principles

Note: Experimentally or naturally infected animals likely to shed pathogens in body secretions or excretions must be separated from non-infected animals.

Summary of Animal Biosafety Level (ABSL)
Animal facilities, like laboratories, may be designated primarily according to the risk group of the microorganisms under investigation as Biosafety Level 1, 2, 3, or 4 by:

1. normal route of transmission,
2. volumes and concentrations to be used,

3. route of inoculation, and
4. route they may be excreted.

With respect to the animals:

1. nature of the animals (i.e., their aggressiveness and tendency to bite and scratch),
2. natural ecto- and endoparasites,
3. zoonotic diseases to which they are susceptible, and
4. possible dissemination of allergens.

As with laboratories, the requirements for design features, equipment, and precautions increase in stringency according to the biosafety level. These guidelines are additive, so that each higher level incorporates the standards of the lower levels. Four biosafety levels are described for activities involving infectious disease work with experimental animals. These four combinations of practices, safety equipment, and facilities are designated **Animal Biosafety Levels (ABSL-1, -2, -3, and -4**) and provide increasing levels of protection to personnel and the environment.

Summary of Animal Facility Containment Levels: Risk Group, Containment Level, Laboratory Practices, and Safety Equipment:

1. ABSL-1: Limited access, protective clothing, and gloves.
2. ABSL-2: ABSL-1 practices + hazard warning signs, Class I or II BSCs for activities that produce aerosols, and decontamination of waste and cages before washing.
3. ABSL-3: ABSL-2 practices + controlled access, BSCs, and special protective clothing for all activities.
4. ABSL-4: ABSL-3 practices + strictly limited access, clothing change before entering, Class III BSCs or positive pressure suits, shower on exit, and decontamination of all wastes before removal from facility.

(ABSL-Animal Biosafety Level; BSC-Biological Safety Cabinet)

Section 6.1 Animal Biosafety Level 1 (ABSL-1)

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

ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See Section 2, Biological Risk Assessment.)

Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

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

A. Standard Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee.
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered.
   Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.
   Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.
   Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security-sensitive agent information should be posted in accordance with the

institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
   Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
   Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
   Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated.
   Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.
    When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

    a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
    b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

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

Decontaminate all potentially infectious materials before disposal using an effective method.

B. Special Practices

None

C. Safety Equipment (Primary Barriers)

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

6. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.
   The wearing of designated personal protection equipment (laboratory coats, gowns, shoe covers, etc.) in the facility is required. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. The animal facility must have a sink for hand washing.
   Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.
   It is recommended that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.
8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Section 6.2 Animal Biosafety Level 2 (ABSL-2)

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

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

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.
The following standard and special practices, safety equipment, and facility requirements apply to ABSL-2:

A. Standard Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each organization must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study, animal protocols must also be reviewed and approved by the IACUC and the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. The safety manual must be available and accessible. Personnel are advised of potential hazards, and are required to read and follow instructions on practices and procedures. Consideration should be given to specific biohazards unique to the animal species and protocol in use.

3. The supervisor must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations.

1. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating the universal biohazard symbol must be posted at the entrance to areas where infectious materials and/ or animals are housed or are manipulated when infectious agents are present. The sign must include the animal biosafety level, general occupational health requirements, personal protective equipment requirements, the supervisor's name (or names of other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Identification of all infectious agents is necessary when more than one agent is being used within an animal room. Security-sensitive agent information and occupational health requirements should be posted in accordance with the institutional policy. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.
6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing. Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials and when handling animals. Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye, face and respiratory

protection should be used in rooms containing infected animals, as dictated by the risk assessment.

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

B. Special Practices

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered

appropriate immunizations for agents handled or potentially present, before entry into animal rooms. When appropriate, a base line serum sample should be stored**.**

2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used. Restraint devices and practices that reduce the risk of exposure during animal manipulations (e.g., physical restraint devices, chemical restraint medications) should be used whenever possible.
3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse. A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented. Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label. Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of content prior to incineration is recommended.
4. Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas. Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

C. Safety Equipment (Primary Barriers)

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered

with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates. Persons having contact with NHPs should assess risk of mucous membrane exposure and wear protective equipment (e.g., masks, goggles, face shields) appropriate for the task to be performed. Respiratory protection is worn based upon risk assessment.
4. Gloves are worn to protect hands from exposure to hazardous materials. A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.
   Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
   Gloves must not be worn outside the animal rooms.
   Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
   Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.
   Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. If the animal

facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning. Floors must be slip-resistant, impervious to liquids, and resistant to chemicals.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.
5. External windows are not recommended; if present, windows must be sealed and resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.
6. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. The cage wash area should be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system

by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance. All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.

12. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
13. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.
14. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Section 6.3 Animal Biosafety Level 3 (ABSL-3)

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

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

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

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

A. Standard Practices

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study, animal protocols must be reviewed and approved by the IACUC5 and the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals.

The safety manual must be available and accessible. Personnel are advised of potential and special hazards, and are required to read and follow instructions on practices and procedures. Consideration must be given to specific biohazards unique to the animal species and protocol in use.

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

Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated.
Persons must wash their hands after removing gloves and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye, face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment. 8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

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

B. Special Practices

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

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

C. Safety Equipment (Primary Barriers)

1. Properly maintained BSCs and other physical containment devices or equipment should be used for all manipulations for infectious materials and when possible, animals. These manipulations include necropsy, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals. The risk of infectious aerosols from infected animals or bedding can be reduced by primary barrier systems. These systems may include solid wall and bottom cages covered with filter bonnets, ventilated cage rack systems, or for larger cages placed in inward flow ventilated enclosures or other equivalent systems or devices.
2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized. Personnel within the animal facility where protective clothing, such as uniforms or scrub suits. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home. Disposable personal protective equipment such as non-woven olefin cover-all suits, wrap-around or solid-front gowns should be worn over this clothing, before entering the areas where infectious materials and/or animals are housed or manipulated. Front-button laboratory coats are unsuitable. Disposable personal protective equipment must be removed when leaving the areas where infectious materials and/or animals are housed or are manipulated. Scrub suits and uniforms are removed before leaving the animal facility. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.
3. All personnel entering areas where infectious materials and/or animals are housed or manipulated wear appropriate eye, face and respiratory protection. To prevent cross contamination, boots, shoe covers, or other protective footwear, are used where indicated. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection when entering areas with potentially high concentrations or airborne particulates.
4. Gloves are worn to protect hands from exposure to hazardous materials.
   A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available. Procedures may require the use of wearing two pairs of gloves (double-glove).
   Gloves are changed when contaminated, glove integrity is compromised, or when otherwise necessary.
   Gloves must not be worn outside the animal rooms.
   Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.
   Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

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

D. Facilities (Secondary Barriers)

1. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking. Access to the animal facility is restricted. Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Entry into the containment area is via a double-door entry, which constitutes an anteroom/airlock and a change room. Showers may be considered based on risk assessment. An additional double-door access anteroom or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility.
2. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility. The sink should be hands-free or automatically operated. If the animal facility has multiple segregated areas where infectious materials and/or animals are housed or are manipulated, a sink must also be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.
3. The animal facility is designed, constructed, and maintained to facilitate cleaning, decontamination and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed, including openings around ducts and doorframes, to facilitate pest control, proper cleaning and decontamination. Walls, floors and ceilings should form a sealed and sanitizable surface. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Flooring is seamless, sealed resilient or poured floors, with integral cove bases. Decontamination of an entire animal room should be considered when there has been gross contamination of the space, significant changes in usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the animal room must be based on the risk assessment.
4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning. Furniture should be minimized. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Equipment and furnishings with sharp edges and corners should be avoided.
5. External windows are not recommended; if present, all windows must be sealed and must be resistant to breakage. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation of the facility should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*. The direction of airflow into the animal facility is inward; animal rooms maintain inward directional airflow compared to adjoining hallways. A ducted exhaust air ventilation system is provided. Exhaust air is discharged to the outside without being recirculated to other rooms. This system creates directional airflow, which draws air into the animal room from "clean" areas and toward "contaminated" areas. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. HEPA filtration and other treatments of the exhaust air may not be required, but should be considered based on site requirements, specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates directional inward airflow be provided at the animal room entry. The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. Alarms should be considered to notify personnel of ventilation and HVAC system failure.
7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas, to facilitate cleaning and minimize the accumulation of debris or fomites.
8. Floor drains must be maintained and filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed in a mechanical cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F. Cages should be autoclaved or otherwise decontaminated prior to removal from ABSL-3 space. The cage wash facility should be designed and constructed to accommodate high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage cleaning process.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. BSCs (Class II, Class III) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or exhausted directly to the outside through a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must supply air in such a manner that prevents positive pressurization of the cabinet or the laboratory room. All BSCs should be used according to manufacturers' specifications. When applicable, equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being

discharged into the animal facility. These HEPA filters should be tested and/or replaced at least annually.

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

THERE ARE CURRENTLY NO ABSL-4 FACILITIES AT USF.

Section 6.4 Invertebrates

The invertebrates that are used for experimental purposes in laboratories are usually the reservoirs or vectors of pathogens or, as in the case of ecological and environmental investigations, may be fortuitously infected with pathogens ingested with their food. They may include members of the following phyla: Annelida, Aschelminthes, Arthropoda, Echinodermata, Mollusca, Platyhelminthes, and Protozoa. As with vertebrates, the animal facility biosafety level will be determined by the risk groups of the agents under investigation or naturally present, but the following additional precautions are necessary with certain arthropods, particularly with flying insects.

1. Separate rooms should be provided for infected and non-infected invertebrates.
2. The rooms should be capable of being sealed for fumigation.
3. Insecticide sprays should be readily available.
4. “Chilling” facilities should be provided to reduce, where necessary, the activity of invertebrates.
5. Access should be through an anteroom containing insect traps and with arthropod-proof screens on the doors.
6. All exhaust ventilation ducts and openable windows should be fitted with arthropod-proof screens.
7. Waste traps on sinks and sluices should not be allowed to dry out.
8. All waste should be decontaminated by autoclaving, as some invertebrates are not killed by all disinfectants.

9. A check should be kept on the numbers of larval and adult forms of flying, crawling, and jumping arthropods.
10. Containers for ticks and mites should stand in trays of oil.
11. Infected or potentially infected flying insects must be contained in double-netted cages.
12. Infected or potentially infected arthropods must be handled in biological safety cabinets or isolators.
13. Infected or potentially infected arthropods may be manipulated on cooling trays.

Section 7 Selected Guidelines for Work with Unique Hazards

Section 7.1 Toxins

General Principles

Biological toxins consist of any toxic substance produced by microorganisms, plants, or animals. They include metabolites of living organisms, degradation products of nonliving organisms, and those materials rendered toxic by the metabolic activity of microorganisms. Examples include botulinum toxins (A-G), tetanus toxin, and staphylococcal enterotoxins (A-F), which are produced by bacteria; tetrodotoxin, and ciguatoxin, which are produced by animals; and ricin toxin, tricothecence mycotoxins, and abrin, which are produced by plants. They can cause acute toxic disease as well as long-term effects. These guidelines specifically address work with exotoxins produced by microorganisms, primarily bacteria but also fungi.

Possession and use of biological toxins must be registered with the IBC.

As toxins are not living organisms, they do not fall neatly into the either category of the "classic" biohazard or the "classic" organic chemical. Toxins are non-replicating and are not communicable between individuals, unlike pathogenic organisms. They are, however, capable of eliciting pathologic effects associated with infectious diseases, and are one of the key virulence factors. Most toxins are highly toxic in minute quantities. There are no short-term exposure limits, ceiling limits, or time weighted averages (TWA), and toxicological data are often limited. There is also no environmental monitoring for toxins currently available. These are important differences distinguishing toxins from chemical hazards. Toxins are generally not volatile or dermally active (some mycotoxins are exceptions), therefore they are most likely to represent a hazard through aerosolization, ingestion, or percutaneous injection. Using features of both chemical and biological safety programs together with a zero exposure approach presents an effective way to mitigate the risk involved in working with biological toxins. Special care must be taken when working with multiple toxins, as toxin-toxin interactions leading to synergistic, zero, and antagonistic effects and sub-threshold combination effects could occur.

Safety Practices for Work with Biological Toxins

Principal Investigators (PIs) are encouraged to read the guidance listed below and to consult with subject matter experts before using any toxin to ensure that appropriate facilities, containment equipment, policies and procedures, personnel training programs, and medical surveillance protocols specific to the toxin and the laboratory are in place. The laboratory facilities, equipment, and procedures appropriate for work with toxins of biological origin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. If both toxins and infectious agents are used, both must be considered when containment equipment is selected and policies and procedures are written. If animals are used, animal safety practices must also be considered.

A. Standard Practices

Standard practices listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

B. Special Practices

Special practices listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Each laboratory should develop standard operating procedures and protocols specific to the toxin(s) used in that laboratory. These should include:
   a. identify the hazards that will be encountered in normal use of the toxin, and those that could be encountered in case of a spill or other accident, and
   b. specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance).
2. Training specific to the toxin(s) used should be required and documented for all laboratory personnel working with toxins, before starting work with the toxin, and at intervals thereafter.
3. An inventory control system should be in place and regularly updated.
4. Toxins should be stored in locked storage rooms, cabinets, or freezers when not in use.
5. Access to areas containing toxins should be restricted to those whose work assignments require access.
6. Preparation of primary containers of toxin stock solutions and manipulations of primary containers of dry forms of toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system approved by the IBC. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the toxin.
7. The user should verify inward airflow of the hood or biological safety cabinet before initiating work.
8. All work should be done within the operationally effective zone of the hood or biological safety cabinet.
9. When toxins are in use, the room should be posted to indicate "Toxins in Use Authorized Personnel Only." Any special entry requirements should be posted on

the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while toxins are in use.

10. All high risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
11. Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Toxins should be transported only in leak/spill-proof secondary containers.
12. Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the toxin before removal from the laboratory for disposal, cleaning, or repair. If decontamination is not possible/practical, materials (e.g., used gloves) should be disposed of as toxic waste. Materials contaminated with infectious agents as well as toxins should also be autoclaved or otherwise rendered non-infectious before leaving the laboratory.
13. The interior of the hood, glove box, or cabinet should be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet should be posted to indicate that toxins are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

Safety Equipment

The safety equipment guidelines listed under BSL-2 and BSL-3 should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. When using an open-fronted fume hood or biological safety cabinet, protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.
2. Eye protection should be worn if an open-fronted containment system is used.
3. Other protective equipment may be required, depending on the characteristics of the toxin and the containment system. For example, use additional respiratory protection if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.
4. When handling dry forms of toxins that are electrostatic:
    a. Do not wear gloves (such as latex) that help to generate static electricity
    b. Use a glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.
5. When handling toxins that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the toxin.
6. Consider both toxin and diluent when selecting gloves and other protective clothing.
7. If infectious agents and toxins are used together in an experimental system, consider both when selecting protective clothing and equipment.

Laboratory Facilities

Laboratory facility recommendations listed under BSL-2 and BSL-3 and OSHA standards should be reviewed and incorporated as appropriate into protocols for work with toxins.

1. Vacuum lines used with systems containing toxins, should be protected with a HEPA filter to prevent entry of toxins into the lines.
2. Sink drains should be similarly protected when water aspirators are used.

Inactivation of Toxins

Biological toxins can be extremely hazardous even in minute quantities. Investigators must ensure that appropriate equipment and safety procedures are in place for the specific toxin used and the type of experiments performed in their laboratory. Guidelines for working with toxins of biological origin can be found in Appendix I of the CDC/NIH Publication *Biosafety in Microbiological and Biomedical Laboratories*.

Decontamination procedures (e.g. autoclaving, chemical disinfectants) should be assessed for each toxin by consulting current literature, as the optimal procedures for each toxin vary widely when working with infectious organisms in conjunction with their toxins, care must be taken to ensure that both the infectious agent and the toxin are neutralized.

For most protein toxins, a sodium hypochlorite, or sodium hypochlorite and sodium hydroxide mixture provides effective decontamination. Surfaces may be decontaminated with a 0.5% solution of sodium hypochlorite. Solid and liquid waste may be decontaminated with a solution of 2.5% sodium hypochlorite and 0.25 N sodium hydroxide. They should be soaked or mixed in a 1:1 ratio and allowed to stand for 16 hours (solid waste) or 8 hours (liquid waste). Again it must be noted that the preferred method is highly variable and should be ascertained for the specific toxin in question. Generally, the higher molecular weight proteinacious bacterial toxins are inactivated by steam sterilization. Steam sterilization should not be used for destruction of any low molecular weight toxins (e.g. mycotoxins, marine, and reptile venoms). (See Appendix VI for toxin inactivation)

Section 7.2 Human and/or Primate Tissue and Cell Culture

Uncharacterized Human Blood or Tissue

All human blood, blood products, body fluids, and tissue have the potential to be infectious (the concept of "Standard Precautions", formerly Universal Precautions) and must be handled using Biosafety Level 2 (BSL-2) practices and procedures.
The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissues.

Persons who are exposed to these materials in the laboratory or other occupational settings are considered to have a potential for exposure to bloodborne pathogens. All employees working with blood and/or body fluids should be enrolled in the institutional Blood Borne Pathogens Program, and should work under the policies and guidelines

established by the USF Exposure Control Plan. Contact the Director of the Health Sciences Center Medical Health Administration at (813) 974-3163.

Tissue Culture

Human or animal pathogens may be associated with cell or organ cultures. Cell cultures known (or suspected) to contain an etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent.

The following cell cultures and tissues require Standard Precautions (i.e., see Appendix VII) and procedures:

1. All cultured cells derived from human sources, including immortalized and "well established" cell lines.
2. All cultured cells derived from non-human primate tissue.
3. All cultured cells exposed to or transformed by a primate (human & non-human) oncogenic virus.
4. All clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.
5. All primate (human & non-human) tissue.
6. All virus-containing primate (human & non-human) cultured cells.
7. All mycoplasma containing cultured cells.

Recommended Practices

Human and non-human primate cells should be handled using Biosafety Level 2 (BSL-2) practices and containment.

Section 7.3 Transmissible Spongiform Encephalopathy (TSE)

Spongiform encephalopathies (Creutzfeldt-Jakob, kuru, and related agents) are fatal prion diseases that have been demonstrated in the brain and spinal cord of infected persons. These agents are resistant to conventional inactivation procedures including chemicals (formalin, alcohol), boiling, dry heat, and irradiation and these agents can be present in fixed tissue from infected persons. Although nerve tissue (brain, spinal cord) is usually more infectious, all tissues from humans and animals infected with these agents should be considered potentially hazardous. Although laboratory-associated infections have not been demonstrated, it is prudent to consider nerve tissue (even fixed tissue) to be potentially infectious. BSL-2 containment and practices are recommended as a minimum for all activities utilizing known or potentially infectious tissues and fluids from naturally infected humans and from experimentally infected animals.

Precautions with Materials that may Contain Prions

The selection of a biosafety level for work with materials associated with TSEs will depend on the samples to be studied, and should be undertaken in consultation with experts. The highest concentrations of prions are found in central nervous system tissue. As there is no method that will ensure decontamination after exposure to prions, it is important to stress the use of disposable instruments whenever possible, and to use a protective covering for the work surface of the biological safety cabinet, which can also be disposed of after use. The main precaution to be taken is to avoid ingestion of

contaminated materials or puncture of the laboratory worker's skin. The following additional precautions should be taken, as the agents are not killed by the normal processes of laboratory disinfection and sterilization.

1. The use of dedicated equipment is highly recommended (i.e., equipment is not shared with other laboratories).
2. Disposable laboratory protective clothing (gowns and aprons) and gloves must be worn (steel mesh gloves between rubber gloves for pathologists).
3. Use of disposable plastic ware, which can be treated and discarded as dry waste, is highly recommended.
4. Tissue processors should not be used because of the problems of disinfection. Jars and beakers should be used instead.
5. All manipulations must be conducted in biological safety cabinets.
6. Great care should be exercised to avoid aerosol production, accidental ingestion, and cuts and punctures of the skin.
7. Formalin-fixed tissues should be regarded as still infectious, even after prolonged exposure to formalin.
8. Bench waste, including disposable gloves, gowns, and aprons, should be autoclaved, followed by incineration.
9. Non-disposable instruments, including steel mesh gloves, must be collected for decontamination.
10. Infectious liquid waste contaminated with prions should be treated with 2 mol/l sodium hydroxide (final concentration) for 1 h followed by autoclaving.
11. Paraformaldehyde vaporization procedures do not diminish prion titers and prions are resistant to ultraviolet irradiation. However, the cabinets must continue to be decontaminated by standard methods (i.e., formaldehyde gas) to inactivate other agents that may be present.
12. Prion contaminated biological safety cabinets and other surfaces can be decontaminated by repeated wetting with 2 mol/l sodium hydroxide for 1 h followed by rinsing with water. High-efficiency particulate air (HEPA) filters should be autoclaved and incinerated at regular intervals.
13. Instruments should be soaked for 1 h in 2 mol/l sodium hydroxide and then rinsed well in water before autoclaving.
14. Instruments that cannot be autoclaved can be cleaned by repeated wetting with 2 mol/l sodium hydroxide over a 1-h period. Appropriate washing to remove residual sodium hydroxide is required.

Section 7.4 Aerosol-Generating Processes

Aerosols (dispersions of particles in air) can result from the use of blenders, mixers, sonicators, cell disrupters, centrifuges, syringes, pipettes, aspirators, and test and centrifuge tube caps. Several well-documented studies have made it clear that great attention must be given to prevent contamination of room air with the suspension of liquid or solid particles containing hazardous materials including radioisotopes and infectious agents (viruses and mycoplasma from "normal" cells), as well as toxic chemicals and carcinogens. Particle size is a factor in determining the path an aerosol will

follow. Particles in the range of 1 to 5 microns present the greatest hazard to the laboratory worker, since they more readily penetrate the respiratory tract than larger particles and are more readily retained than smaller or larger particles. Many laboratory procedures produce aerosols with particles in this range. Particles larger than 10 microns fall out on surfaces or are impinged on materials with an opposite electrostatic charge. In the respiratory tract, larger particles do not penetrate into the lower spaces but are removed by interception and impaction in the upper respiratory tract and subsequently expelled or swallowed. Large droplets that fall out on surfaces dry quickly and secondary aerosols of the dry particles can be created by air currents or laboratory activity. Significant settling of larger particles from an aerosol can occur in five minutes; however, most of the remaining small particles require 30 minutes to an hour to settle, assuming that fresh currents of air do not prevent their settling. This is why it is best to wait before cleaning up a spill of infectious virus, or other biologically hazardous material. Besides the direct effects of aerosols, they may contaminate surfaces of the skin or equipment and subsequently enter the body as a result of hand-to-mouth contact and ingestion or through abrasions of the skin. In addition to avoiding the creation of an aerosol, three general approaches are recommended to decrease the hazards of aerosols associated with research on tumor specimens, cell and virus cultures and concentrates, and toxic chemical materials:

- Reduce the extent or concentration of the aerosol.
- Contain the aerosol in a primary barrier system.
- Use personal respiratory protection and protective laboratory clothing.

Examples of Some Aerosol-Generating Processes

- Forced expulsion of the last drop of liquid or mixing of liquid by alternately sucking and blowing with the pipette, creating splashes and bubbles.
- Removing the cap or stopper from bottle after vigorous shaking to mix, wash, or re-suspend material.
- Blending materials to disrupt cells, release enzymes or viruses, to homogenize suspensions, etc., without aerosol tight cover seals or leak-proof rotor bearings.
- Sonic disruption of cells or organelles.
- Grinding tissue with mortar and pestle, glass tissue grinder, or ball mill.
- Pouring hazardous materials from one container to another (e.g., decanting supernatants).
- Sterilizing a wire loop or needle in a flame, creating splatter.
- Withdrawing a syringe needle as from a vaccine bottle or following inoculation of experimental animals.
- Weighing dry hazardous materials.
- Opening a freeze-dried preparation.
- Removing plugs from flasks and tubes.
- Handling cages with contaminated bedding that held infected animals or large animals in open areas or unventilated cages.

Measures to Decrease Hazards from Aerosols

The below are general tips (for equipment specific procedures to reduce airborne hazards see laboratory equipment section):

- Use gravity flow of liquid with pipette calibrated for mark-to-mark drain-to-tip delivery and with pipette tip in contact with container wall.
- Use swirling motion rather than shaking; allow aerosol to settle for a few minutes after bubbles disappear before removing cap or stopper.
- Use special safety containers with seals to prevent escape of aerosols; use drain/siphon system to remove contents without removing cover.
- Use cup or chamber that is aerosol tight; allow aerosol to settle before opening cup. Place sonicator in fume hood or biosafety cabinet.
- Use slow speeds; use a clear plastic or inflatable glove bag to further contain the operation within the safety cabinet; allow aerosol to settle before removing cover.
- Use transfer pipettes or closed siphon or vacuum technique.
- Gradually dry loop or needle near flame, or use specially designed incinerator for loops and needles.
- Use sterile cotton gauze to enclose needle; if experiment permits, use disinfectant with cotton or gauze.
- Use draft-free, low-humidity enclosure for balance; discharge static electricity; use tared weighing containers not open weighing dishes or papers.
- If material is in an ampoule, nick the ampoule with a file, cover its neck with sterile gauze.
- If material is in a rubber-stoppered bottle, first relieve vacuum with a hypodermic needle.
- If material is to be dissolved or suspended in liquid, introduce the liquid with a syringe and cover needle with gauze wetted with disinfectant.
- Avoid disturbing cage contents; it is recommended to use liquid disinfectants to dampen cage bedding before cleaning in conjunction with use of PPE.

Section 8 Personal Protective Equipment (PPE)

Choice of PPE should be selected based on the specific work, exposure conditions that will be encountered, and the anticipated level of risk.

Section 8.1 General Principles

PPE acts as a barrier to minimize the risk of exposure from accidental ingestion, absorption, inhalation, and parenteral introduction (accidental inoculation via: hypodermic needle, sharp instruments or broken glass, cuts and scratches, or animal scratches/bites). PPE should be worn when working in the laboratory. At a minimum the USF Institutional Biosafety Officer (IBO) recommends:

- Lab Coat
- Eye Protection

- Appropriate Gloves
- Closed Toe Shoes

A thorough risk assessment will aid in the determination of additional PPE to be used.

Before leaving the laboratory, protective clothing should be removed, and hands should be washed.

- Safety glasses, face shields (visors), or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects, and/or sources of artificial ultraviolet radiation.
- Lab coat - protective laboratory clothing is worn to protect exposed skin surfaces and personal clothing.
- Personal Protective Equipment (lab coats, etc.) should not be worn outside of the laboratory (e.g., canteens, coffee rooms, offices, libraries, staff rooms, toilets).
- Protective laboratory clothing should not be stored in the same lockers or cupboards as street clothing.
- Open-toed and/or heel-less footwear should not be worn in laboratories.

Section 8.2 Laboratory Coats, Gowns, Coveralls, and Aprons

The purpose of lab clothing is:

- to serve as a barrier for exposures
- to keep street clothing, forearms, or other exposed surfaces free of contamination
- to protect one's personal clothing
- to prevent contamination of laboratory tissue cultures/specimens/products from the normal flora present on the skin

Laboratory coats should be:

- fully buttoned
- long-sleeved

For BSL-3 laboratories:

- solid-front or wrap-around gowns or suits with non-absorbent coating
- elastic-cuffed

Additional criteria for selecting clothing are:

- comfort,
- appearance,
- closure types and location,
- antistatic properties, and
- durability.

The wearing of lab coats is considered to be standard microbiological practice for BSL-1 and -2 laboratories. Laboratory coats made of 100% cotton are flame resistant and non-reactive to many chemicals.

Laboratory dress code should discourage the wearing of shorts and open-toed and/or heel-less shoes.

It is a good laboratory practice to remove lab coats or gowns before leaving the laboratory to minimize the spread of contamination outside the laboratory. It is important to realize that wearing these items (especially lab coats) to the cafeteria, libraries, meetings, or to other buildings provides a mechanism for spreading contamination to others as well as to oneself. Lab coats should be left in the laboratory and must not be taken home for washing.

Section 8.3 Gloves

Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to "sharps" injuries. Gloves are available that provide protection against a variety of hazards, including infectious agents, chemicals, and radioactive material. Unfortunately, there is no single glove type that provides adequate protection for all hazards (or even all chemicals).

Gloves prevent exposure of the skin, and any cuts, dermatitis, etc. that may be present, to biohazardous materials. Both latex and nitrile exam gloves will prevent exposure to microorganisms. However latex gloves do not generally provide adequate protection against liquid chemicals; additionally, many people develop latex allergies(such as dermatitis) as a result of wearing latex gloves, particularly those with powder. Thin nitrile gloves are an alternative to latex examination gloves that provide similar dexterity and increased chemical resistance.

Contamination control requires that gloves be removed and hands thoroughly washed after exiting a Biological Safety Cabinet (BSC), before touching non-contaminated laboratory areas and equipment (e.g., clean areas, phones, computers, door knobs, pencils, pens), after handling infectious materials, and prior to exiting the laboratory. Always check gloves for pinholes prior to use and wash hands after removing gloves. Used disposable gloves should be discarded with biohazardous wastes.

Section 8.4 Goggles, Safety Glasses, and Face Shields

Eye and face protection:

- Prevention of splashes into the eyes, nose, and mouth (mucous membrane exposure), and onto the skin. Microbial infection can occur as a result of splashes.
- Prescription glasses can be manufactured with special frames that meet ANSI Z87.1 standards and comply with OSHA regulations as safety glasses.
- Goggles provide protection against biological hazards. Goggles with indirect venting provide a good barrier against such splashes.
- Face shields should be full face (i.e., chin length face), and are held in place by head straps or caps. A face shield can be worn in addition to goggles (some face

shields do not provide adequate eye protection by themselves) to provide further protection.

Section 8.5 Respirators

Use of N-95 particulate respirators requires a risk assessment when working with certain infectious agents. The IBC will make the final determination if certain laboratory activities require respiratory protection to prevent inhalation of infectious agents. Respiratory protection may be used when carrying out high-hazard procedures (e.g., BSL-3 lab). In addition, respirators prevent the inhalation of aerosolized microorganisms (inhalation exposure) when safety equipment or engineering controls designed to contain infectious aerosols, such as a biosafety cabinet, is not available or feasible. The choice between mask and respirator, and type of respirator will depend on the type of hazard. **Note: no filter other than a HEPA filter will provide protection against microorganisms. Fully self-contained respirators with an integral air supply provide full protection.**

OSHA Regulations, as well as good safety practice, require that personnel be medically evaluated, specifically trained, and fit tested prior to wearing respiratory protective equipment on an annual basis. The wearer of respirators, including "dust masks" need to contact Environmental Health and Safety (EH&S) at 974-4036 to determine the need to enroll and complete the USF Respirator Program which includes a mandated physical exam, fit testing and training on an annual basis. If respiratory protective equipment is required or if there are questions about the respiratory protection program contact EH&S (http://usfweb2.usf.edu/eh&s/)

SECTION 9 SAFE HANDLING OF LABORATORY EQUIPMENT

Human error, poor laboratory techniques, and misuse of equipment are the cause of laboratory accidents, injuries, and work-related infections. This section provides a guideline of technical methods that are designed to avoid or minimize the most commonly reported problems of this nature.

Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biosafety hazards. This section deals with basic principles related to equipment suitable for laboratories of all biosafety levels.

Section 9.1 Essential Biosafety Equipment

This following list is not all inclusive:

- Biological Safety Cabinet (BSC)

- Pipetting Aids (automatic pipettors)
- Disposable Loops
- Loop Micro-incinerators
- Autoclaves (manual or automatic)
- Spatter Shield
- Leak Proof Vessels for collection and transport of infectious materials
- Sharps Disposal Containers
- Transport Containers (between laboratories, institutions)
- Screw-capped Bottles
- Vacuum Line Protection

1. **Biological safety cabinets** are to be used whenever:
   a. infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet
   b. there is an increased risk of airborne infection
   c. procedures with a high potential for producing aerosols are used; these may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure, intranasal inoculation of animals, and harvesting of infectious tissues from animals and eggs.
2. **Pipetting aids** – to avoid mouth pipetting. Many different designs are available.
3. **Plastic disposable transfer loops -** Alternatively, electric transfer loop incinerators may be used inside the biological safety cabinet (BSC) to reduce aerosol production.
4. **Screw-capped** tubes and bottles.
5. **Autoclaves** to decontaminate infectious materials.
6. **Plastic disposable Pasteur pipettes**, whenever available, to avoid glass.

Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods (usually by a certified examiner) before being put into use. Recertification should take place at regular intervals, according to the manufacturer's instructions.

Section 9.2 Biological Safety Cabinets

General Principles

Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment, and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks, and diagnostic specimens.

Note: Horizontal and vertical outflow cabinets (e.g., "clean-air work stations", "laminar flow cabinets") are not biological safety cabinets and should not be used as such.

Class I Biological Safety Cabinet

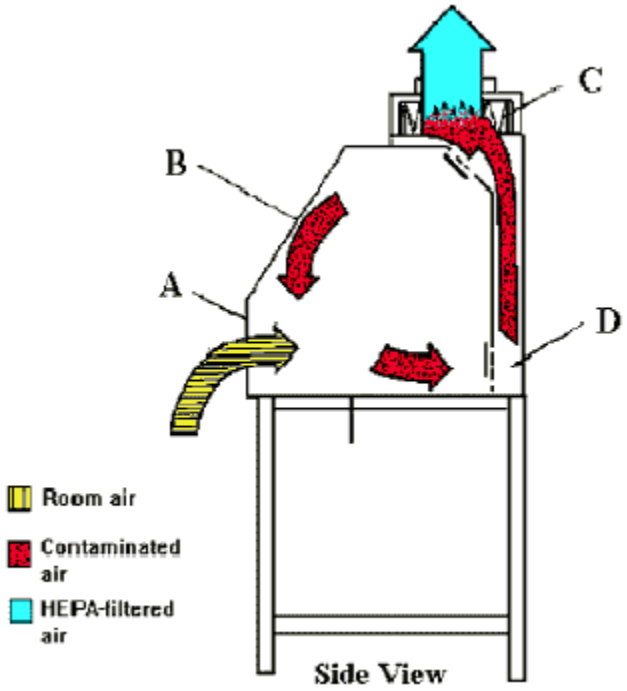
A ventilated cabinet for personnel and environmental protection, having an un-recirculated inward airflow away from the operator that exhausts all air either into the laboratory or to the outside after filtration through a HEPA filter. Class I cabinets are suitable for work where no product protection is required.

Room air is drawn in through the front opening at a minimum velocity of 75 fpm; it passes over the work surface and is discharged from the cabinet through the exhaust duct. The directional flow of air whisks aerosol particles that may be generated on the work surface away from the laboratory worker and into the exhaust duct. The front opening allows the operator's arms to reach the work surface inside the cabinet while he or she observes the work surface through a glass window. The window can also be fully raised to provide access to the work surface for cleaning or other purposes. The air from the cabinet is exhausted through a HEPA filter:

a. into the laboratory and then to the outside of the building through the building exhaust;
b. to the outside through the building exhaust; or
c. directly to the outside.

The HEPA filter may be located in the exhaust plenum of the BSC or in the building exhaust. Some Class I BSCs are equipped with an integral exhaust fan, whereas others rely on the exhaust fan in the building exhaust system. The Class I BSC was the first recognized BSC (Note: Class I BSCs are currently being manufactured on a limited basis; many have been replaced by Class II BSCs.). Because unsterilized room air is drawn over the work surface through the front opening, it is not considered to provide consistently reliable product protection.

Class I, Biosafety Cabinet



A. front opening
B. sash
C. exhaust HEPA
D. exhaust plenum

Class II Biological Safety Cabinets

A ventilated cabinet for personnel, product, and environmental protection having an open front with inward airflow for personnel protection, downward HEPA filtered laminar airflow for product protection, and HEPA filtered exhausted air for environmental protection.

The Class II BSC was designed not only to provide personnel and environmental protection but also to protect work surface materials from contaminated room air. Class II BSCs, of which there are four types (A1, A2, B1, and B2), differ from Class I BSCs by allowing only HEPA-filtered (sterile) supply air to flow over the work surface. The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3.

Class II Type A1 Biological Safety Cabinet (formerly designated Type A)

- Exhaust approximately 30% of total air handled and recirculate 70% in cabinet
- Minimum inflow air velocity is 75 fpm (0.38 m/s) through the front opening
- May exhaust HEPA filtered air back into the room or through a canopy (to the outdoors)
- Audiovisual alarm required when connected to a building exhaust system

Type A1 cabinets are not suitable for work with volatile toxic chemicals and volatile radionuclides.

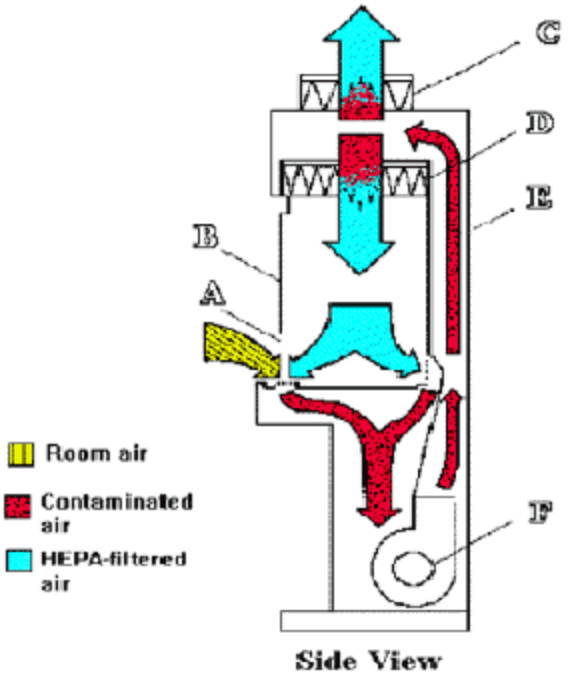
The Class II type A1 BSC: An internal fan draws room air (supply air) into the cabinet through the front opening and into the front intake grill. The inflow velocity of this air should be at least 75 fpm at the face of the front opening. The supply air then passes through a supply HEPA filter before flowing downwards over the work surface. As the air flows downwards it "splits" about 6–18 cm from the work surface, one half of the downwards flowing air passing through the front exhaust grill, and the other half passing through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward air flow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Owing to the relative size of these filters, about 70% of the air re-circulates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside. Air from the Class II A1 BSC exhaust can be re-circulated to the room or discharged to the outside of the building through a thimble connection to a dedicated duct or through the building exhaust system. Re-circulating the exhaust air to the room has the advantage of lowering building fuel costs because heated and/or cooled air is not being passed to the outside environment.

Class II Type A2 (formerly designated Type B3)

- Exhaust approximately 30% of total air handled and recirculate 70%
- Minimum inflow air velocity is 100 fpm (0.51 m/s) through the front opening
- Audiovisual alarm required when connected to a exterior system
- May exhaust HEPA filtered air back into the room or through a canopy

Type A2 cabinets used for work with minute quantities of volatile toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies must be exhausted through properly functioning exhaust canopies.

Class II, Type A, Biosafety Cabinet



A. front opening
B. sash
C. exhaust HEPA filter
D. rear plenum
E. supply HEPA filter
F. blower

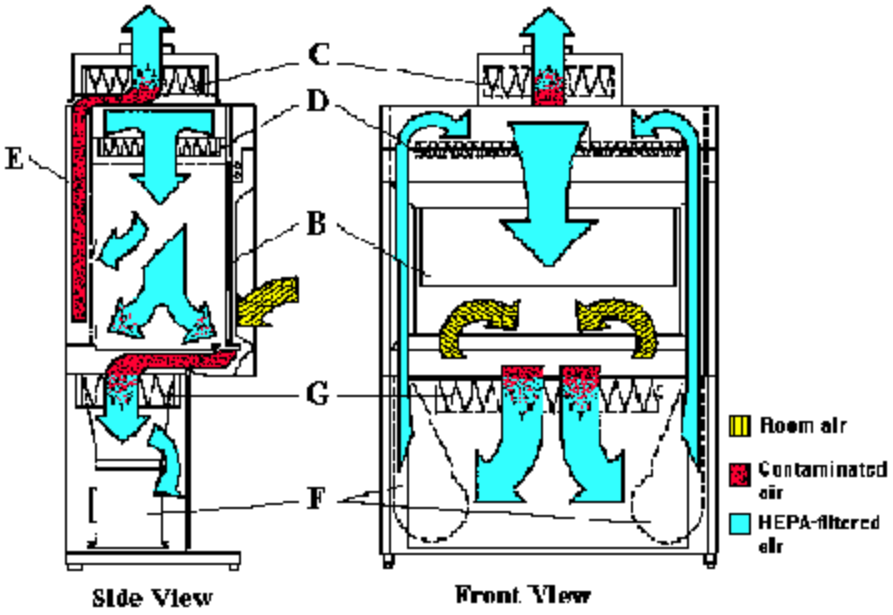
Class II B1 Biological Safety Cabinet

Connection of cabinet exhaust to building exhaust air system is required.

- Exhaust approximately 70% of total air handled and recirculate 30%
- Unique in that the air in the back of the cabinet is exhausted to the outdoors through a dedicated exhaust plenum and the air in the front is recirculated
- Minimum inflow air velocity is 100 fpm (0.51 m/s) through the front opening
- Must be hard ducted to the outside for the cabinet to function
- Requires audiovisual alarm when exhaust loses 20% of volume - must shut down cabinet blower within 15 sec

Type B1 cabinets may be used for work treated with minute quantities of volatile toxic chemicals and tracer amounts of radionuclides required as an adjunct to microbiological studies if work is done in the direct exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

Class II, Type B1, Biosafety Cabinet



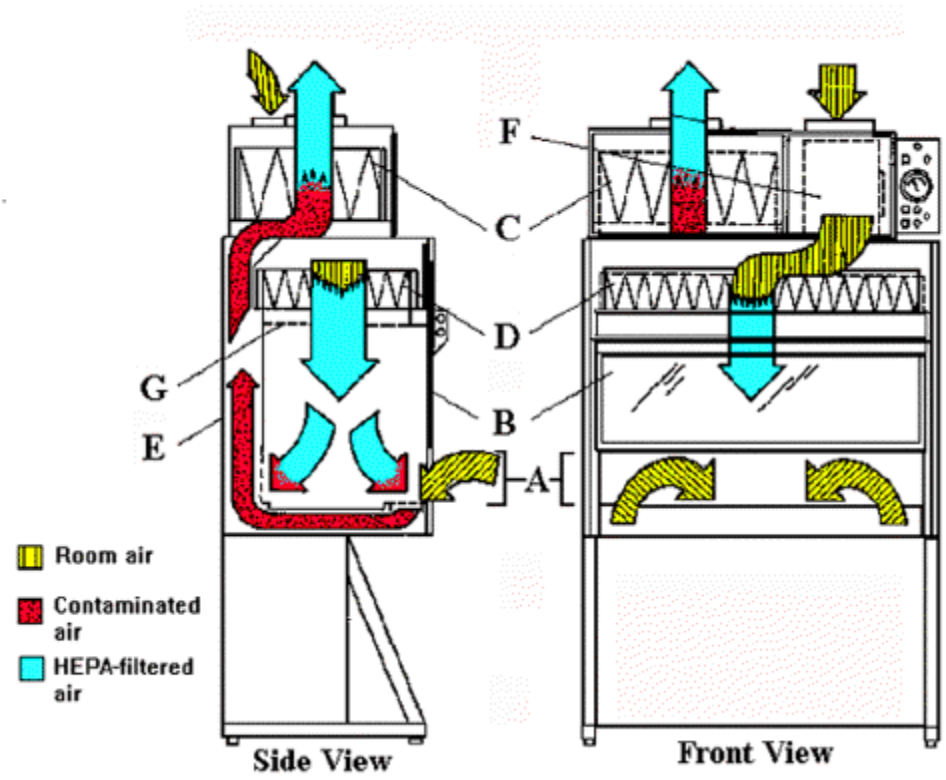
A. front opening
B. sash
C. exhaust HEPA filter
D. supply HEPA filter
E. negative pressure exhaust plenum
F. blower
G. additional HEPA filter for air supply
Note: The cabinet exhaust needs to be connected to the building exhaust.

Class II Type B2 cabinets (sometimes referred to as "total exhaust")

- maintain a minimum average inflow velocity of 100 ft/min (0.5 m/s) through the work access opening;
- have HEPA filtered downflow air drawn from the laboratory or the outside air (i.e., downflow air is not recirculated from the cabinet exhaust air);
- exhaust all inflow and downflow air to the atmosphere after filtration through a HEPA filter without recirculation in the cabinet or return to the laboratory;

Type B2 cabinets may be used for work with volatile toxic chemicals and radionuclides required as adjuncts to microbiological studies.

The Class II, Type B2 BSC.
(Connection to building exhaust system required)

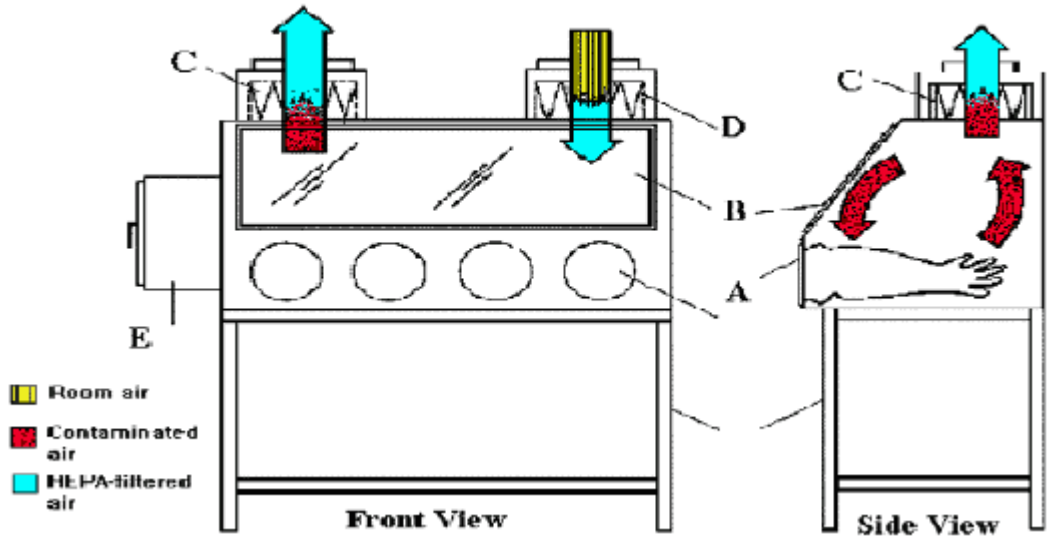


A. front opening
B. sash
C. exhaust HEPA filter
D. supply HEPA filter
E. negative pressure exhaust plenum
F. supply blower
G. filter screen
Note: The carbon filter in the building exhaust is not shown. The cabinet exhaust needs to be connected to the building exhaust system.

Class III Biological Safety Cabinet

This type provides the highest level of personnel protection and is used for Risk Group 4 agents. All penetrations are sealed "gas tight." Supply air is HEPA-filtered and exhaust air passes through two HEPA filters. Air flow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure (about 124.5 Pa). Access to the work surface is by means of heavy duty rubber gloves, which are attached to ports in the cabinet. The Class III BSC should have an attached pass-through box that is sterilizable and is equipped with HEPA-filtered exhaust. The Class III cabinet may be connected to a double-door autoclave used to decontaminate all materials entering or exiting the cabinet. Several glove boxes can be joined together to extend the work surface. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories.

Class III, Biosafety Cabinet



A. glove ports with O-ring for attaching arm-length gloves to cabinet
B. sash
C. exhaust HEPA filter
D. supply HEPA filter
E. double-ended autoclave or pass-through box

Summary of Types of Biosafety Cabinets

Type of Biosafety Cabinet	Minimum Intake Air Velocity cfpm	Percent of Air Recirculated	Percent of Air Exhausted from Cabinet
Class I	75	---	100 - Exhaust Direct to Outside
Class II A1	75	70	30
Class II A2	100	70	30
Class II B1	100	30	70
Class II B2	100	0	100
Class III	100	---	100 - Exhaust Direct to Outside

Biological Safety Cabinet Air Connections

A “thimble” or “canopy hood” is designed for use with Class II A1 and II A2 vented to the outside BSCs. The thimble fits over the cabinet exhaust housing, sucking the cabinet exhaust air into the building exhaust ducts. A small opening, usually 2.5 cm in diameter,

is maintained between the thimble and the cabinet exhaust housing. This small opening enables room air to be sucked into the building exhaust system as well. The building exhaust capacity must be sufficient to capture both room air and the cabinet exhaust. The thimble must be removable or be designed to allow for operational testing of the cabinet. Generally, the performance of a thimble-connected BSC is not affected much by fluctuations in the air flow of the building. Class II B1 and II B2 BSCs are hard-ducted (i.e., firmly connected without any openings to the building exhaust system or, preferably, to a dedicated exhaust duct system). The building exhaust system must be precisely matched to the air flow requirements specified by the manufacturer in both volume and static pressure. Certification of hard-duct connected BSCs is more time-consuming than that for BSCs that re-circulate air to the room or which are thimble-connected.

Recommendations for Using Biological Safety Cabinets in the Laboratory

Location

Ideally, BSCs should be situated in a location remote from traffic and potentially disturbing air currents. Whenever possible clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance and clearance; clearance above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operators

- To maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets, arms should be moved in and out slowly, perpendicular to the front opening.
- Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms.
- The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material Placement in BSC

- The front intake grill of Class II BSCs must not be blocked with paper, equipment, or other items.
- Materials to be placed inside the cabinet should be surface-decontaminated with 70% alcohol.
- Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes.
- All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill.
- Aerosol-generating equipment (e.g., mixers, centrifuges) should be placed towards the rear of the cabinet.
- Bulky items, such as biohazard bags, discard pipette trays, and suction collection flasks should be placed to one side of the interior of the cabinet.

- Active work should flow from clean to contaminated areas across the work surface.
- The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier, and can compromise both personnel and product protection.

Operation and Maintenance of BSC
Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to "purge," (i.e., to allow time for contaminated air to be removed from the cabinet environment). All repairs/maintenance made on BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

Ultraviolet Lights use in BSC
Ultraviolet lights are not required in BSCs. If they are used, they must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure.

Open Flames use in BSC
Open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the air flow patterns and can be dangerous when volatile, flammable substances are also used. To sterilize bacteriological loops, micro-burners or electric "furnaces" are available and are preferable to open flames.

Spills within BSC
A copy of the procedures for handling biohazardous spills should be posted, read, and understood by everyone who uses the laboratory. When a spill of biohazardous material occurs within a BSC, cleanup should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected and/or autoclaved. Spill Notification: Notify the Principal Investigator and for all spills regarding BSL-2 or BSL-3 agents notify the IBO (or Biosafety Staff) at:

(During normal work hours)
(813) 974-0954, E-mail to: fmoulvi@usf.edu
(813) 974-5091, E-mail to: dhoweth@usf.edu
(813) 974-5110, E-mail to: pmackley@usf.edu

(After normal work hours)
University Police (Tampa Campus) – **911** from campus phone; or **(813) 974-2628**
University Police (St. Petersburg Campus- **3-4140** from campus phone; **or 727-553-4140**
The police will contact the Institutional Biosafety Officer regarding biohazardous spills.

Annual Certification
The functional operation and integrity of each BSC should be certified to performance standards at the time of installation and annually thereafter by qualified technicians, according to the manufacturer's instructions.

Cleaning and Disinfection of BSCs
- All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed (residual culture media may provide an opportunity for microbial growth).
- The interior surfaces of BSCs should be decontaminated before and after each use. (work surfaces and interior walls should be wiped with an appropriate disinfectant)
- The final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.
- If hard ducted, it is recommended that the cabinet be left running. If not, it should be run for 5 min in order to purge the atmosphere inside before it is switched off.

Decontamination of BSCs
BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination should be performed by a qualified professional.

Personal Protective Equipment
Personal protective clothing should be worn whenever using a BSC. Laboratory coats are acceptable for work being performed at BSL-1 and -2. A solid front, back-closing laboratory gown provides better protection and should be used at BSL-3. Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator's wrists. Masks and safety glasses may be required for some procedures.

Alarms in BSCs
BSCs can be equipped with one of two kinds of alarm. Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the operator has moved the sash to an improper position. Corrective action for this type of alarm is returning the sash to the proper position. Air-flow alarms indicate a disruption in the cabinet's normal air-flow pattern. This represents an immediate danger to the operator or product. When an air-flow alarm sounds, work should cease immediately and the PI/laboratory supervisor should be notified. Manufacturers' instruction manuals should provide further details. Training in the use of BSCs should cover this aspect.

Section 9.3 Equipment Related Hazards and How to Reduce Its Risk

Use of Centrifuges

Centrifuges may produce aerosols, splashing, and tube breakage. The following recommendations are for safe operating practices to reduce/eliminate these biological hazards:

1. Centrifuges should be operated according to the manufacturer's instructions.
2. Centrifuges should be placed at such a level that workers of less than average height can see into the bowl to place trunnions and buckets correctly.
3. Centrifuge tubes and specimen containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use.
4. Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation.
5. The buckets must be loaded, equilibrated, sealed, and opened in a biological safety cabinet.
6. Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.
7. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer's instructions.
8. Distilled water or alcohol should be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
9. Sealable centrifuge buckets (safety cups) must be used for microorganisms of Risk Groups 2 or higher.
10. When using angle head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak.
11. The interior of the centrifuge bowl should be inspected periodically for staining or soiling at the level of the rotor. If staining or soiling is evident then the centrifugation protocols should be re-evaluated.
12. Centrifuge rotors and buckets should be inspected periodically for signs of corrosion and for hair-line cracks.
13. Buckets, rotors, and centrifuge bowls should be decontaminated after each use.
14. After use, buckets should be stored in an inverted position to drain the balancing fluid.
15. Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the cabinet air flow if the centrifuge is placed in a traditional open-fronted Class I or Class II biological safety cabinet. Enclosing centrifuges in Class III safety cabinets prevents emitted aerosols from dispersing widely. Open buckets or rotors after aerosols have settled (30 min) or in a biological safety cabinet. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.
16. Close the centrifuge top during operation.
17. Allow the centrifuge to come to a complete stop before opening.

18. Disinfect weekly and immediately following any spill or breakage the surfaces of the centrifuge head, bowl, trunnions, and buckets. Use 70% alcohol, 2% glutaraldehyde, or any registered mycobacteriacide. **Note: bleach might corrode components.**

Ultra Centrifuges (in addition to the above):

- Clean rotors, lids, adapters, and associated parts with 1% non-alkaline detergent, rinse with distilled water, and dry with a soft cloth. Encrusted material should be removed with a twist bristle brush and 1% non-alkaline soap solution.
- Lubricate weekly all O-rings with vacuum grease and metal rotor threads with anti-galling grease.
- Make sure that rotors are locked to the spindle and that buckets are properly seated on their pins. Only use the rotor handle tool to tighten ultra-speed lids.
- Do not use rotors which have been dropped or struck against a hard surface.
- Contact your centrifuge representative for specific information.
- Install a HEPA filter between the centrifuge and the vacuum pump.
- Connect the vacuum pump exhaust to a disinfectant trap.

Use of Tissue Grinders

1. Glass grinders should be held in a wad of absorbent material in a gloved hand. Plastic (PTFE) grinders are safer.
2. Tissue grinders should be operated and opened in a biological safety cabinet.
3. If manual tissue grinders are used, hold tube in a wad of absorbent material.

Inoculating Loops

Flaming inoculating loops can result in spatter and release of aerosols and droplets. The use of a cooled loop for insertion into a culture is recommended; ensure that the loop is completely closed; the shank should be no more than 6 cm in length (to avoid vibrations); Use of an electric micro-incinerator or a hot bead sterilizer will effectively control spatter resulting from sterilization of inoculating loops. Alternatively, use disposable pre-sterilized plastic loops.

Use of Homogenizers, Shakers, Blenders, and Sonicators

The greatest risk when using any of these devices is the creation of aerosols.

1. Domestic (kitchen) homogenizers should not be used in laboratories as they may leak or release aerosols. Laboratory blenders and stomachers are safer.
2. Safety blenders are designed to prevent leakage from the bottom of the blender jar (i.e., from rotor bearings and O-ring gaskets) and to withstand sterilization by autoclaving.
3. They also provide a cooling jacket to avoid biological inactivation. Avoiding glass blender jars prevents breakage.
4. Caps and cups or bottles should be in good condition and free from flaws or distortion. Caps should be well-fitting and gaskets should be in good condition.
5. Pressure builds up in the vessel during the operation of homogenizers, shakers, and sonicators. Aerosols containing infectious materials may escape from between the cap and the vessel. Plastic, in particular, polytetrafluoroethylene

(PTFE) vessels are recommended because glass may break, releasing infectious material and possibly wounding the operator.
6. When in use, homogenizers, shakers, and sonicators should be covered by a strong transparent plastic casing. This should be disinfected after use. Where possible, these machines should be operated, under their plastic covers, in a biological safety cabinet.
7. Aerosols must be allowed to settle for five minutes before opening the blender jar (or grinder or sonicator container).
8. At the end of the operation the containers should be opened in a biological safety cabinet.
9. Culture stirrers, shakers, and agitators may produce aerosols, splashing, and spillage. It is recommended to operate them in a biological safety cabinet or specially designed primary containment. In addition use heavy-duty screw-capped culture flasks, fitted with filter-protected outlets, if necessary, and well secured.
10. Sonicators and ultrasonic cleaners may create aerosols, impaired hearing, and/or dermatitis. Ensure insulation is present to protect against sub-harmonics. In addition, wear gloves for protection against high-frequency plus detergent action on skin. Moreover, hearing protection may be needed for people using sonicators.

Lyophilizers

When using freeze-dryers (lyophilizers) the biohazards of concern include production of aerosols and direct contact contamination.

1. It is recommended that o-ring connectors be used to seal the unit throughout.
2. Carefully inspect all glass vacuum vessels for surface scratches. Use only glassware designed for vacuum work.
3. When opening ampoules of lyophilized cultures, avoid hasty opening by snapping the neck, which can lead to the sudden inrush of air and dispersal of contents. Make a file mark near the middle of the cotton plug and apply a red-hot glass rod to crack the glass; allow time for the air to seep into the ampoule and gently remove the top and plug; add liquid for re-suspension slowly to avoid frothing. Lyophilizer vacuum pump exhaust should be filtered through HEPA filters or vented into a biosafety cabinet when used with infectious agents and use air filters to protect vacuum lines.
4. Polypropylene tubes should be used in place of glass ampoules for storing biohazardous material in liquid nitrogen. Ampoules can explode, causing eye injuries and exposure to the biohazardous material.
5. Provide an all-metal moisture trap and a vapor condenser.

Incubators

Incubators, water baths, and Warburg baths can become the inadvertent and undesired repositories of microorganisms. Although they may present a hazard to laboratory workers, most often they are a source of contamination of laboratory cultures. Besides the moist surfaces, rubber gaskets, the humidity trough (if present), and fan mechanism are areas in which contaminating microorganisms concentrate. It is recommended that an antimicrobial agent, such as Zepharin Chloride be added to the humidity source water; do not use sodium azide. This is because sodium azide forms explosive compounds with

some metals. In addition, it is recommended that the inner panels, trays, and the other removable parts should be autoclaved and the gaskets and non-removable parts wiped thoroughly with 70% ethanol every two months.

Use of Pipettes and Pipetting Aids

Pipetting aids should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean.

1. A pipetting aid should always be used. **Pipetting by mouth is prohibited.**
2. All pipettes should have cotton plugs to reduce contamination of pipetting devices.
3. Plugged (aerosol-resistant) pipette tips should be used when manipulating microorganisms and cell cultures.
4. Air should never be blown through a liquid containing infectious agent.
5. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
6. Liquids should not be forcibly expelled from pipettes.
7. Mark-to-mark pipettes are preferable to other types as they do not require expulsion of the last drop.
8. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for 18-24 h before disposal.
9. A discard container for pipettes should be placed within the biological safety cabinet, not outside it.
10. Syringes fitted with hypodermic needles must not be used for pipetting. Blunt cannulas should be used instead of needles. There are devices for opening septum-capped bottles that allow pipettes to be used and avoid the use of hypodermic needles and syringes.
11. To avoid dispersion of infectious material accidentally dropped from a pipette, a disinfectant-soaked cloth or absorbent paper should be placed on the working surface; this should be autoclaved or discarded as infectious waste after use.
12. Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard.

Section 9.4 Protection of Vacuum System when Filtering Biohazardous Materials

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into primary collection flasks is a common laboratory procedure.

1. A HEPA filter provides an effective barrier to protect the vacuum system.
2. Flexible tubing is used of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum.
3. Flasks should be of appropriate size to contain the amount of fluid aspirated.

4. Flasks contain an appropriate disinfectant solution. Use an antifoam additive to prevent foam production, if allowed to reach the filter, foam will shut off the vacuum.
5. If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter should be autoclaved before the filter is discarded.

The apparatus is shown in Figure 1:

- two suction flasks (A & B)
- HEPA filter (C)
- vacuum source (D)
- rubber stoppers
- flexible vacuum tubing
- glass tubing
- glass sparger (aerosol passing through the collection flask is dispersed in small bubbles so that adequate contact is made with the disinfectant solutions)

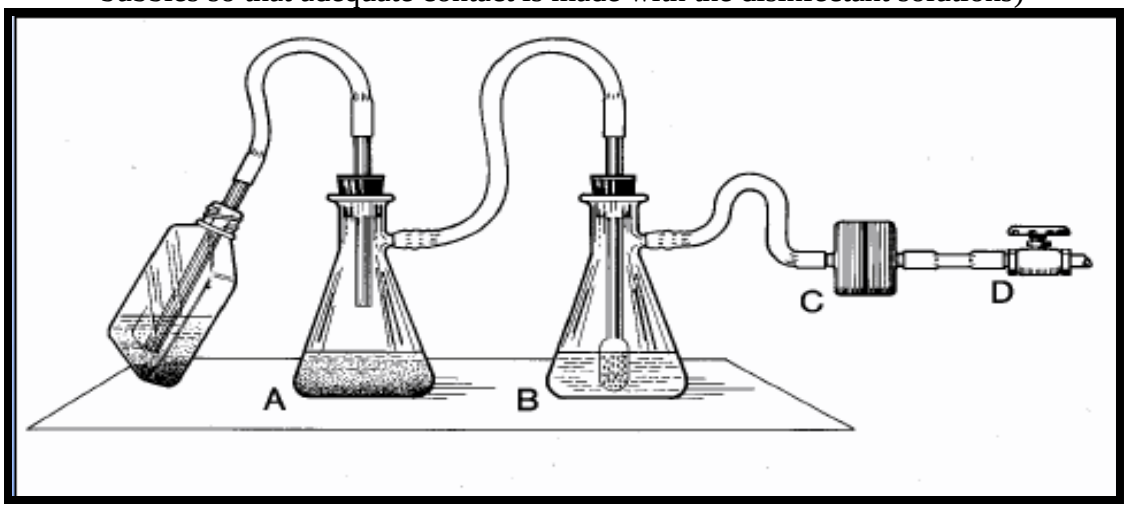


Figure 1 *(Office of Health and Safety, Centers for Disease Control and Prevention, 1600 Clifton Road N.E., Mail Stop F05 Atlanta, Georgia 30333, USA Last Modified: 1/2/97)*
One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. A glass sparger in flask (B) minimizes splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

Section 9.5 Autoclave Operating Procedures

The following procedures are recommended by the Biosafety Office.

What Materials Should Be Autoclaved?

The following materials are recommended to be autoclaved:

- Culture and stocks of infectious agents (bacteria, viruses, fungi, etc.)
- Reusable items to be sterilized: plastic pipette tips, pipettes, surgical instruments, and scrubs
- Animal tissue specimens and cages of potentially pathogenic animal carcass(es)

Autoclave Cycles

There are three basic autoclave cycles:

1. **Gravity** or "Fast Exhaust" Cycle: Used to sterilize dry goods, glassware, etc. This cycle charges the chamber with steam and holds it at a set temperature for a set period of time. At the end of the cycle a valve opens and the chamber rapidly returns to atmospheric pressure. Drying time may also be added to the end of the cycle.
2. **Liquid** or "Slow Exhaust" Cycle: Used to prevent sterilized liquids from boiling, steam is exhausted slowly at the end of the cycle, allowing the liquids (which will be super-heated) to cool.
3. **Pre-Vacuum** Cycle: For porous materials, animal bedding, etc. This cycle partially evacuates the chamber prior to introducing steam for greater steam penetration. Pre-vacuum cycles are not available on all machines and not suitable for liquids.

Written Procedures

A written sterilization procedure should be in place for each workplace. For IBC registered studies the IBC may modify the procedure.

Use the following Personal Protective Equipment (PPE):

- heat-resistant autoclave gloves for loading and unloading autoclave;
- fluid-resistant gloves to eliminate contact with contaminated wastes;
- lab coat to protect your apparel; and
- splash goggles if a splash hazard is present.

Parameters

- Appropriate parameters (temperature and pressure settings) for sterilization may be contained in the unit-specific operations manual.
- Monitor the autoclave process for proper cycle and length of time.
- Cycle and time depend on the material being sterilized.

(For example, liquids require the use of slow exhaust and, while most loads require cycle times of 15 to 30 minutes at 121^0 C, longer times may be needed to properly stabilize special loads. The decontamination of biomedical waste may regularly require 60 minutes at 121^0 C.)

Protocol

The following procedures are typical for most autoclaves on campus:

- Identify standard treatment containers and proper load placement.
- If any warning lights activate during a cycle, the operator should refer to the instruction/operating manual and follow specified instructions.
- If for any reason the integrity of the sterilization process is in question, the load should be considered contaminated and should be reprocessed.
- The autoclave and work areas shall be cleaned after every use and the work area shall be disinfected, as needed.

- Ensure no broken glass is in autoclave. It may cut hands or waste bags and may compromise the door seal.
- Model-specific preventive maintenance should be performed as recommended by the manufacturer.

Containers and Packaging for Autoclaving

- Items should be autoclaved in autoclave bags and a rigid secondary container (typically polypropylene or stainless steel).
- Never place sharps (e.g., syringes with needles, broken glass) in a biohazard bag. All sharps need to be disposed of in sharps containers.
- Density of the load affects steam penetration.
- Autoclave bags are not to be overloaded or purposely compacted.
- Biohazardous items should be put into appropriate polypropylene autoclave bags.
- **Do Not** tightly close autoclave bags during the sterilization process.

Loading the Autoclave

- Properly load the autoclave.
- Add one cup of water to create additional steam that drives residual air from the bag.
- Allow the steam to circulate freely throughout the chamber (i.e., do not overload the chamber with bags that exceed the autoclave's capacity.
- **Do Not** mix "clean" and "contaminated" items together during the same cycle (they require different heat exposure times).

Unloading the Autoclave

- Wait until the chamber pressure gauge reads zero before attempting to open the autoclave.
- Slowly unlock and open the door.
- Open door slightly - Stand back and allow steam to escape through the open door.
- Wait for several minutes before removing the bag from the autoclave to allow the chamber and any residual liquids to cool.
- Be aware of molten agar or solutions that may have collected in the secondary container during the cycle.
- Use special caution when autoclaving containers that may have become pressurized.
- Never autoclave a sealed container of liquids as this may result in an explosion of super-heated liquid during the cycle or when the container is opened.

Autoclave Training and Operation

Principal Investigators and/or supervisors must train their staff for operation of autoclaves. Qualified autoclave users should understand the time, temperature, and pressure relationships required for proper materials decontamination. Biosafety Staff are available for additional questions. Please call 813-974-0954 for assistance.

Autoclave Maintenance

Follow manufacturer recommended routine maintenance procedures, for repair, use manufacturer warranty, or a maintenance contract, if possible. For autoclaves out of warranty, or maintenance contract is not available call Physical Plant maintenance staff.

Autoclave Usage Tips

- Regularly inspect your autoclave components for proper operation. If a problem is found, promptly notify your supervisor. **DO NOT OPERATE AN AUTOCLAVE UNTIL IT HAS BEEN PROPERLY REPAIRED**.
- Never place sealed containers in an autoclave. Large bottles with narrow necks can simulate sealed containers if filled with too much liquid.
- Don't autoclave items containing solvents, volatile, or corrosive chemicals (phenol, trichloroacetic acid, ether, chloroform, etc.) or any radioactive materials.
- After loading and starting the autoclave, processing time starts ***AFTER*** the autoclave reaches normal operating conditions of 121^0 C (250^0 F) and 15 psi pressure.
- Decontamination conditions vary with type of load therefore processing times will vary according to the conditions. A minimum of 30 minutes is needed to decontaminate biological waste.
- At the end of a decontamination cycle make sure that the pressure in the autoclave chamber is zero before opening the door. Slowly crack-open the autoclave door and allow the steam to gradually escape from within the autoclave.
  CAUTION: Opening the autoclave door too quickly may result in glassware breakage and/or steam burns on your skin.
- Allow materials inside the autoclave to cool for 10 minutes before removing them from the autoclave.
- After autoclaving, dispose of as a biohazardous solid waste.
- Always follow written lab procedures; however, dry goods typically require about 30 minutes sterilization, plus about 20 minutes drying time (dry time may need to be increased for enclosed items such as pipette tips or bottles with lids).
- Average liquid sterilization times (add an additional 10 to 20 minutes for crowded items):

• <500 ml, 30 minutes	• 500 ml - 1 L, 40 minutes	• 2 L - 4 L, 55 minutes	• >4 L, 1 hour or more

- Not all plastics can be autoclaved. Polypropylene and polycarbonate will survive, but polyethylene and high density polyethylene will not. The different types of plastic can be identified by looking for initials imprinted on the bottom of containers (PP=polypropylene, PC=polycarbonate, PE=polyethylene, HDPE=high density polyethylene). If you are unsure about a new container, place it in an autoclave safe container the first time.
- To prevent the bottoms of bottles from breaking, place them in a tub with 1 to 2 inches of water.

Section 10 Prudent Practices and Good Techniques

Section 10.1 Biohazard Spill Kits

It is recommended that a Biohazard Spill Kit be available in each area where there is potential for spills/releases of a biohazardous nature. Kits may be commercially prepared and purchased through safety supply vendors or be put together by individual laboratories. These kits should include, but are not limited to, such items as:

- Concentrated disinfectant (e.g. bleach)
- Paper towels or another suitable absorbent
- Spray bottle (for mixing 10% bleach)
- Alternatively a commercially produced 10% bleach solution
- Biohazard/Autoclave bags
- Sharps container(for contaminated broken glass or needles)
- PPE (gloves, goggles/safety glasses, lab coat)
- Forceps, tongs or dust pan and broom to pick up broken glass (Should be autoclavable or disposable)

This kit may be stored where chemical spill kits are located or where first aid kits are stored. Sample guidelines for a spill cleanup are indicated in a later section.

Section 10.2 Signs and Labels

The purpose of signs and labels is to establish a uniform system for indicating the presence of biohazardous areas to personnel and visitors. Anyone entering areas where biohazardous materials are used must be aware of the potential hazards. Biohazard signs (see below) should be posted on doors to rooms where microorganism or biological toxins that cause disease in humans are used. The following areas require posting:

- Entrances to laboratories that use agents classified as BSL-2 or BSL-3.
- Animal rooms housing infected animals are to be posted with "BIOHAZARD" signs.

Biohazard door signs may be requested from Research Integrity & Compliance biosafety staff. In addition the PI must post the following information on the door for BSL-2 labs:

- The biosafety level of the laboratory.
- If applicable, infectious agents used in that area.
- Special requirements for entering the area.
- List the name and telephone number for the Principal Investigator to facilitate contact in case of emergency.

Orange or orange-red biohazard labels should be placed on containers and storage units (refrigerators, incubators, waste containers, etc.) that are used for microorganism(s) or biological toxins causing human diseases. Contaminated equipment should be labeled as well. Labels recommended by this section shall include the following legend:

These labels shall be fluorescent orange or orange-red or predominantly so, with lettering and symbols in a contrasting color.

Section 10.3 Access

- Laboratory doors should be kept closed when work is in progress. This in turn limits access to only authorized personnel
- Children under the age of 16 years should not be authorized or allowed to enter laboratory working areas.
- Persons at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as, individuals who are immunosuppressed, immunodeficient, or undergoing immunosuppressive therapy, must be made aware of the risks associated with work in BSL-2 or higher laboratory.
- Animals not involved in the work of the laboratory should not be permitted in the laboratory.
- "No Smoking," "No Eating," and "No Drinking" signs should be prominently displayed outside the laboratory.

Visitors

- Visitors may be permitted in the **BSL-2** laboratories when approved by the PI and accompanied by a laboratory worker approved for access.
- Visitor admission into the **BSL-3** laboratories will only be allowed with prior approval of the Principal Investigator and/or the Institutional Biosafety Officer (IBO).
- Exposure to infectious agents will be kept to a minimum (i.e., when no work is being conducted in the lab).

Section 10.4 Recommended Practices

- Plan and organize materials/equipment before starting work.
- Whenever there is a risk of aerosolization, work should be conducted in a biological safety cabinet (BSC).

- The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.
- All spills, accidents, and overt or potential exposures to infectious materials and/or recombinant or synthetic nucleic acid molecules must be reported to the PI/laboratory supervisor. A written record of such accidents and incidents should be maintained.
- Materials must not be placed in the mouth. Labels must not be licked.
- Centrifuge materials containing infectious agents in unbreakable, closable tubes. Use a rotor with sealed heads or screw-capped safety cups. After centrifugation, open the tubes in a biological safety cabinet.
- Cover counter tops where hazardous materials are used with plastic-backed disposable paper to absorb spills.
- Packing and transportation (e.g., infectious agents or diagnostic specimens) must follow applicable national and/or international regulations.
- Foods or drinks for human consumption should not be stored anywhere in the laboratory working areas.
- Personnel should be advised of special hazards and required to read the safety or operations manual and follow standard practices and procedures. The laboratory supervisor should make sure that all personnel understand these practices and procedures. A copy of the safety or operations manual should be available in the laboratory.
- All laboratory personnel should be familiar with the emergency spill protocol and the location of clean-up equipment.

Avoiding Injection of Infectious Materials

Accidental inoculation with hypodermic needles (needle-sticks), glass Pasteur pipettes, broken or chipped glassware, and other "sharps" can be avoided through careful practices and procedures.

1. Glassware should be replaced with plastic-ware whenever possible.
2. Needle-stick accidents can be reduced by taking particular care, and minimizing the use of syringes and needles; for many techniques, syringes with blunt cannulas may be used instead.
3. Simple devices are available for opening septum-stoppered bottles so that pipettes can be used.

Avoiding Ingestion of Infectious Materials and Contact with Skin and Eyes

1. Large particles and droplets (> 5 μm in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator. Disposable gloves must be worn. Laboratory workers should avoid touching their mouth, eyes, and face.
2. Food and drink must not be consumed or stored in the laboratory.
3. There should be no gum-chewing in the laboratory.
4. Cosmetics should not be applied in the laboratory.
5. The face, eyes, and mouth must be shielded or otherwise protected during any operation that may result in the splashing of potentially infectious materials.

Section 10.5 Biological Material Storage

It is recommended that every item must be labeled or coded by some reasonable method. Researchers should be able to explain their coding system and identify all samples. It is recommended that storage units housing BSL-2 agents or higher or biological toxins should display a biohazard sticker or sign. Stickers can be obtained from the Biosafety Office (813) 974-0954.

Storage of Ampoules Containing Infectious Materials
It is recommended that ampoules containing infectious materials should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal. If very low temperatures are required, ampoules should be stored only in the gaseous phase above the liquid nitrogen. Otherwise, infectious materials should be stored in mechanical deep-freeze cabinets or on dry ice. Laboratory workers should wear eye and hand protection when removing ampoules from cold storage. The outer surfaces of ampoules stored in these ways should be disinfected when the ampoules are removed from storage.

Opening of Ampoules Containing Lyophilized Infectious Materials
Care should be taken when ampoules of freeze-dried materials are opened, as the contents may be under reduced pressure and the sudden inrush of air may disperse some of the materials into the atmosphere. Ampoules should always be opened in a biological safety cabinet. The following procedures are recommended for opening ampoules.

1. First decontaminate the outer surface of the ampoule.
2. Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
3. Hold the ampoule in a wad of alcohol-soaked cotton to protect hands before breaking it at a file scratch.
4. Remove the top gently and treat as contaminated material.
5. If the plug is still above the contents of the ampoule, remove it with sterile forceps.
6. Add liquid for re-suspension slowly to the ampoule to avoid frothing.

Section 11 Decontamination: Disinfection and Sterilization

Basic conceptual information regarding decontamination is provided to develop more specific and standardized procedures to suit the needs of the various levels of biohazards involved in a particular laboratory. In this regard, the following general principles apply to all known classes of microbial pathogens, with the notable exception of prions, which are dealt with separately. The specific requirements for decontamination for biosafety

will depend on the type of experimental work and the nature of the infectious agent(s) being handled.

Section 11.1 Methods of Decontamination

Decontamination is the reduction of contaminants to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means viable microorganisms are still present.

In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

In order to select the proper method and tools, it is important to consider the impact of physical and chemical disinfectants on the products, materials, environment, and personnel.

There are four main categories of physical and chemical means of decontamination:

- Chemical-liquid
- Chemical-gasses and vapors
- Heat
- Radiation

Definitions -

Antimicrobial: An agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic: A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied on body surfaces.

Biocide: A general term for any agent that kills unicellular and multicellular organisms.

Bacteriostat: Inhibits the growth of bacteria.

Chemical germicide: A chemical or a mixture of chemicals used to kill microorganisms.

Decontamination: Any process for removing and/or killing microorganisms.

Disinfectant: A chemical or mixture of chemicals used to kill microorganisms, but not necessarily their spores. Disinfectants are usually applied on inanimate surfaces or objects.

Disinfection: A physical or chemical means of killing microorganisms, but not necessarily their spores.

Microbiocide: A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide," "germicide" or "antimicrobial."

Sterilization: A process that destroys and/or removes all classes of microorganisms and their spores.

Anionic Detergents: (soaps) have free negative ions that produce curd when combined with calcium and magnesium in hard water.

Cationic Detergents: (quaternary ammonium) contain positively charged ions which remain suspended in solution.

Section 11.2 Chemical Decontamination: Liquid

The IBC requires 10% bleach as the primary disinfectant, all others as a secondary disinfectant unless approved by the IBC. (1:10 dilution of standard household bleach (5.25% sodium hypochlorite) to water. This needs to be made fresh at least weekly and preferably stored in opaque containers)

There is an ever-increasing number and variety of products. Formulations must therefore be carefully selected for specific needs, and stored, used and disposed of as directed by the manufacturer.

The selection of liquid disinfectant is based on assessment of the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are the agents of choice for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved.
Variables to remember when disinfecting:

- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be effective.
- Type and number of microorganism(s) present - Higher concentrations of microorganisms require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat.
- Presence of organic material and dirt - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature/humidity - Increased exposure/contact time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time. Particular care is needed in the use and storage of such chemicals in tropical regions, where their shelf-life may be

reduced because of high ambient temperatures. Many germicides can be harmful to humans and the environment.

Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. Basically, the chemical disinfectants fall into the following categories: acids/alkalis; alcohols; chlorides; formaldehyde; glutaraldehyde; iodine; mercurial; phenolics; and quaternaries.

Note: if not specified we recommend a 10-20 minute contact time for the chemical disinfectants.

A summary of practical disinfectants is provided as a guidance document in Appendix V.

For listings of EPA's registered antimicrobial products effective against certain blood borne/body fluid pathogens, Mycobacteria tuberculosis (tubercle bacteria), human HIV-1 virus, Hepatitis B or Hepatitis C virus as well as products classified as sterilizers and products used for medical wastes-visit the website at:
http://www.epa.gov/oppad001/chemregindex.htm

Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g., with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions.

As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

- Sodium hypochlorite (household bleach) has an available chlorine content of 5.25%, or 52,500 ppm.
- It is effective against vegetative bacteria, fungi, and most viruses.
- A 5000-ppm available chlorine solution is preferred for general use all-purpose laboratory disinfectant. Excess organic materials inactivate chlorine compounds. This type of solution is made by diluting household bleach 1:10 with water.
- Air and light inactivate diluted solutions, so solutions should be freshly made in order to maintain adequate available chlorine concentrations.
- They are strong oxidizers, thus are very corrosive to metal surfaces, as well as to skin, eyes, and respiratory tract. Always use appropriate personal protective equipment when using these compounds.

- A stronger solution of chlorine is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter.
- Bleach should be made up in cold water in order to prevent breakdown of the disinfectant.

Bleach Solutions:
1/10 dilution of 5.25% bleach ~ 5,250 ppm
1/0 straight 5.25% bleach ~ 52,500 ppm

Note: Chlorine gas is highly toxic. Bleach must therefore be stored and used in well-ventilated areas only. Also, bleach must not be mixed with acids in order to avoid the rapid release of chlorine gas. Many byproducts of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, and in particular bleach, should be avoided.

Chloramines

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial concentrations are therefore required for efficiencies equivalent to those of hypochlorites. On the other hand, chloramine solutions are not inactivated by organic matter to the same extent as hypochlorite solutions, and concentrations of 20 g/l are recommended for both "clean" and "dirty" situations. Chloramine solutions are virtually odor-free. However, items soaked in them must be thoroughly rinsed to remove any residue of the bulking agents added to chloramine-T (sodium tosylchloramide) powders. Chloramines can also be used to disinfect water for drinking when used at a final concentration of 1–2 mg/l available chlorine.

Formaldehyde

Formaldehyde (HCHO) is a gas that kills all microorganisms and their spores, at temperatures above 20 °C. Formaldehyde is not active against prions. It is relatively slow-acting and needs a relative humidity level of about 70%. It is marketed as the solid polymer, paraformaldehyde, in flakes or tablets, or as formalin, a solution of the gas in water of about (37%), containing methanol (100 ml/l) as a stabilizer. Both formulations are heated to liberate the gas, which is used for decontamination and disinfection of enclosed volumes such as safety cabinets and rooms. Effective concentration is a 5-8% solution of formalin (formaldehyde in water; made by diluting a 37% solution). Formaldehyde liquid (5% formalin in water) may be used as a liquid disinfectant. Its toxic nature (TLV: 1 ppm) reduces the desirability of this solution for general use. Formaldehyde solutions are active in the presence of organic matter and do not corrode metal.

Note: Formaldehyde is a suspected carcinogen that can cause respiratory problems at very low concentrations, and has an irritating odor. Also a sensitizer, so a potential exists for developing allergic reactions. It has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-

ventilated areas. Applicable regulations on chemical safety and the MSDS must be consulted prior to its use.

Glutaraldehyde

Like formaldehyde, glutaraldehyde (OHC (CH2) 3CHO) is also active against vegetative bacteria, spores, fungi, and lipid/non-lipid containing viruses. It is non-corrosive and faster acting than formaldehyde. However, it takes several hours to kill bacterial spores. It is generally supplied as a solution with a concentration of about 20 g/l (2%) and most products need to be "activated" (made alkaline) before use by the addition of a bicarbonate compound supplied with the product. The activated solution can be reused for 1–4 weeks depending on the formulation, type, and frequency of its use. Dipsticks supplied with some products give only a rough indication of the levels of active glutaraldehyde available in solutions under use. Glutaraldehyde solutions should be discarded if they become turbid. Cidex™, Sporicidin™, and 3M Glutarex™ are commercially prepared glutaraldehyde disinfectants that are used routinely for cold surface sterilization of clinical instruments.

Note: Glutaraldehyde is toxic and an irritant to skin and mucous membranes, and contact with it must be avoided. It must be used in a fume-hood or in well-ventilated areas. It is not recommended as a spray or solution for the decontamination of environmental surfaces. Applicable regulations on chemical safety must be consulted prior to its use.

Phenolic compounds

Phenolic compounds (o-phenophenoate-base compounds), a broad group of agents, were among the earliest germicides. However, results of more recent safety concerns restrict their use. They are active against vegetative bacteria and lipid containing viruses and, when properly formulated, also show activity against mycobacteria. They are not active against spores and their activity against non-lipid viruses is variable. Many phenolic products are used for the decontamination of environmental surfaces and some (e.g., triclosan and chloroxylenol) are among the more commonly used antiseptics. Triclosan is common in products for hand-washing. It is active mainly against vegetative bacteria and safe for skin and mucous membranes. They are not as adversely affected by organic loads as other disinfectants. Cresols, hexachlorophene, alkyl- and chloro-derivatives, and diphenyls are more active than phenol itself. Available commercial products are Amphyl™, O-syl, Tergisyl, Dettol™, Lysol™, Vesphene™, L-Phase, and Expose. However, in laboratory-based studies, bacteria made resistant to low concentrations of triclosan also show resistance to certain types of antibiotics. The significance of this finding in the field remains unknown. 1.0-5.0% solutions containing 0.5-2.0% phenol are effective against lipoviruses. Phenols are corrosive and may leave a sticky, gummy residue. Phenolic compounds are irritating to the skin and eyes and are relatively toxic (Phenol TLV: for skin is 5 ppm).

Note: Phenolic compounds are not recommended for use on food contact surfaces and in areas with young children. They may be absorbed by rubber and can also penetrate the skin.

Alcohols

Ethanol (ethyl alcohol, C2H5OH) and 2-propanol (isopropyl alcohol, (CH3)2CHOH) have similar disinfectant properties. Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. They are active against vegetative bacteria, fungi, and lipid-containing viruses but not against spores and are less active against non-lipid viruses. For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water; higher or lower concentrations may not be as germicidal. Absolute alcohols are not as effective indicating that some water is required in the disinfection process. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. However, they evaporate fast and therefore have limited exposure time.

Mixtures with other agents are more effective than alcohol alone. Alcohol compatibly combines with other disinfectants (quaternaries, phenolics, and iodine) to form tinctures, extending alcohol's biocidal action (e.g., 70% (v/v) alcohol with 100 g/l formaldehyde and alcohol containing 2 g/l available chlorine).

A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches, and biosafety cabinets, and to soak small pieces of surgical instruments. The contact time on skin should be no less than 10 s and on environmental surfaces no less than 3 min. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses.

Note: Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labeled to avoid their accidental autoclaving.

Iodine and Iodophors

The action of these disinfectants is similar to that of chlorine, although they may be slightly less inhibited by organic matter. Iodophors, organically bound iodine compounds, (Wescodyne diluted to 1:10) are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans. Although these show poor activity against bacterial spores, they are recommended for general use (70 to 150 ppm total iodine). They are effective against vegetative bacteria, fungi, and viruses. They are stable in storage if kept cool and tightly covered. Most iodophors have a built-in indicator. If the solution is brown or yellow, it is still active. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. Iodophors can be readily inactivated and iodophor stains can be readily removed with solutions of sodium thiosulfate. Polyvidoneiodine is a reliable and safe surgical scrub and preoperative skin antiseptic. Iodine should not be used on aluminum, silver, silver-plate, or copper.

Wescodyne™, Betadyne™, Povidone-Iodine, and other iodophors are commercially available iodine-based disinfectants, which give good control when the manufacturer's instructions for formulation and application are followed.

Note: Iodine can be toxic. Organic iodine-based products must be stored at 4–10 °C to avoid the growth of potentially harmful bacteria in them.

Hydrogen Peroxide and Peracids

Like chlorine, hydrogen peroxide (H2O2) and peracids are strong oxidants and can be potent broad spectrum germicides. They are also safer than chlorine to humans and the environment. Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5-10 times its volume with sterilized water. However, such 3-6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action, and to make it less corrosive. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid (CH3COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Note: Hydrogen peroxide and peracids can be corrosive to metals such as aluminum, copper, brass, and zinc, and can also decolorize fabrics, hair, skin, and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

Quaternary Ammonium Compounds

Many types of quaternary ammonium compounds (quats) are used as mixtures and often in combination with other germicides, such as alcohols. The efficacy of quaternary ammonium compounds still generates considerable controversy. Quats are effective in destroying ordinary vegetative bacteria and lipid containing virus but are not effective against pseudomonas, proteus, and other gram-negative bacilli. Also, quats are not effective against bacterial spores at the usual use concentrations of 1:750. Certain types (e.g., benzalkonium chloride) are used as antiseptics. The mode of action of the "quats" is through membrane damage and leakage, followed by protein denaturation. They are relatively nontoxic and acceptable for general disinfection, such as decontaminating food equipment or for general cleaning. Basically these compounds are not suitable for any type of terminal disinfection. Commercially available products include Roccal™ (concentration 0.4-0.8% [v/v]), OstroSan™, Zephirin, CDQ, and A-3.

Note: The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness, and anionic detergents. Care is therefore needed in selecting agents for pre-cleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

Section 11.3 Chemical Decontamination: Vapors & Gases

Vapors and gases are primarily used to decontaminate BSCs and associated systems; bulky or stationary equipment not suited to liquid disinfectants; instruments or optics which might be damaged by other decontamination methods; and rooms, buildings and associated air-handling systems. Agents included in this category are glutaraldehyde and formaldehyde vapor, ethylene oxide gas, peracetic acid and hydrogen peroxide vapor, and chlorine dioxide gas. These sterilants are used in hospitals and commercial facilities where closed systems controlling temperature, humidity, and concentration are required to achieve sterilization using these agents.

Biological safety cabinets are decontaminated using paraformaldehyde heated to decomposition in order to release formaldehyde gas. This procedure should be performed only by personnel trained in this procedure due to the explosive nature of formaldehyde.

Of the sterilants listed above, ethylene oxide (ETO) has wide use as an alkylating agent with very broad biocidal activity including spores and viruses. It is believed that the oxide ring reacts with free amino, sulfhydryl, and hydroxyl groups on proteins. ETO is highly flammable and needs an inerting agent when used in a sterilizer. Additionally, beta-propiolactone behaves similarly to ETO. Instruments and optics that may be damaged by other sterilization methods, rooms, buildings, and air-handling systems in particular are also sterilized using these sterilants.

Chlorine dioxide is a strong and fast-acting germicide, often reported to be active at levels lower than those needed by chlorine as bleach. To obtain an active solution for laboratory use it is generally necessary to mix two separate components, hydrochloric acid (HCl) and sodium chlorite (NaClO2). Stability can be an important issue with this germicide, and materials compatibility and corrosiveness must also be considered when selecting products based on it.

All of these sterilants are extremely toxic, and are regulated under OSHA and EPA regulations. When used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Great care must be taken during use because of the hazardous nature of many of these compounds. Contact USF Environmental Health and Safety (EHS) at 974-4036 if these compounds are to be used.

Section 11.4 Heat Sterilization

Heat is the most common among the physical agents used for the decontamination of pathogens. There are three types of heat sterilization; wet, dry, and incineration. Wet or dry heat can be used to kill microorganisms. “Dry” heat, which is totally non-corrosive, is used to process many items of laboratory ware which can withstand temperatures of 160° C or higher for 2–4 hours. Burning or incineration (see below) is also a form of dry heat. “Moist” heat is most effective when used in the form of autoclaving. The use of dry heat

is discouraged because of its unpredictable variation. Similarly, microwave, ultraviolet, and ionizing radiation are unsuitable in a laboratory setting.

Dry Heat

Dry heat is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of viable organisms on impermeable non-organic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. Sterilization of glassware by dry heat ovens can usually be accomplished at 160-170° C for periods of 2-4 hours. Dry heat sterilizers should be monitored on a regular basis using appropriate biological indicators (spore strips or tubes).

Incineration

Incineration is another effective means of decontamination by heat. As a disposal method incineration has the advantage of reducing the volume of the material prior to its final disposal. Ideally the temperature in the primary chamber should be at least 800° C and that in the secondary chamber at least 1000° C. However, there are no incinerators at USF. Incineration is available through a contract disposal company.

Wet Heat Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. (link to: Autoclave Use)

Effective Autoclave Use

Autoclaves must be used properly to effectively decontaminate potentially biohazardous materials. The following elements all contribute to autoclave effectiveness.

- **Temperature and Pressure**: Adequate chamber temperature is at least 121°C (250°F). Adequate chamber pressure is 15 psi.
- **Time**: Adequate autoclaving time is a minimum of 30 minutes, measured after the temperature of the material being sterilized reaches 121°C and 15 psi pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load.
- **Contact**: Steam saturation of the load is essential for effective decontamination. To ensure steam saturation, autoclave bags should be left partially open during autoclaving to allow steam to penetrate into the bag. Adding a small amount of water to the bag helps ensure heat transfer to the items being decontaminated. Water must not be added if it will cause biohazardous materials to splash out of the bag. Materials should be loosely packed in the chamber for easy steam penetration and air removal. Bags should allow the steam to reach their contents.
- **Containers**: Autoclavable items must be placed in leak-proof containers. Plastic bags must be placed inside a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are appropriate secondary containers. Plastic bags and pans must be autoclavable, so that the plastic will not melt.
- **Indicators**: Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C, but cannot measure the length of time spent at

121°C. Biological indicators (such as Bacillus stearothermophilus spore strips) and certain chemical indicators (such as Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms. A simple chemical indicator, measuring temperature only, should be used in every load to monitor the effectiveness of individual autoclave runs (temperature only). Once a month, either a biological indicator (such as Bacillus stearothermophilus spore strips) or a more complete chemical indicator, measuring both time and temperature (such as Sterigage), should be used. The indicator must be buried in the center of the load to validate adequate steam penetration. Results should be recorded in a log book.

Examples of Different Autoclaves

- Gravity Displacement Autoclaves - Steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA filter.
- Pre-Vacuum Autoclaves - These machines allow the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134° C and the sterilization cycle can therefore be reduced to 3 min. They are ideal for porous loads, but cannot be used to process liquids because of the vacuum.
- Fuel-Heated Pressure Cooker Autoclaves - These should be used only if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas, electricity or other types of fuels. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat reduced. The pressure and temperature rise until the safety valve operates at a preset level. This is the start of the holding time. At the end of the cycle the heat is turned off and the temperature allowed to fall to 80° C or below before the lid is opened.

Recommended Precautions in the Use of Autoclaves

The following rules can minimize the hazards inherent in operating pressurized vessels:

1. Responsibility for operation and routine care should be assigned to trained individuals and a preventive maintenance program should include regular inspection of the chamber, door seals, and all gauges and controls by qualified personnel.
2. The steam should be saturated and free from corrosion inhibitors or other chemicals, which could contaminate the items being sterilized.
3. All materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration; the chamber should not be tightly packed or steam will not reach the load evenly.
4. For autoclaves without an interlocking safety device that prevents the door being opened when the chamber is pressurized, the main steam valve should be closed and the temperature allowed to fall below 80° C before the door is opened.

5. Operators should wear suitable gloves and visors for protection when opening the autoclave, even when the temperature has fallen below 80° C.
6. In any routine monitoring of autoclave performance, biological indicators or thermocouples should be placed at the center of each load. Regular monitoring with thermocouples and recording devices in a "worst case" load is highly desirable to determine proper operating cycles.
7. The drain screen filter of the chamber (if available) should be removed and cleaned daily.
8. Care should be taken to ensure that the relief valves of pressure cooker autoclaves do not become blocked by paper, etc. in the load.

Section 11.5 Radiation

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use. Non-ionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria, and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means. Because of the low penetrating power of UV, microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV lamps in biosafety cabinets are not recommended for sole method of decontamination. Ultraviolet sources are used in biological safety cabinets for partial contamination control. **This form of control is extremely limited due its poor penetrating power, susceptibility to air movement, requirement for long contact time periods, and has not been documented as an effective control method due to the fact that UV lamp intensity or destructive power decreases with time. All UV lamps should be checked monthly with a UV meter or monitoring strip. Frequent cleaning every few weeks is necessary to prevent accumulation of dust and dirt on the lamp, which also reduces its effectiveness drastically. Thus chemical liquids should be used as primary method of decontamination in BSCs.**

Section 11.6 Lab Decontaminating: Recommended Procedures & Practices

Pre-Cleaning and Cleaning Laboratory Materials

In practical terms, cleaning is the removal of visible dirt and stains. This is generally achieved either by (a) brushing, vacuuming, or dry dusting; or (b) washing or damp mopping with water containing a soap or detergent. Where the risk of human or animal contact with pathogen-contaminated materials is high and subsequent decontamination is needed, pre-cleaning is routinely carried out. This is necessary because dirt and soil can shield microorganisms and can also interfere with the killing action of chemical germicides. In such cases, pre-cleaning is essential to achieve proper disinfection or sterilization. Also, many germicidal products claim activity only on precleaned items. Pre-cleaning must be carried out with care to avoid exposure to infectious agents, and materials chemically compatible with the germicides to be applied later must be used. It

is quite common to use the same chemical germicide for pre-cleaning and disinfection. Since heavily soiled items cannot promptly be efficiently disinfected or sterilized, it is equally important to understand the fundamentals of pre-cleaning.

Local Environmental Decontamination

Decontamination of the laboratory space, its furniture, and its equipment requires a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 10 % bleach solution. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H2O2) make suitable substitutes for bleach solutions. Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating paraformaldehyde or boiling formalin. All openings in the room (e.g., windows, doors) should be sealed with masking tape or similar before the gas is generated. Fumigation should be conducted at an ambient temperature of at least 21° C and a relative humidity of 70%. The gas should be in contact with the surfaces to be decontaminated for at least 8 hours. After fumigation the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Fumigation needs to be conducted by trained professional personnel. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde. Fumigation of spaces with vapors of solution of hydrogen peroxide has been reported but requires further study.

Note: Formaldehyde is a dangerous and irritant gas and is a suspected carcinogen. Full-face respirators with air supply may be necessary.

Decontamination of Biological Safety Cabinets

A complete decontamination of a BSC and/or its HEPA filters should only be done by a trained and certified vendor.

Hand-Washing and Hand Decontamination

Suitable gloves should always be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. The use of germicidal soaps is recommended.

- Hands should be thoroughly lathered with soap, using friction, for at least 10-20 seconds, rinsed in clean water, and dried using a clean paper or cloth towel (if available, warm-air hand-dryers are also recommended).
- Automatic, foot- or elbow-operated faucets are required for BSL-3 facilities.

Decontamination of Prion-Containing Materials

Prions, also referred to as “unconventional” infectious agents or “agents of transmissible spongiform encephalopathies,” are believed to contain protein only. As mentioned previously, they can cause Creutzfeldt-Jakob disease in humans, scrapie in sheep, bovine spongiform encephalopathy in cattle, etc. These infectious agents are unusually resistant to inactivation by most physical and chemical agents and materials suspected of containing them require special processing before reuse or disposal. To date, available data indicate that prions can be inactivated by a solution of 2 mol/l sodium hydroxide

(NaOH) containing 4.0 mol/l guanidinium hydrochloride (HNC(NH2)2.HCl) or guanidinium isocyanate (HNC(NH2)2.HNCO) and sodium hypochlorite (NaOCl) (> 2% available chlorine) followed by steam autoclaving at 132° C for 4.5 hours. Incineration is also an effective means of dealing with prion-contaminated materials.

Preparing Lab Equipment for Service, Transfer, or Disposal

- Laboratory equipment is commonly taken out of service for repairs, transfer to a different lab, lab close-out, storage, or disposal. Regardless of the reason a piece of equipment is removed from service, it **must be** cleaned and decontaminated to protect workers servicing the equipment, others that may come in contact with the equipment, and the environment.
- Insuring that equipment is cleaned and decontaminated is the responsibility of the Principal Investigator or the designated lab supervisor. If these individuals are no longer at the university, it becomes the responsibility of the departed Principal Investigator's department.
- Equipment will not be accepted for disposal/recycle/reuse unless it has been properly cleaned and decontaminated.

Please consult with the IBO at (813) 974-0954.

Procedures for Biological Decontamination

These procedures are to be followed regardless of the reason that a piece of equipment is taken out of service (either temporarily or permanently):

- Assess the type of contamination that may be present. This may require interviewing several individuals including those in other labs sharing a piece of equipment.
  Note: Contact the USF Radiation Safety Officer at (813) 974-1194 for radioisotope decontamination assistance.
- Remove all contents.
- If biological material is to be disposed of as waste, place the material in a red biohazard bag for pick up.
- Wearing gloves-clean all surfaces with warm soapy water.
- Sanitize with a 1:10 bleach solution **(1:10 dilution of standard household bleach (5.25% sodium hypochlorite) to water. This needs to be made fresh at least weekly and preferably stored in opaque containers)**. For equipment that will be returned to service, rinse surfaces after 10 minutes contact time as bleach is corrosive.
- For biological safety cabinet decontamination contact the Biosafety Office at (813) 974-0954.

Equipment Needing Repair:

- Contact the service company to determine if they require written verification of decontamination before they will service equipment. Certifying that equipment has been properly decontaminated is the responsibility of the PI.
- Consult the equipment manual for cleaning/decontamination procedures, policies, and chemical compatibility.

- Consult with Institutional Biosafety Officer (813) 974-0954 for any questions or information on choice of decontaminants.

When a service person (University or outside contractor) needs to work on equipment in the laboratory:

- Prepare a working area which is clean and free of hazards.
- Clear enough space for easy access around the equipment.
- Remove any hazardous items stored near, on, or under the equipment.
- Inform the individual of potential hazards in the laboratory.
- Provide personal protective equipment if necessary.

Section 12 Biomedical Waste Management and Disposal

Laboratory waste may be potentially infectious and must be handled appropriately to prevent possible harm to personnel and/or the environment. Biomedical waste, also called biohazardous or infectious waste, is defined as any solid or liquid waste which may present a threat of infection to humans. All applicable rules and regulations of local, state, and federal agencies are to be followed in the handling, treatment, and disposal of biomedical waste. All biomedical waste must be packaged, contained, and located in a manner that protects and prevents the waste from release at any time. Biomedical waste is under Environmental Health and Safety. Information related to this can be found on their website at http://www.usf.edu/administrative-services/environmental-health-safety/hazardous-waste/biomedical-waste.aspx

Section 12.1 USF Biomedical Waste Management Plan

This subsection is excerpted from the USF biomedical waste polices (source: USF's Environmental Health and Safety's Biomedical Waste Policies). For up to date information please contact EH&S.

Biomedical waste is generated by research, instructional, and clinical activities at the University of South Florida (USF) System. The management of biomedical waste in the State of Florida is mandated by Chapter 64E-16, Florida Administrative Code (F.A.C.), and in section 381.0098, Florida Statutes. All areas within USF that generate biomedical waste are required to comply with the requirements of the USF Biomedical Waste Operating Plan. Departments may choose to implement a more stringent, site-specific Biomedical Waste Operating Plan that serves their operational needs and must comply with at least the minimum set forth by the USF Biomedical Waste Operating Plan.

III. RESPONSIBILITIES

A. The Division of Environmental Health and Safety (EH&S) has the overall responsibility for the Biomedical Waste Program, including the following tasks:

- Contract Management: Ensures that waste is picked up regularly in accordance with the Florida Administrative Code (FAC) 64E-16. This also includes maintenance of shipping manifests, invoices and other contract documents.
- Problem Resolution: Resolves problems between the University and vendors. Complaints or requests for special services should be directed to EH&S who in turn coordinates with the vendors.
- Inspection Coordination: Coordinates Department of Health biomedical waste inspections and provides assistance to the Health inspector. Prepares corrective action reports and forwards them to the Department of Health.
- Training: Offers training sessions to USF faculty, staff, and students.

B. Principle Investigators, Instructors, and Clinical Supervisors are responsible for supervising biomedical waste practices in their respective areas. This includes:

- Ensuring that all biomedical waste is handled and disposed of in accordance with the requirements of the USF Biomedical Waste Operating Plan.
- Maintaining training documentation for all affected personnel.

IV. DEFINITION

Biomedical waste is defined by the Florida Administrative Code (FAC) 64E-16 as any solid or liquid waste which may present a threat of infection to humans. Examples of biomedical waste are as follows:

- Body fluids (include lymph, semen, vaginal secretions, cerebrospinal, synovial, pleural, peritoneal, pericardial and amniotic fluids)
- Blood and blood products (human and primate; whole blood, serum plasma and blood products)
- Blood components which include devices which retain visible blood adhering to inner surfaces, such as IV tubing.
- Animals, animal parts/tissues and animal blood that contain human disease-causing agents
- Used absorbent materials such as bandages, gauze, or sponges which are saturated with blood or body fluids
- Needles and needle-syringe units (whether infectious or not)
- Scalpels, razor blades, hard plastic or glass contaminated with tissues, blood, blood products, or body fluids

V. SEGREGATION OF BIOMEDICAL WASTE

Biomedical waste must be separated from all other waste streams at the point of origin as per the requirements of 64E-16 of the Florida Administrative Code (FAC). Once separated, the waste must be placed in either a sharps container or a red bag. Each individual location is required to have an adequate number of sharps containers and red bags to dispose of the biomedical waste generated.

Mixed chemical and biomedical waste

Biomedical waste mixed with chemical waste, as defined in Chapter 62-730, F.A.C., must be managed as hazardous waste. Any biomedical waste that is mixed with chemical waste must be separated if possible. Any questions pertaining to mixed chemical and biological waste disposal should be directed to EH&S. ***Mixed radioactive and biomedical waste***

Biomedical waste mixed with radioactive waste must be managed in accordance with the provisions of Chapter 64E-16, F.A.C. Any questions pertaining to mixed radioactive and biological waste should be directed to Research Integrity and Compliance (RI&C).

Uncontaminated Laboratory Wastes

The following list of materials may be placed into the regular trash if not contaminated with biomedical waste:

- Non-infectious pipettes
- Scalpels, razors, glass or plastic not containing tissues, blood, blood products, blood components or body fluids
- Non-infectious tubes, tubing or other glass or plastic containers not containing tissues, blood, blood products, blood components or body fluids (e.g. centrifuge tubes, microcentifruge or Eppendorf tubes, curettes and capped tubes)
- Non-infectious intact or broken glassware or plastic ware**. It is important to note that broken glass must be placed into appropriate cartons labeled "Broken Glass". In no case may non-infectious needles or syringes be placed in these cartons.**

These materials must be packaged to prevent sharp points or edges from protruding through regular trash bag containers and do not present a threat of infection to humans.

VI. CONTAINMENT

These minimum containment standards must be followed according to Chapter 64E-16, F.A.C:

- Red bags must meet the standards set forth by FAC 64E-16. Generators of biowaste shall purchase red bags from vendors who certify that their bags meet the applicable standards and maintain a copy of the certification on file in their department.
- Sharps containers shall meet the requirements of FAC 64E-16. Generators of biowaste shall purchase sharps containers from vendors who meet the above standards.
- Place all sharps into sharps containers at the point of origin. Sharps containers must be sealed and labeled prior to disposal by the biomedical waste transporter.
- Red bags must be placed into an outer container at the point of origin prior to disposing of any biomedical waste. The outer container must be rigid, leak-resistant and puncture-resistant. Reusable outer containers shall be constructed of smooth, easily cleanable materials and shall be decontaminated after each use. Red bags must be sealed and labeled prior to disposal by the biomedical waste transporter.
- Ruptured or leaking packages of biomedical waste must be placed into a larger container.

VII. LABELING

All sealed biomedical red bags and sharps containers must be labeled with the following information:

- Facility Name (e.g. USF)
- Facility Address or department physical address
- Facility Phone or department phone number
- Facility Contact or responsible person

If a sealed red bag or sharps container is placed into a larger red bag prior to transport, labeling the exterior bag is sufficient. Outer containers are labeled by the biomedical waste transporter with their name, address, registration number, and 24-hour phone number.

VIII. STORAGE

Biomedical waste must not be stored for more than 30 days after the first non-sharps item of biomedical waste is placed into a red bag or sharps container, or when a sharps container that contains only sharps is sealed.
Access to indoor biomedical waste storage areas must be restricted through the use of locks, signs, or location. Locate away from pedestrian traffic and maintain in a sanitary condition. The area should be constructed of smooth, easily cleanable materials that are impervious to liquids and vermin/insect free.
Outdoor storage areas also must be conspicuously marked with a six-inch international biological hazard symbol and must be secured from vandalism.

IX. TRANSPORT

Biomedical waste pickups are conducted at least weekly by the USF biomedical waste transporter. Pick-up times may vary by locations. The USF biomedical waste transporter and treatment facility is:
Stericycle, Inc.
4245 Maine Avenue
Lakeland, FL 33840

X. TRAINING FOR PERSONNEL

Biomedical waste training is required annually by paragraph 64E-16.003(2) (a), F.A.C. for all personnel that handle biomedical waste. EH&S provides biomedical waste training on a quarterly basis through the Division of Research Integrity & Compliance Biosafety Core Course or by request. Training can also be provided by your department. The main components of the training must cover:

- Definition and Identification of Biomedical Waste
- Segregation
- Storage
- Labeling
- Transport
- Procedure for Decontaminating Biomedical Waste Spills
- Contingency Plan for Emergency Transport
- Procedure for Containment
- Treatment Method

Each facility must maintain records of employee training. Training records must be kept for participants in all training sessions for a minimum of three (3) years and must be available for review by Department of Health (DOH) inspectors.

Training is provided by EH&S

XI. PENALTIES

Violation of any provision of Chapter 64E-016, F.A.C., may result in denial, suspension or revocation of the university's biomedical waste permits or an administrative fine of up to $2500 per day for each violation of this chapter or other enforcement action authorized by law.

XII. SPILL CONTINGENCY PLAN

- Surfaces contaminated with spilled or leaked biomedical waste must be cleaned with a solution of industrial strength detergent to remove visible soil before being disinfected by one of the following methods:
- Steam for a minimum of 30 seconds.
- Rinse for at least three (03) minutes with a hypochlorite solution containing 100 parts per million (ppm) available free chlorine (note: one tablespoon per two (02) gallons of water is approximately 100 ppm available free chlorine), or rinse for at least three (3) minutes with an iodine solution containing 25 ppm available iodine.
- Use a chemical germicide that is registered by the Environmental Protection Agency (EPA) as a hospital disinfectant and following recommended dilutions and directions. Liquid waste created by these chemical disinfecting operations shall be disposed of into the sanitary sewage system.
- Employees cleaning spills of biomedical waste must wear appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection.

XIII. GENERATOR LOCATIONS

A list of the generator locations can be found in the USF Biomedical Waste Plan kept within the USF EH&S office. This list is available for review. Please contact Environmental Health & Safety at 813-974-4036.

Section 12.2 Guidelines for Solid Biohazardous Waste

- Non-sharp infectious waste must be placed in red biohazard bags that are labeled with the words “Biohazardous Waste” or with the international biohazard symbol and the word “Biohazard.” Full bags should be tied to prevent leakage or expulsion of contents during future storage, handling, or transport.
- The biohazardous bags in turn should be placed in closable, leak-proof containers/receptacle. When transporting bags a second leak-proof container or bag which is closable should be placed over the outside of the first and closed to prevent leakage during handling, storage, and transport.
- Laboratory and veterinary waste that contain human disease-causing agents shall be treated as biohazardous waste and placed in red bags for disposal.

The waste contractor that collects the biohazardous waste from collection sites is Stericyle, Inc.

If the specimens are derived from animal sources (e.g., animal carcasses, animal parts/tissues, and animal blood), place the solid waste in a red biohazard bag, and place the materials in cold storage within the Division of Comparative Medicine’s Vivarium waste disposal site or other approved dedicated site.

Section 12.3 Guidelines for Liquid Biohazardous Waste

Liquid biohazardous waste includes all blood and liquid waste from humans or animals and all other liquid biohazardous waste (A known or potential biohazard)such as microbial cultures and/or recombinant or synthetic nucleic acid molecules). Collect liquid waste in closeable, rigid plastic, leak proof containers labeled with the universal biohazard symbol.

All liquid waste must be autoclaved or treated with a disinfectant prior to disposal. If biohazardous materials are to be chemically disinfected, contact the Institutional Biosafety Officer (813) 974-0954 for more information regarding the disinfectant of choice, its contact time, and methods of disposal. Liquid waste treated with small quantities of bleach or other household disinfectants can be disposed of by flushing directly to the sanitary sewer. Liquid waste treated with other chemical disinfectants must be disposed of as hazardous chemical waste through EH&S (813) 974-4036
Chemically treated articles should not be autoclaved because of the vapor hazards of chemical disinfectants

Section 12.4 Guidelines for Sharps

Sharps include **all** syringes, lancets, scalpels, and other similar medical instruments (whether contaminated or not), as well as contaminated Pasteur pipettes, broken glass, and other instruments or materials that can cut or puncture personnel.

Syringes and other sharps shall be segregated from solid or liquid biohazardous waste and placed in a properly labeled sharps container. Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are:

1. Closable;
2. Puncture resistant;
3. Rigid;
4. Leak proof, on sides and bottom; and
5. Labeled or color-coded in accordance with this standard.

During use, containers for contaminated sharps shall be:

1. Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., in BSCs);
2. Maintained upright throughout use; and
3. Replaced routinely.

Sharps containers must be designed so that sharps can be safely introduced into the container but not easily retrieved (**old coffee cans, etc. are not acceptable**). Containers must be red in color and labeled with the universal biohazard symbol.

- All sharps containers should be disposed of when 2/3 - 3/4 full by securing the lid (tape securely) and placed in the biohazard waste stream for pickup by Stericycle Inc. disposal services.

- Do not shake up the container to try to fit more materials into it. Shaking the container aerosolizes the materials contained within.

When moving containers of contaminated sharps from the area of use, the containers shall be:

1. Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, or transport.
2. Placed in a secondary container if leakage is possible. The second container shall be:
   a. Closable;
   b. Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and
   c. Labeled or color-coded in accordance with this standard.

Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.

Stericycle Inc. disposal services make regularly scheduled pickups at several locations on and off campus. These include the College of Medicine (MDC building), Bioscience Facility (BSF building), College of Engineering (ENG building), the College of Public Health (CPH building), Interdisciplinary Science Building (ISA), Natural and Environmental Sciences (NES), Byrd Institute, and others. For information regarding site pickup locations at your facility and their schedules please contact the Biohazardous Waste Coordinator, Environmental Health and Safety (EH&S), 813-974-4036. Rooms or areas used to store biohazardous waste shall have restricted access and be designated in the written operating plan regarding biomedical/biohazardous waste.

Needles

The majority of laboratory biohazard injuries are due to hypodermic needles. There is special concern over needle use and disposal. Here are some basic guidelines to prevent injuries:

- Avoid using needles and syringes whenever possible.
- Do not bend, break, or otherwise manipulate needles.
- Do not recap needles. Do not remove needles from syringes.
- Throw away the entire syringe-needle combination. Don't take it apart.
- Place needles, syringes, scalpel blades, Pasteur pipettes, and pipette tips into an appropriate sharps container.

Recommendations to Prevent Needle-sticks

1. Hypodermic needles: Accidental inoculation, aerosol, or spillage.
   a. Do not recap or clip needles.
   b. Use a needle-locking type of syringe to prevent separation of needle and syringe, or use a disposable type where the needle is an integral part of the syringe unit.
   c. Use good laboratory techniques:
      - fill the syringe carefully to minimize air bubbles and frothing of inoculum;

- avoid using syringes to mix infectious liquids; if used, ensure that the tip of the needle is held under the surface of the fluid in the vessel and avoid excessive force;
- wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant before withdrawing the needle from a rubber-stoppered bottle;
- expel excess liquid and air bubbles from the syringe vertically into a cotton pledget moistened with an appropriate disinfectant or into a small bottle containing cotton.

d. Use a biological safety cabinet (BSC) for all operations with infectious material.
e. Restrain animals while they are being inoculated. Use blunt needles or cannulas for intranasal or oral inoculation. Use a biological safety cabinet (BSC).

Section 12.5 Autoclave Use for Biohazardous Waste

Introduction

Pre-treating infectious waste prior to disposal in waste stream is recommended for BSL-2 labs, but required for BSL-3 labs. When using autoclave for decontaminating waste prior to placing in the biomedical waste stream some general procedures are recommended. For details on autoclave procedures see Section 9.6 – (Link to: Decontamination Using an Autoclave)

Steam sterilizers (autoclaves):

- produce superheated steam under high pressure.
- must be properly used to be effective.
- packaging, including the size of containers, stacking containers above one another, overloading an autoclave, and their distribution in the autoclave influence the temperature to which the material is subjected and the contact time.
- containers must have good steam permeability and must be arranged in the autoclave in a manner that promotes free steam circulation (tight-fitting containers do not permit steam penetration).
- autoclaves should receive routine inspection to determine the need for maintenance and repair.
- autoclave door gaskets may become distorted if the door is tightly shut for prolonged periods resulting in leaks - doors should be kept open or loosely closed except when the autoclave serves as a barrier between clean and dirty areas.

Autoclave Testing

Autoclaves should 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) confirm that the load has reached the appropriate temperature for sterilization; however, they must not be used as the sole indicator of sterility.

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.

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 that are used to deactivate other material).
- Every month-recommended for BSL-3 facility

Method: A commercially available test indicator kit that uses bacterial spores *Bacillus stearothermophilus* that are rendered unviable at 250^0 F or 121^0 C. For the test, ampoules of *B. stearothermophilus* are autoclaved along with a load of waste. Upon completion of the cycle, the ampoules are incubated for 48 hours and then observed for any sign of growth. Growth 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.

Autoclave Record Keeping

Maintain the following records:

1. Maintenance records
2. Operations log (each load of deactivated material shall be logged as follows):
    a. Date, time, and operator's name;
    b. Type and approximate amount of waste (lbs or kgs);
    c. Confirmation of sterilization
        i. Record the temperature, pressure, and length of time the load is sterilized. **Note**: 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
        ii. Save the autoclave printout, if the autoclave has a working printer.

Section 12.6 Laundry

All lab coats must be cleaned, and/or disposed of as biohazardous waste. Apparel contaminated with blood or other potentially infectious materials should be handled as little as possible and decontaminated, preferably by autoclaving or chemical cleaning, before being sent to the laundry for cleaning. Contaminated laundry shall be handled as

little as possible with a minimum of agitation. Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be taken home.

Disposable contaminated laundry shall be placed and transported in bags or containers labeled as biohazardous waste.

Section 13 Training

Human error and poor technique can compromise the best of safeguards to protect the laboratory worker. A safety-conscious staff, well informed about the recognition and control of laboratory hazards, is the key to the prevention of laboratory-acquired infections, incidents, and accidents. For this reason, continuous in-service training in safety measures is essential. An effective safety program begins with the Principal Investigator (PI), whose responsibility it is to ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures are an integral part of new employees' introduction to the laboratory.

Section 13.1 Institutional Requirement

Training in proper microbiological techniques is required for anyone working with recombinant DNA, infectious agents, Select Agents, and biological toxins or who works in a laboratory where these materials are used/stored.

The assurance of proper training is the responsibility of the Principal Investigator (PI) or laboratory supervisor of the facility in which the hazard is used. We recommend that the PI/Lab Supervisor supplement the training courses by conducting practical demonstrations and assessments of competence in the laboratory.

Training Requirements

There are three types of Biosafety training requirements:

- **Core** – Must be completed by those who have not completed it previously
- **Continuing Education** – Must be completed annually.
- **Special Topics** – Required for persons involved in certain types of work.

Courses Offered

Core Course
USF Biosafety Training Course – This is required for those who have not completed it previously; **One-time completion is mandatory.** It may be repeated in subsequent years to meet the annual requirement for continuing education. More details are available on the Biosafety Course registration page at http://www.research.usf.edu/dric/biosafety/core-course/register.asp.

Continuing Education Courses

Biological Safety Cabinet Course – This meets the annual continuing education requirement for those who have completed the core course in a previous year. This web-based training course can be accessed worldwide from the Internet. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/safety-cabinets-course/register.asp.

Personal Protective Equipment (PPE) Course - This course provides information about general personal protection equipment (PPE) available for use in biomedical laboratories. Topics include basic PPE types, protection levels, limitations, material types, selection, use and care, hazard recognition, policy, and resources available. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/ppe-course/register.asp.

Autoclave Course - Learn the safe use of autoclave equipment from the following information: an overview of autoclaves in biomedical laboratories, purpose and function, what can and cannot be autoclaved, potential hazards, primary and secondary containers, loading and unloading, determining cycle times, and testing effectiveness. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/autoclave-course/register.asp.

Disinfectant Course –Learn to select and use the correct disinfectants: Definition of Key Terms (Decontamination, Disinfection, and Sterilization), Characteristics of effective disinfectants, Categories of disinfectants, How to select the right disinfectant, Primary and Secondary Containers, How to use disinfectants effectively, Spill Management. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/disinfectants-course/register.asp .

IBC Overview Course – Learn about the Institutional Biosafety Committee (IBC): an overview of the NIH requirement for an IBC, the IBCs duties, the IBC responsibilities, the review process, etc. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/ibc-overview-course/register.asp

Introduction to Viral Vectors Course – Learn about viral vectors: an overview of basic virology of viral vectors, hazards associated with the use of viral vectors in research and animal laboratories, risk assessment of viral vectors, and hoe to incorporate safety features when using viral vectors in research. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes

about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/viral-vectors-course/register.asp

Working Safely with Centrifuges – Learn the principles of using centrifuges, recognize potential hazards and risk of accidents for working with centrifuges and how to incorporate safety practices when working with centrifuges in research based on the identified risk factors. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/centrifuge-safety-course/register.asp

Human Gene Transfer Studies– Learn what you need to know about Human Gene Transfer Studies Basic Principles for Human Gene Transfer (HGT). NIH role in HGT study oversight. NIH submission requirements for HGT studies. Institutional oversight of HGT studies. HGT review process IBC Monitoring of HGT protocols. Biosafety issues relating to the HGT Protocols. Reporting requirements for HGT protocols. This meets the annual continuing education requirement for those who have completed the core course in a previous year. It takes about 45 minutes to complete. To receive credit, you must pre-register on-line at http://www.research.usf.edu/dric/biosafety/human-gene-transfer/register.asp

USF Biosafety Training Course – This live core course may be repeated to meet the annual requirement for continuing education. More details are available on the Biosafety Course registration page at http://www.research.usf.edu/cs/biosafetyregister.htm.

Special Topics
Shipping Biohazardous Materials – All USF personnel who ship and/or receive potentially infectious biological agents are required to complete this course biannually. This training is a self-paced training module that can be reviewed on your own (average allotted time of 2-3 hours) and testing is conducted at our office location. For more information or to schedule time to complete the course, please go to http://www.research.usf.edu/dric/biosafety/shipping.asp

Section 13.2 Components of the Core USF Biosafety Course

USF core training includes principles and practices of biosafety dealing with the following highly hazardous procedures that are commonly encountered by all laboratory personnel:

- Employees will be introduced to Risk Group (RG), Biosafety Levels (BSL), and to USF guidelines (USF Laboratory Biosafety Manual).
- Principal Investigators (PI) play the key role in training their immediate staff in good laboratory techniques.
- Training in proper microbiological techniques is required for anyone working with recombinant DNA (recombinant or synthetic nucleic acid molecules), infectious agents, Select Agents, and biological toxins or who works in a laboratory where these materials are used and/or stored. This is provided by the PI

- Decontamination and spill cleanup procedures.
- Inhalation risks (i.e., aerosol production), such as Grinding, blending, vigorous shaking or mixing; vortexing, sonic disruption, inoculating animals intranasally, harvesting infected tissues from animals, using high concentrations or large volumes of infectious agents and centrifugation;
- Ingestion risks;
- Absorption, via splashes onto exposed skin;
- Risks of percutaneous exposures, through the use of syringe and needle techniques;
- Animal handling that may result in bites and scratches;
- Handling of blood and other potentially hazardous pathological materials; and
- Decontamination and disposal of infectious material.
- The exact training required for a particular person will depend on the hazards to which he or she is exposed and the experimental procedures to be used.
- The assurance of proper training is the responsibility of the Principal Investigator (PI) or laboratory supervisor of the facility in which the hazard is used.
- Other specialized training as deemed appropriate by the IBC and/or the IBO.

Although the USF Biosafety Staff will provide the Principles and Practices of Biosafety Course and other specific courses, we recommend that the PI/Lab Supervisor supplement the training courses by conducting practical demonstrations and assessments of competence in the laboratory. Biosafety Staff conducts training in a modular format that includes the following topics: Principles of Biosafety, Biosafety Levels, Biological Safety Cabinets, Decontamination and Spill Response, Gloves and Hand Washing, Institutional Biosafety Committee, Recombinant DNA and CDC/USDA Select Agents and Toxins.

General Lab safety training and hazardous/biomedical waste training are also required and offered by Environmental Health and Safety. (http://www.usf.edu/administrative-services/environmental-health-safety/)

Personnel responsible for the packaging and shipping of infectious agents (e.g., microorganisms, blood samples, clinical samples) for research and/or pathological testing are required by federal and international regulations to receive training every two years. Research Integrity & Compliance (813) 974-0954 offers this training. You can register for this course on the registration page.

Section 13.3 Ancillary Staff Training

The basic biosafety principles are provided to Physical Pant Staff (housekeeping and maintenance) through a joint training initiative with the Radiation Safety Office. Training is provided annually.

Section 14 Occupational Health

Section 14.1 Blood Borne Pathogens

All USF faculty, staff and volunteers who have the potential for occupational exposure to bloodborne pathogens **with the exception of employees, faculty, staff and volunteers at USF Health** must comply with the "USF Exposure Control Plan"

If you are USF Health then you must comply with OSHA Bloodborne Pathogen training and Exposure Control Plan (OSHA Blood Borne Pathogen (BBP) Training for USF Health Sciences Center).

Section 14.2 Standard Precautions

Standard Precautions (formerly Universal Precautions) apply to blood and to other body fluids containing visible blood. Blood is the single most important source of HIV, HBV, and other bloodborne pathogens in the occupational setting. Standard Precautions also apply to tissues and to the following fluids: semen, vaginal secretions, cerebrospinal fluid (CSF), synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid.

The USF program is administered by the USF Health Medical Health Administration. Please contact that office at (813) 974-3163 for information Refer to Appendix VII for the Code of Federal Regulations: 29 CFR 1910.1030

Personal Protective Equipment:

A. Gloves
- Indicated when touching blood, bloody body fluids, and items or surfaces soiled with blood or body fluids.
- Inspect gloves frequently for holes, tears, or deterioration.
- Double gloving decreases the chances of inoculation by 50%.
- Do not wash or re-use gloves.
- Wash hands after removing gloves.

B. Face shields/goggles
- Indicated when droplets or splashes to mucous membranes are anticipated.
- Goggles must have side protectors to prevent eye splashes.

C. Gowns/lab coats/aprons
- Are indicated when direct contact with potentially infectious material is likely.
- Should be removed when leaving patient room/work area.

D. Masks
- Are indicated when droplets or airborne transmission is likely.
- The N 95 disposable respirator mask is preferable.

E. Handwashing
- Wash for 20 seconds with friction and lather if hands are visibly soiled.
- Wash after removing gloves and when leaving work area.
- Alcohol gel is an acceptable alternative to soap and water if hands are not visibly soiled.

Decontamination, disposal, and transportation of biohazardous material:

A. Blood spills

- Put on gloves.
- Remove any sharp objects with forceps.
- Saturate the spill with one part of chlorine bleach to nine parts water (make fresh weekly).
- Let stand 15-20 minutes.
- Wipe up the spill with a paper towel.
- Discard gloves and paper towels into a red biohazardous bag.

B. Biohazardous bags

- Red, biohazardous bags are indicated for non-sharps waste that is contaminated and distinguishable from general waste.
- Red bags are to be 2-ply thickness and labeled with a biohazardous symbol.

C. Transportation of potentially infectious specimens

- Place the specimen in a sealed container (e.g., specimen cup, test tube).
- Place the sealed container in a secondary sealed container (e.g., freezer storage bag, plastic cooler) and line with absorbent material.
- Label the outside container with the biohazard symbol.

Section 14.3 Health and Safety in the Care and Use of Animals

- Occupational health and safety principles require that personnel caring for and/or using animals know the hazards associated with their work, understand how these hazards are controlled, have safe practices, and use protective supplies and equipment.
- Before beginning research involving animals, all personnel must be familiar with the health risks associated with the species involved in their work.

Please access the **Division of Comparative Medicine Occupational Health & Safety** website for further information.

Section 14.4 Department of Defense/Department of the Army Sponsored Biological Defense Programs

Governing Rule: 32 CFR Part 627.8, 627.9 & 10(a)

Target Audience: All employees who are required to work under a Biological Defense Program research grant sponsored by the Department of Defense/Department of the Army.

Requirements: 32 CFR Part 627.10(a) “Before assignment to work with etiologic agents, personnel will be evaluated by the appropriate medical personnel with respect to their assignment & will be evaluated in the medical surveillance program described in 627.8.”

32 CFR Part 627.8 includes the following:

1. Medical examinations by a licensed medical doctor prior to employment, at least every 3 years thereafter, and upon termination of duties requiring access to laboratories where etiologic agents are used. When full medical exams are not given annually, health professionals will perform annual health screenings

32 CFR Part 627.9 includes the following:

2. If applicable, baseline serum samples will be collected and stored for their biologically useful lifetime, but not longer than 40 years.
3. All personnel assigned duties in work areas where etiological agents are used will be evaluated to determine their suitability for their assigned tasks by the medical doctor and only those who are physically and mentally capable of working in BSL-3 containment areas will be assigned to those areas.
4. For immunization of at-risk personnel, the latest edition of the American College of Physicians' Guide for Adult Immunizations and recommendations of Health and Human Services (HHS) publication (NIH 88-8395) will be followed.
5. Spills and mishaps which result in observable, known, or potential exposures to a known or potential biohazard and/or recombinant or synthetic nucleic acid will be immediately reported to the supervisor, safety officer, and responsible medical personnel. Appropriate medical evaluation, surveillance, and treatment will be provided and records maintained for 40 years.

Scope of Services:

1. Medical Component

The Program Consists of:

a. Evaluation of hazards in the workplace.
b. Physical exam(s).
c. Maintenance of all clinical and program records. All clinical records are confidential and access is restricted to medical/clinical staff.
d. Annual medical follow-up.
e. In the event of a workplace incident, follow-up care will be provided after the initial emergency care.

2. Program Coordination

The Institutional Biosafety Officer (IBO), Research Integrity & Compliance will coordinate the medical surveillance program, provide logistical support, ensure compliance with 32CFR Part 627.8, 627.9 & 10(a), and serve as a liaison for program medical and research staff.

3. Principal Investigator (PI) Responsibility

Each PI will be responsible for the following:

a. Identify all current laboratory personnel subject to the provisions of 32 CFR Part 627.8, 627.9 & 10(a) and supply their names to the IBO, Research Integrity & Compliance.

b. Identify all potential laboratory hires and supply the information to the IBO prior to their employment in order to complete their medical evaluation in accordance with 32 CFR Part 627.8, 627.9 & 10(a).
c. Identify all laboratory personnel whose duties will terminate and who will no longer require access to the laboratory with etiological agents. Supply this information to the IBO prior to staff termination so that a medical evaluation is performed in accordance with 32 CFR Part 627.8, 627.9 & 10(a).
d. Immediately report spills and mishaps to the IBO and medical staff, which result in observable, known, or potential exposures to a known or potential biohazard and/or recombinant or synthetic nucleic acid.

Section 14.5 Immunoprophylaxis

The IBC follows the recommendations of the Centers for Disease Control and Prevention (CDC). The USF Health Medical Heath Administration follows the Public Health Service Advisory Committee for Immunization Practices (ACIP) for vaccination of at-risk personnel.

Vaccinations may be required or recommended, as needed. Particular attention is given to individuals who are or may become immunocompromised or immunosuppressed, as recommendations for vaccine administration may be different than for immunologically competent adults. Various other factors may also be taken into account (e.g., pregnancy, history of allergy, or HIV status) during evaluations conducted by USF Health Medical Health Administration.

When considering the need for immunization in association with the review of the registration application, a risk assessment will be conducted by the IBC.

Section 15 Transport and Shipment of Infectious Substances

Section 15.1 On-Campus Transport of Biohazardous Materials

Any biohazardous materials transported between laboratories or buildings on campus should be in double containment with absorbent material placed between the primary and secondary containers to absorb the contents of a spill in case of an accident to prevent release of the materials into the environment. Transport containers should be labeled with the universal biohazard symbol, emergency contact, and the identity of the material inside.

The following are guidelines for double containment:

- The container should be preferably of plastic rather than glass.

- The container should be robust and should not leak when the cap or stopper is correctly applied.
- No material should remain on the outside of the container.
- Containers should be correctly labeled to facilitate identification.
- To avoid accidental leakage or spillage secondary containers, such as coolers, should be fitted with racks so that the specimen containers remain upright.

In all cases of transport:

1. The tubes should be capped (screw cap and taped shut).
2. Place in a test tube rack.
3. Place inside a sealed, puncture-resistant, unbreakable secondary container.
4. Enclose with a biohazard label and emergency contact information.
5. The specimens' identity should be labeled on the tubes.
6. The secondary container must contain sufficient absorbent material to absorb the contents of the tube in case of a spill or leak.

Opening Packages

Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt Standard Precautions, particularly when dealing with broken or leaking containers. Specimens should be unpacked in a biological safety cabinet. Disinfectants should be readily available.

Leaking Specimen Containers

- Inform the PI and IBC.
- Use disposable gloves to handle the material in the biological safety cabinet.
- Decontaminate the container by placing it in an autoclave pan and pouring a 10% bleach solution over it.
- Transfer the amount of specimen needed for culture to a sterile tissue culture flask and inactivate the remaining material with bleach and autoclave.

Section 15.2 Off-Campus Transport of Biohazardous Materials by Commercial Carriers

All *off-campus* transport of biohazardous materials *by commercial carriers* must comply with federal and state shipping and permitting requirements, as described in the following sections. Off-campus includes across town to a collaborative research facility, out of town within the state, out of state in the U.S., and out of the country. Shipment (packaging, labeling, etc.) of biohazardous agents must comply with Federal and State regulations (U.S. Department of Transportation [US DOT] regulations and International Air Transport Association [IATA]).

Any product that is or contains a hazardous material must be transported according to the requirements outlined in the Department of Transportation's (DOT) Title 49 CFR regulations. This includes hazardous biological agents. To comply with DOT regulations, all hazardous materials must be properly classified, packaged, documented, and handled.

If the regulatory requirements for hazardous materials shipments are not met, citations and fines may be levied. The regulations also require **Hazardous Materials Shipping Training** for personnel involved in the transportation of hazardous materials.

All North American airlines and carriers such as FedEx, use the IATA regulation (also referred to as the Dangerous Goods Regulation or DGR) as their standard. Meeting the conditions of this standard will ensure meeting the provisions of the other U.S. regulations. Personnel responsible for the packaging and shipping of infectious agents (e.g., microorganisms, blood samples, clinical samples) for research and/or pathological testing are required by federal and international regulations to receive training every two years. Research Integrity & Compliance (813) 974-0954 offers this training. You can register for this course on the registration page.
..
Note: Any biological materials for which a state or federal permit or license is required, also requires registration with the Biological Safety Office. Additional important links regarding the packaging and shipment of biological Materials can be found in Appendix VIII.

Section 15.3 Import, Export, and Transport Permits

Permit Requirements
Special federal permits may be required for importing and/or transporting human pathogens, animal pathogens, animals or animal products, plant pathogens or plant pests, and plants or plant products. Permit requirements should be verified well in advance of needing the material in question, because some permits can take several weeks to obtain. Contact the USF's Biosafety Office (biosafety@research.usf.edu) for assistance regarding shipping and/or required permits for biological materials.

Animals, Plants, Introduction of Genetically Modified Organisms: The USDA, through its Animal and Plant Health Inspection Service (APHIS), regulates transport of materials that could potentially harm U.S. agricultural products, such as livestock or crops. For this reason, APHIS permits may be required for import, export, and/or transport of animal or plant pathogens, import or export of animals, animal products, plants or plant products, or introduction of genetically modified organisms into the environment. The information contained on the APHIS web site and the Biosafety Office can help determine if a permit is required and assist with the application process. USDA Animal and Plant Health Inspection Service Import-Export Directory Animal and Plant Health Inspection Service (APHIS)

Human Pathogens or Biological Toxins: The Department of Health and Human Services, through the CDC, regulates transport of biological materials that could cause illness in humans. These regulated biological materials include pathogenic bacteria or viruses, toxins from biological sources (e.g., tetanus toxin, aflatoxin), blood, or tissues suspected of containing diseases transmissible to humans and certain animals, and insects that may harbor disease causing organisms. The information contained on the CDC web site and the Biosafety Office can help determine if a permit is required and assist with the

application process. The Centers for Disease Control and Prevention's Importation Permits for Etiologic Agents: CDC -Etiologic Agent Import Permit Program

Shipment to Canada - There is an Agriculture Canada permit required for the shipment of most biological materials to Canada. Human pathogens require a Health Canada permit. Zoonotic agents require both. Please contact the USF's Biosafety Office (biosafety@research.usf.edu) for further information.

Other International Shipments - There is a validated export license required for the shipment of any organism or toxin that appears under Export Classification Number (ECCN) 1C351, 1C354 to any foreign destination. For additional information contact the USF's Office of Export Control website (exportcontrol@usf.edu) or visit the website for U.S. Department of Commerce, Office of Export Licensing at http://www.access.gpo.gov/bis/index.html.

Section 15.4 Off-Campus Transport of Biohazardous Materials by Non-Commercial Routes

All off-campus transport of biohazardous materials *by non-commercial routes* must comply with the Guidelines for Transport of Infectious Materials by Non-Commercial Routes.

- We recommend that USF personnel should transport biohazardous materials by non-commercial routes only in university vehicles. Note: Personal vehicles may have insurance coverage limitations.
- Transport by non-commercial routes may only be done within the state of Florida.

Section 16 Contingency Plans and Emergency Procedures

Emergency plans should be tailored for a given biohazardous situation. The PI/laboratory supervisor should prepare instructions specifying immediate steps to be taken. These instructions should be displayed prominently in the laboratory and periodically reviewed with personnel. A written contingency plan for dealing with laboratory emergencies is a necessity in any facility that works with or stores Risk Group 3 microorganisms (containment laboratory – Biosafety Level 3). Federal, state, and local health authorities should be involved in the development of the emergency preparedness plan.

No single plan will apply to all situations but the following general principles should be considered.

Contingency Plan
The contingency plan should provide operational procedures for:

- precautions against fire, flood, etc.

- biohazard risk assessment
- accident-exposure management and decontamination
- emergency evacuation of people from the premises
- emergency medical treatment of exposed and injured persons
- clinical management of exposed persons

In the development of this plan, the following items should be considered for inclusion:

- identification of high-risk organisms
- location of high-risk areas (e.g., laboratories, storage areas, animal facilities)
- identification of at-risk personnel and populations
- identification of responsible personnel and their duties (e.g., Institutional Biosafety Officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services)
- lists of treatment and isolation facilities that can receive exposed or infected persons
- transport of exposed or infected persons
- lists of sources of immune serum, vaccines, drugs, special equipment, and supplies
- provision of emergency equipment (e.g., protective clothing, disinfectants, decontamination equipment)

Section 16.1 Emergency/Exposure/Accident Response

This section outlines the basic procedures for dealing with exposures to a known or potential biohazard and/or recombinant or synthetic nucleic acid molecules that may be encountered in a research laboratory. An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials and/or recombinant or synthetic nucleic acid molecules. A person who sustains a known or potential exposure incident must remove their gloves/PPE and treat the affected area immediately by following the appropriate exposure incident response below. All biosafety incidents MUST BE REPORTED TO BIOSAFETY via phone (974-0954 or 813-469-1625) or email at biosafety@usf.edu.

Accidental Injection, Cuts, and Abrasions

a. The affected individual should remove protective clothing, wash the area with soap and running water for at least 15 min. If contaminated material is splashed or sprayed into the face contaminating the eyes, nose or mouth: flush the eyes for 15 minutes, rinse mouth out with clean water and be sure not to swallow, wash down face being sure that the nasal cavities have been rinsed as much as possible ,
b. Inform the lab director/PI and IBO (974-0954 or 813-469-1625) about the cause of the exposure and the microorganisms or the recombinant or synthetic nucleic acid molecules involved.
c. If exposed to a Blood Borne Pathogen contact the USF Health Medical Health Administrator (813-974-3163) during regular working hours and the on call

Infectious Disease physician at 974-2201 during non-regular working hours. See emergency contact list

d. If medical treatment is needed, go to a nearby medical facility.
e. In addition, all injuries should be reported to USF Workers' Compensation group at (813) 974-5775. For USF employees follow the link below for an approved medical facility: http://usfweb2.usf.edu/human-resources/employee-relations/workers-comp.asp
f. PPE and clothing should be considered contaminated and disposed or treated appropriately.

Accidental Ingestion of Potentially Hazardous Material and/or recombinant or synthetic nucleic acid molecules

a. Inform the lab director/PI and IBO (974-0954 or 813-469-1625)
b. Go to a nearby medical facility.
c. The physician should be informed of the material ingested
d. Should be reported to USF Workers' Compensation group at (813) 974-5775. (Further instructions may be found at the Workers' Compensation website.)

Animal Bites and Scratches

The following emergency response procedures shall be followed when a worker has been exposed to zoonotic agents and/or recombinant or synthetic nucleic acid molecules via a needle-stick, cut, animal bite, or scratch, via mucous membrane contact, or via non-intact skin contact.

Worker

The exposed site must be washed immediately:

- If needle-stick, cut, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.
- If mucous membrane (eyes, nose, mouth) or non-intact skin contact (cuts, rash, eczema, or dermatitis), flush with water at the nearest faucet or eye wash station for at least 15 mins.
- Inform the lab director/PI and IBO (974-0954 or 813-469-1625)
- The worker must follow the reporting requirements. It is the responsibility of each individual to report all work related injuries and/or work related illnesses immediately to their supervisor. Comparative Medicine's Standard Operating Procedures - Reporting/Tracking Work Related Injury/Illness is found at: http://www.research.usf.edu/cm/SOP's/S029_5_Procedures_for_Reporting_Injury_Illness_5_09.pdf
- If an injury requires an employee to seek non-emergency medical care:
  - **Between the hours of 8:00 a.m. and 5:00 p.m.** the manager/supervisor reports the injury/illness, prior to obtaining medical treatment, to USF Worker's Compensation Specialist at 813-974-5720 or 813-974-5775.
  - On a **weekend, holiday, or before 8:00 a.m. or after 5:00 p.m.** the manager/supervisor reports the injury/illness, prior to obtaining medical treatment, to AmeriSys at 800-455-2079.

- Completes as much as possible of the "***Supervisor's Accident Investigation Report Form***".
- Sends a copy of the Supervisor's Accident Investigation Report Form with the employee seeking medical treatment.
- Faxes a copy of the Supervisor's Accident Investigation Report Form to USF Worker's Compensation at 813-974-7535.

- If an injury requires an employee to seek emergency medical care, call 911 or go directly to an emergency room. As soon as possible, the manager/supervisor:
  - Ensures the emergency room is aware that the incident is work related.
  - Reports the incident to a USF Worker's Compensation Specialist at 813-974-5720 or 813-974-5775 Mon.-Fri., between the hours of 8:00 a.m. and 5:00 p.m.
  - Reports the incident to AmeriSys at 1-800-455-2079, if emergency medical care is required on a weekend, holiday, or before 8:00 a.m. or after 5:00 p.m.

Potentially Hazardous Aerosol Release

a. All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice. USF employees follow the link below for an approved medical facility and Workers' Compensation information: http://usfweb2.usf.edu/human-resources/employee-relations/workers-comp.asp
b. The laboratory supervisor and the IBO should be informed at once.
c. No one should enter the room for at least 30 mins, to allow dispersement of aerosols via the ventilation system and heavier particles to settle.
d. During this time, signs should be posted indicating that entry is restricted.
e. After 30 mins, decontamination should proceed, in consultation with the IBO. Appropriate protective clothing and respiratory protection must be worn if applicable.

Broken and Spilled Infectious Substances, including Cultures

a. Broken objects contaminated with infectious substances and/or recombinant or synthetic nucleic acid molecules, including vials or containers, or spilled infectious substances, and/or recombinant or synthetic nucleic acid molecules,including cultures, should be covered with a cloth or paper towels.
b. Disinfectant should then be poured over these and left for at least 20 min.
c. The cloth or paper towels and the broken material may then be cleared away; glass fragments should be handled with forceps or dust pans.
d. The contaminated area should then be swabbed with disinfectant.
e. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant for 24 hours.
f. Cloths, paper towels, and swabs used for cleaning up should be placed in a biohazardous waste container.
g. Proper PPE must be worn for all these procedures.

Breakage of Tubes Containing Potentially Hazardous Material in Centrifuges <u>not having</u> Sealable Buckets

a. If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed for 30 min.
b. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed for 30 min.
c. In both instances, the USF IBO should be informed.

Strong gloves (e.g., thick rubber), covered if necessary with suitable disposable gloves, should be worn for all subsequent operations.

d. Forceps, or cotton held in the forceps, should be used to retrieve glass debris.
e. All broken tubes, glass fragments, buckets, trunnions, and the rotor should be placed in non-corrosive disinfectant known to be active against the organisms concerned and left for 24 hours.
f. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered after 60 minutes.
g. The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, left overnight and then swabbed again, washed with water and dried.
h. All materials used in the clean-up should be treated as infectious waste.

Breakage of Tubes <u>Inside</u> Sealable Buckets (safety cups)

a. All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet.
b. If a breakage is suspected, the cap should be opened, left loose and the bucket autoclaved, if appropriate.

Section 16.2 Biological Spill Response

The guidelines are intended to assist the principal investigator, laboratory supervisor, and other responsible individuals who may be involved in the cleanup of biological spills. This guide outlines the basic procedures for dealing with some of the biological spills of a known or potential biohazard and/or recombinant or synthetic nucleic acid molecules that may be encountered in a research laboratory. All lab personnel should refer to the laboratory specific spill response procedures before initiating their experiments.

Biosafety Level 1 (BSL-1) Spill

- Notify others in the area, to prevent contamination of additional personnel and environment.
- When a BSL-1 spill occurs outside the lab (e.g., hallways, common rooms, corridors) report these BSL-1 spills to: (**1) Lab Director (2) USF IBO (813) 974-0954.**
- Remove any contaminated clothing and wash exposed skin with soap and water.

Clean-up of BSL-1 Spill

- Wearing gloves and lab coat, cover spill with paper towels, pour disinfectant around the spill allowing it to mix with spilled material. Allow suitable contact time, at least 15 min.
- Pick up any pieces of broken glass with forceps and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard bag.
- Wash hands with soap and water.

Biosafety Level 2 (BSL-2) Spill

- Notify others in the laboratory regarding the spill
- Close door, and post with a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
- Wash all exposed skin with soap and water.
- Inform Supervisor and/or Lab director **and USF IBO (813) 974-0954.**

Clean-up of BSL-2 Spill

- Allow aerosols to disperse and or settle for at least 30 minutes before reentering the laboratory (if spill outside biological safety cabinet). Assemble clean-up materials (disinfectant, paper towels, biohazard bags, and forceps).
- Put on protective clothing (lab coat, facemasks/face protection, utility gloves, and booties if necessary).
- Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use a higher concentration of disinfectant as it is diluted by the spill. Allow at least a 20 minute contact time.
- Pick up any sharp objects with forceps, tongs, or autoclavable dust pan and brush and discard in a sharps container.
- Clean up the disinfectant and spill using mechanical means, such as an autoclavable broom and dustpan, since there may be sharps under the paper towels, and place the materials into a sharps container.
- Smaller pieces of glass may be collected with cotton or paper towels held with forceps. If no sharps were involved in the spill discard the materials into an autoclave bag.
- Wipe surrounding areas (where the spill may have splashed) with disinfectant.
- Spray the area with 10% household bleach solution and allow air-drying (or wiping down with disinfectant-soaked towels after a 20-minute contact time).
- Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
- Wash hands and exposed skin areas with soap and water.

Emergency Services: Who to Contact

The telephone numbers and addresses of the following should be prominently displayed:

- **University Police - 911**
- The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)

- Principal Investigator and/or Lab Director
- Laboratory supervisor
- Responsible technician
- Institutional Biosafety Officer – (813) 974-0954
- Radiation Safety Officer – (813) 974-1194
- Environmental Health & Safety – (813) 974-4036
- Blood borne pathogen exposures contact – (813) 974-3163 or
  USF Health Infectious Disease Physician on call – (813) 974-2201
- Workers compensation - (813) 974-5775
- USF Physical Plant – (813) 974-2845

Persons requiring immediate emergency care should seek it. Preparation of paperwork will be secondary to obtaining prompt medical attention.

Emergency Equipment
It is recommended the following emergency equipment be available:

- First-aid kit, including universal and special antidotes
- Appropriate fire extinguishers
- Biological spill kit (if applicable)
- Chemical Spill kit (if applicable)
- Appropriate PPE (if applicable)

Section 16.3 Near Miss

There are often incidents that did not result in an injury or an illness, but which might easily have done so. Near misses are narrowly avoided occurrences that could have resulted in staff being exposed to a biological agent and/or recombinant or synthetic nucleic acid molecules. The Biosafety program is tracking these Near Misses as they may identify situations where implementation of practices and precautions may be able to prevent similar future incidents. Fill out a USF Incident and Near Miss Reporting Form to document the recognition of a hazard, to document the change that is made to prevent a recurrence of the potential of being exposed to biohazard/ and/or recombinant or synthetic nucleic acid molecules and to share what you have learned with others.

Section 17 Select Agents

Background

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have identified certain bacteria, viruses, toxins, rickettsia, and fungi that pose a potential threat to public health or welfare. These organisms are considered **Select Biological Agents and Toxins; High Consequence**

Livestock Pathogens and Toxins. A Select Agent may not be possessed or used in the United States, received from outside the United States, or transferred within the United States by any individual(s), academic institution, or other legal entity unless such activities are conducted for a lawful purpose and in accordance with the federal law.

The University of South Florida (USF), in order to comply with the USA PATRIOT Act of 2001, and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, is required to register the possession, use, and transfer of select biological agents and toxins. If you wish to initiate studies involving these agents, you must be registered with the Centers for Disease Control and Prevention (CDC) or the USDA/APHIS through the Office of Research Integrity & Compliance and begin by declaring your intentions to the USF Institutional Biosafety Officer at (813) 974-0954.

Registration with the USF IBC

Researchers planning to work with any Select Agent material must also complete a USF *Registration Document for the Use of Infectious Agents and Biological Toxins* and submit it to the Institutional Biosafety Committee (IBC) for review. This registration document is available on our Web Site at: http://www.research.usf.edu/dric/biosafety/forms.asp

Frequently Asked Questions:

Q. Where can I find a listing of Select Agents and Toxins?

A. The complete list of agents and toxins are posted on our Web Site at http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html

Q. According to the listings, my study will involve Select Agents. What do I Do?

A. The staff will work with you to complete the necessary steps to register your request with the CDC or the USDA/APHIS and with the USF Institutional Biosafety Committee (IBC). **PLAN WELL IN ADVANCE AS THE REGISTRATION PROCESS MAY TAKE FOUR TO SIX MONTHS OR MORE TO COMPLETE.**

Some of the requirements are as follows:

- Registration is valid only for the specific select agents and toxins, the particular activities and locations involved, and the specific individuals approved to handle or use the regulated materials listed on the application.
- The PI must collaborate with the USF Institutional Biosafety Officer to develop and implement agent-specific plans for biosafety, site-specific plans for security (e.g., inventory control, access control, cyber security, and emergency response).
- As part of the federal registration process the Responsible Officer, Alternate Responsible Officer, PIs, and staff will undergo a security risk assessment that includes a background check and finger printing.
- Only those individuals who have documented a legitimate need to handle or use agents or toxins and who have appropriate training and skills to handle such agents will be granted access to the agents.

- There are specific requirements for record keeping, notification of transfer, theft, loss, or destruction.
- The laboratory will be inspected by the CDC or USDA before final federal approval is granted.
- The Responsible Officer must conduct regular inspections of the laboratory where select agents/toxins are used or stored.
- There are certain criteria that exclude personnel from working with Select Agents. (see details below: USA Patriot Act - Restricted Person)

Shipping Select Agent and Biological Toxins:
The Centers for Disease Control and Prevention (CDC) regulates the possession, use, and transfer of Select Agents and Toxins that have the potential to pose a severe threat to public health and safety. The CDC Select Agent Program oversees these activities and registers all laboratories and other entities in the United States of America that possess, use, or transfer a Select Agent or Toxin.

The Department of Health and Human Services rule has specific requirements regarding shipping Select Agents and Toxins:

- Facilities sending out or receiving certain designated Select Agents, such as certain specified viruses, bacteria, rickettsia, fungi, and biological toxins, are now required to apply for and receive a Site Registration Number from the CDC before any shipments occur. Substantial criminal penalties apply to both individuals and organizations that do not comply with the regulation requirements.
- Separate paperwork must not only be completed for each laboratory on campus that will be sending out and/or receiving shipments of any of the Select Agents covered by this regulation, but also for each Select Agent used. The paperwork consists of an extensive application packet requiring completion every three years. Registered laboratories are subject to inspection by outside agencies. The IBO can help determine whether the Select Agent rule applies to specific projects and whether registration is necessary. The Centers for Disease Control and Prevention's Laboratory Registration and Select Agent Transfer Program may be accessed at: http://www.selectagents.gov/

USA PATRIOT ACT

On October 25, 2001 in response to the terrorist attacks of 9/11/2001, Congress expanded the restrictions on the use and possession of potential bioterrorist agents by enacting the USA Patriot Act of 2001. The Act places restrictions on who can possess "Select Agents" and how the agents are to be protected from unauthorized use.

A "Restricted Person" is (as defined in the USA PATRIOT Act) an individual that meets any one of the following criteria:

- A person who is under indictment for a crime punishable by imprisonment for a term exceeding 1 year;
- A person who has been convicted in any court of a crime punishable by imprisonment for a term exceeding 1 year;
- A person who is fugitive from justice;
- A person who is an unlawful user of any controlled substance;
- A person who is an alien illegally or unlawfully in the United States;
- A person who has been adjudicated as a mental defective or has been committed to any mental institution;
- A person who is an alien (other than an alien lawfully admitted for permanent residence) who is a national of a country as to which the Secretary of State has made a determination that such country has repeatedly provided support for acts of international terrorism; currently these countries are:
  - Cuba
  - Iran
  - Syria
  - Sudan
- A person who has been discharged from the Armed Services of the United States under dishonorable conditions.

Section 18 Laboratory Security

Researchers and their laboratory personnel should be aware of their responsibilities to maintain a secure research environment. Be aware of your surroundings and apply a level of personal vigilance in maintaining the security of USF labs. It is recommended that research labs take the following steps to provide security against any unauthorized entry as well as to remain in compliance with federal, state, and USF regulations.

1. Lock all doors to laboratories when they are unoccupied to prevent unauthorized entry and theft of potentially harmful materials stored in the laboratory.
2. Lock all equipment (e.g., freezers, cabinets, incubators, scintillation counters) that may contain hazardous materials and are located in hallways or common use laboratories.
3. Always secure hazardous materials when they are not being used.
4. Maintain an inventory of all hazardous materials including chemical, biological, and radiological materials. Track hazardous material use and report any missing

inventory to USF Police at 974-2628 (911 in an emergency) and Research Integrity & Compliance at 974-0954.
5. Be aware of your surroundings. Report any suspicious person or activity and any unusual inquires about hazardous materials to the USF police at 974-2628 (911 in an emergency) and the Institutional Biosafety Officer at 974-0954.
6. Ensure emergency contact information posted on laboratory doors is current and is updated on a regular basis.
7. Limit access to laboratories where hazardous materials are used and stored to authorized laboratory personnel.
8. Know who is in the laboratory area. It is recommended that visitors be escorted during their visit to the laboratory. Access for visitors (students, visiting scientists, etc.) should be limited to hours when regular employees are present.
9. Know what materials are being brought into the laboratory area. Packages containing specimens, bacterial or virus isolates, or toxins should be opened in a biological safety cabinet (BSC).
10. Know what materials are being removed from the laboratory area. Biological materials/toxins for shipment to other laboratories should be packaged and labeled in compliance with all applicable local, federal, and international shipping regulations.
11. Review safety policies and procedures regularly. Laboratory supervisors should ensure that all laboratory workers and visitors understand security requirements and are trained and equipped to follow established procedures. Review safety policies and procedures whenever an incident occurs or a new threat is identified.
12. Control access to areas where biological agents or toxins are used and stored. Only workers required to perform a job should be allowed in laboratory areas, and workers should be allowed only in areas and at hours required to perform their particular job. Areas where research is conducted utilizing Select Agents and other sensitive materials are required to have all spaces locked and secured at all times.
13. Access for routine cleaning, maintenance, and repairs should be limited to hours when regular employees are present. Freezers, refrigerators, cabinets, and other containers where stocks of biological agents are stored should be locked when they are not in direct view of workers (e.g., when located in unattended storage areas).

These steps are reasonable and easy to implement. By following these simple recommendations, we can do our part to heighten security and safety in our laboratories and maintain a secure research environment.

Section 19 Safety Rules for Ancillary Staff

Before engineering, maintenance, cleaning personnel, etc. enter the lab premises they should be properly trained in exposure avoidance/reduction methods (PPE, etc.) and as they interact with the laboratory staff, it is essential that they should perform their duties and follow appropriate safety rules.

Engineers and maintenance staff should not enter Biosafety Level 3 (BSL-3) containment laboratories except after clearance by the IBO, and under the supervision of the laboratory supervisor or his designated representative. The staff should receive training as outlined below.

The following guidelines are designed to aid in the prevention of laboratory-acquired infections by Engineering, Building Maintenance Services, and House Keeping personnel:

1. Follow the instructions of the laboratory staff.
2. Wash your hands often and always before leaving the laboratory.
3. Do not eat or drink, or apply cosmetics in any laboratory. Use the staff room or the restroom.
4. Do not dust or clean any work benches without the permission of the laboratory staff.
5. If you have an accident of any kind, or knock over or break any bottle, tube, jar, or piece of equipment, tell your supervisor or one of the laboratory staff at once.
6. Do not attempt to clear up after any accident without permission.
7. Do not pick up broken glass with your fingers. Use a dustpan and brush, tongs, tweezers, or forceps.
8. Do not enter any room that has a “Restricted Entry or Authorized Entry Only” sign on the door (e.g., BSL-3 Lab) unless authorized to do so and accompanied by a laboratory staff representative.

APPENDICES

The following appendices
are intended to provide convenient reference information
about a wide range of biosafety-related matters

APPENDIX I - Biosafety Checklist

This checklist is intended to assist in assessments of the biosafety status of biomedical laboratories.

Biosafety Level 2 Laboratory

Biosafety Level 2	**Yes**	**No**	**N/A**	**Comments**
A. Standard Microbiological Practices				
1. Access limited when experiments in progress.				
2. Persons wash hands after work w/cultures & removing gloves, before leaving lab.				
3. Eating, drinking, storing food, etc. prohibited.				
4. Mouth pipetting prohibited; pipettors used.				
5a. Sharps handling policies in place. 5b. Sharps disposed in biohazardous Sharps containers. 5c. Broken glassware is only handled by mechanical means. 5d. Plastic ware is substituted for glassware whenever possible. 5e. Disposable needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated prior to disposal. 5f. Syringes that "re-sheath" the needle or needleless systems are used when appropriate.				
6. Splashes & aerosols are minimized.				
7a. Work surfaces disinfected after completion of				

work and after any spill. 7b. Biohazard spill cleanup kit available.				
8. Regulated waste disposed properly.				
9. A biohazard sign, PI/Emergency contact information, and biosafety level are posted on entry doors to lab.				
10. Insect & rodent control program in place.				
11. PI ensures personnel receive appropriate training.				

B. Special Practices:	Yes	No	N/A	Comments
1. Laboratory staff is advised of hazards.				
2. Laboratory staff is provided medical surveillance and appropriate immunizations if applicable.				
3. Policy in place regarding baseline serum for at risk personnel, as appropriate.				
4. A laboratory-specific biosafety manual must be prepared and adopted as policy.				
5. The PI must ensure that laboratory staff demonstrates proficiency in standard and special microbiological practices.				
6. Infectious agents must be placed in a durable, leak proof container during collection, handling, storage and transport.				
7. Policies for containing and decontaminating spills are in place. Laboratory equipment decontaminated after spills, before repair, maintenance, or removal from lab.				
8. Policies for accidental exposures are in place.				

9. Animals & plants not involved in work not permitted in lab.				
10. Experimental procedures that generate aerosols should be conducted within the biosafety cabinet.				
11. Equipment and storage areas for use with biohazard are properly labeled. Agents are properly labeled.				
C. Safety Equipment (Primary Barriers)	Yes	No	N/A	Comments
1. Biosafety cabinet is certified annually.				
2a. Lab coats/gowns designated for lab use is worn by all personnel for work with biohazardous agents. 2b. Remove lab coats/gowns before leaving for non-lab areas. 2c. Dispose Lab clothing appropriately or deposit for launder on-site. Lab coats/gowns are not taken home for laundering.				
3. Eye and Face protection is available for procedures with potential of aerosols/splashes generation with biohazardous agents that is not conducted in the BSC cabinet or other containment device.				
4a. Gloves must be worn when working with Biohazardous agents. 4b. Gloves must not be worn outside the laboratory in non-lab areas. 4c. Change gloves when contaminated or integrity compromised. 4d. Do not wash or reuse disposable gloves.				
5. Eye, face and respiratory protection should be used in rooms containing infected animals, as				

determined by risk assessment.				
D. Laboratory Facilities (Secondary Barriers)	Yes	No	N/A	Comments
1. Laboratory doors should be self-closing and have locks.				
2. Labs have hand wash sink.				
3. Lab is easily cleaned. No carpets or rugs.				
4. Lab furniture is sturdy. Spaces accessible for cleaning. Bench top impervious to water and resistant to chemicals. Chairs used in laboratory covered with a non-porous material.				
5. Windows that open to exterior not recommended. If they do open to the exterior, must be fitted with fly screens.				
6. BSCs located away from doors, heavily traveled areas, etc, to maintain airflow.				
7. Vacuum lines protected by disinfectant traps or HEPA filters or equivalent.				
8. Eyewash readily available.				
9. Recommended inward flow of air without recirculation to spaces outside of the lab.				
10. Air from class II BSC can be re-circulated into the lab if cabinet tested and recertified annually or can also be connected to lab exhaust system by either thimble (canopy) or direct (hard) connection.				
11. Illumination is adequate, avoiding glares and reflections that could impede vision.				
E. Toxin Use Items	Yes	No	N/A	Comments
1. Laboratory personnel are trained use of the				

specific toxin.				
2. Toxin specific Chemical Hygiene Plan must be available in the lab.				
3. Inventory System in place to account for toxin use and disposition.				
4. Toxins are stored in locked access box.				
5. When toxins are in use, the room must be clearly posted "Toxins in Use—Authorized Personnel Only." Unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used.				
6. A certified BSC, chemical fume hood or glove box is available for manipulation of the dry toxin.				
F. Institutional Biosafety Committee	Yes	No	N/A	Comments
1. IBC review and approval of agent(s).				
2. Changes/modifications reported to IBC.				
3. USF Biosafety training for all staff in date.				
4. If Shipping biohazardous agents, appropriate DOT/IATA training completed and applicable permits in place.				
5. Reviewed lab spaces to determine that they are complete and accurate.				

Other Comments:

Date _____ Signature of Inspector_____

Date _____ Reviewed by _____Title _____

Biosafety Level 3 Laboratory

Biosafety Level 3				
A. Standard Microbiological Practices	**Yes**	**No**	**N/A**	**Comments**
1. Institutional policies being enforced that control access to the laboratory.				
2. Persons wash hands after work w/cultures & removing gloves, before leaving lab.				
3. Eating, drinking, storing food, etc. prohibited.				
4. Mouth pipetting prohibited; pipettors used.				
5a. Sharps policies in place. 5b. Sharps disposed in biohazardous Sharps containers. 5c. Broken glassware is only handled by mechanical means. 5d. Plastic ware is substituted for glassware whenever possible. 5e. Disposable needles are not bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated prior to disposal. 5f. Syringes that "re-sheath" the needle or needleless systems are used when appropriate.				
6. Splashes & aerosols are minimized.				

7a. Work surfaces disinfected after completion of work and after any spill, disinfectants effective. 7b. Biohazard spill cleanup kit available.				
8. Waste decontaminated and disposed in effective manner. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory suite (e.g., autoclave, chemical disinfection)				
9. A biohazard sign, PI/Emergency contact information, biosafety level, and required procedures for entering and exiting the laboratory are posted on entry doors to lab.				
10. An effective integrated pest management program is required.				
11a. PI ensures personnel receive appropriate training. 11b. Personnel must receive annual updates and additional training when procedural or policy changes occur. 11c. All laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. 11d. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.				
B. Special Practices:	**Yes**	**No**	**N/A**	**Comments**
1. Laboratory staff is advised of potential hazards.				
2. Laboratory staff is provided medical surveillance and appropriate immunizations if applicable.				
3. Policy in place regarding baseline serum for at risk personnel, as appropriate.				
4a. A laboratory-specific biosafety manual must be prepared and adopted as policy.				

4b. The biosafety manual must be available and accessible.				
5. The PI must ensure that laboratory staff demonstrates proficiency in standard and special microbiological practices prior to work with BSL-3 agents.				
6. Infectious agents must be placed in a durable, leak proof container during collection, handling, storage and transport.				
7a. Laboratory equipment decontaminated routinely, after spills, before repair, maintenance, or removal from lab. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.				
8a. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to polices. 8b. All such incidents must be reported to the laboratory supervisor and biosafety officer. 8c. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.				
9. Animals & plants not involved in work not permitted in lab.				
10a. Open manipulation w/ agents in BSC and or other containment devices. No work with agents on open bench. 10b. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.				
11. Equipment and storage areas for use with biohazard are properly labeled. Agents are properly labeled.				
C. Safety Equipment (Primary Barriers)	**Yes**	**No**	**N/A**	**Comments**
1. All procedures conducted in BioSafety Cabinet (BSC). BSC is certified annually.				
2a. Protective laboratory clothing with a solid-front such				

as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory 2b. Lab coats/gowns worn & not removed from lab. 2c. Reusable clothing decontaminated before laundering. 2d. Clothing is changed when contaminated 2e. Gloves worn when handling agents, animals or equipment.				
3a. Eye and Face protection is used for anticipated splashes or sprays of infectious agent. 3b.Eye wear must be disposed when contaminate with other lab waste or decontaminated prior to reuse				
4a. Gloves worn when handling agent, animals, or equipment. Change gloves frequently, accompanied by handwashing. 4b. Gloves must not be worn outside the laboratory in non-lab areas. 4c. Change gloves when contaminated or integrity compromised. 4d. Do not wash or reuse disposable gloves. 4e. BSL-3 laboratory workers should: Wear two pairs of gloves when appropriate.				
5. Eye, face and respiratory protection should be used in rooms containing infected animals.				
D. Laboratory Facilities (Secondary Barriers)	**Yes**	**No**	**N/A**	**Comments**
1a. Laboratory doors must be self closing and have locks in accordance with the institutional policies. 1b. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. 1c. Access to the laboratory is restricted to entry by a series of two self-closing doors. 1d. A clothing change room (anteroom) may be included				

in the passageway between the two self-closing doors.				
2. Each lab room contains hand-free handwashing sink located near exit door.				
3a. Lab must be designed so that it can be cleaned and decontaminated. 3b. Carpet and rugs not permitted. Surfaces cleanable (walls, floors, ceiling). 3c. Seams & penetrations sealed. 3d. Walls and ceiling sealed smooth finish for easy cleaning and decontamination. 3e. Floor slip resistant. 3f. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.				
4a. Lab furniture is appropriate for loading and use. 4b. Spaces between cabinet, benches and equipment accessible for cleaning. 4c. Benchtops impervious to water and resistant to chemicals. 4d. Chairs used in laboratory covered with a non-porous material.				
5. All windows in the laboratory must be sealed.				
6. BSCs located away from doors, heavily traveled areas, etc, to maintain airflow pattern.				
7a. Vacuum lines protected by HEPA filters or equivalent. 7b. Filters must be replaced as necessary				
8. Eyewash readily available inside lab.				
9a. A ducted air ventilation system is required. Negative pressure airflow into laboratory. Under failure conditions the airflow will not be reversed. The laboratory exhaust air must not re-circulate to any other				

area of the building. The laboratory building exhaust air should be dispersed away from occupied areas or exhaust must be HEPA filter. 9b. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. 9c. Audible alarms should be considered to notify personnel of air flow disruption				
10a. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy)connection or a direct (hard) connection. 10b. Provisions to assure proper safety cabinet performance and air system operation must be verified 10c. BSCs should be certified at least annually to assure correct performance.				
11. A method of decontaminating all laboratory waste should be available preferably within the laboratory. If contaminated waste leave lab, they are sealed & not transported in public corridor. Large pieces of equipment should be decontaminated before removal from the laboratory.				
12. Aerosol producing equipment (e.g., continuous flow centrifuges) are contained in devices that exhaust through HEPA filters. These HEPA filters should be tested annually.				
13a. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory. 13 b. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out				

capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (With appropriate decontamination procedures) capability. 13c. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually				
14. BSL3 facility & operational procedures documented. Facility tested for verification prior to operation. Facilities re-verified, at least annually against these procedures.				
15. Illumination is adequate, avoiding glares and reflections that could impede vision.				
16. Autoclaving procedures verified. If yes, explain how.				
E. Institutional Biosafety Committee	**Yes**	**No**	**N/A**	**Comments**
1. IBC review and approval of agent(s).				
2. Changes/modifications reported to IBC.				
3. USF Biosafety training for all staff in date.				
4. If Shipping biohazardous agents, appropriate DOT/IATA training completed and applicable permits in place.				

APPENDIX II - CDC/USDA Select Agents

Except for exclusions listed in Appendix II, the viruses, bacteria, fungi, toxins, genetic elements, recombinant nucleic acids, and recombinant organisms specified in this list are Department of Health and Human Services (HHS) Select Agents and Toxins, United States Department of Agriculture (USDA) High Consequence Livestock Pathogens, or HHS/USDA Overlap Agents. Animal and Plant Heath Inspection Service (APHIS) Regulated Plant Pathogens are listed in Appendix III.

HHS SELECT AGENTS AND TOXINS

- Abrin
- Botulinum neurotoxins*
- Botulinum neurotoxin producing species of Clostridium*
- Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
- *Coxiella burnetii*
- Crimean-Congo haemorrhagic fever virus
- Diacetoxyscirpenol
- Eastern Equine Encephalitis virus[1]
- Ebola virus*
- *Francisella tularensis**
- Lassa fever virus
- Lujo virus
- Marburg virus*
- Monkeypox virus[1]
- Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
- Ricin
- Rickettsia prowazekii
- SARS-associated coronavirus (SARS-CoV)
- Saxitoxin
- South American Haemorrhagic Fever viruses:
    - Chapare
    - Guanarito
    - Junin
    - Machupo
    - Sabia
- Staphylococcal enterotoxins A,B,C,D,E subtypes
- T-2 toxin
- Tetrodotoxin
- Tick-borne encephalitis complex (flavi) viruses:
    - Far Eastern subtype

  - Siberian subtype
- Kyasanur Forest disease virus
- Omsk hemorrhagic fever virus
- Variola major virus (Smallpox virus)*
- Variola minor virus (Alastrim)*
- *Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

- *Bacillus anthracis **
- *Bacillus anthracis Pasteur strain*
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei**
- *Burkholderia pseudomallei**
- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus[1]

USDA SELECT AGENTS AND TOXINS

- African horse sickness virus
- African swine fever virus
- Avian influenza virus[1]
- Classical swine fever virus
- Foot-and-mouth disease virus*
- Goat pox virus
- Lumpy skin disease virus
- *Mycoplasma capricolum[1]*
- *Mycoplasma mycoides[1]*
- Newcastle disease virus[1,2]
- Peste des petits ruminants virus
- Rinderpest virus*
- Sheep pox virus
- Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

- *Peronosclerospora philippinensis (Peronosclerospora*
- *sacchari)*
- *Phoma glycinicola (formerly Pyrenochaeta glycines)*
- *Ralstonia solanacearum*
- *Rathayibacter toxicus*
- *Sclerophthora rayssiae*
- *Synchytrium endobioticum*

- *Xanthomonas oryzae*

*Denotes Tier 1 Agent

[1]Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus , west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, and Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3, provided that the individual or entity can verify that the agent is within the exclusion category.

[2] A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

Genetic Elements, Recombinant and/or Synthetic Nucleic Acids, and Recombinant and/or Synthetic Organisms:

1. Nucleic acids that can produce infectious forms of any of the select agent viruses listed.
2. Recombinant and/or Synthetic nucleic acids that encode for the functional form(s) of any of the toxins listed if the nucleic acids:
    a. Can be expressed *in vivo* or *in vitro,* or
    b. Are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*.
3. Select Agents or toxins which have been genetically modified.

Other Restrictions

- Experiments that involve the deliberate transfer of, or selection for, a drug resistance trait to select agents that are not known to acquire the trait naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture.
- Experiments involving the deliberate formation of synthetic or recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] < 100 ng/kg body weight.

Exclusions

1. Any select agent or toxin that is in its naturally occurring environment provided the select agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
2. Non-viable select agents or nonfunctional select toxins.

3. Low pathogenic strains of avian influenza virus.
4. Any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus.
5. All subspecies of Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia) and all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia).
6. South America genotypes of Eastern Equine Encephalitis virus (EEEV).
7. Any subtypes of Venezuelan Equine Encephalitis virus (VEEV) except for subtypes IAB or IC.
8. The West African clade of Monkeypox virus.
9. Genetic elements or sub-units of agents or toxins, if the genetic elements or sub-units are not capable of causing disease.
10. The medical use of toxins for patient treatment.
11. The following toxins (in the purified form or in combinations of pure and impure forms) if the aggregate amount under the control of a principal investigator does not, at any time, exceed the amount specified:
    - 100 mg of Abrin
    - 0.5 mg of Botulinum neurotoxins
    - 100 mg of Short, paralytic alpha Conotoxins
    - 1,000 mg of Diacetoxyscirpenol
    - 100 mg of Ricin
    - 100 mg of Saxitoxin
    - 5 mg of Staphylococcal enterotoxins (subtypes A, B, C, D and E)
    - 100 mg of Tetrodotoxin
    - 1,000 mg of T-2 toxin

The administrator may exclude from this list attenuated strains of HHS Select Agents or Toxins upon a determination that they do not pose a severe threat to the public health and safety.

Please check the CDC Website for latest updates regarding Sect Agents and Biological Toxins at http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html
.

APPENDIX III - APHIS Plant Pathogens

Animal and Plant Health Inspection Service (APHIS) Regulated Plant Pathogens

- Peronosclerospora philippinensis (Peronosclerospora sacchari)
- Phoma glycinicola (formerly Pyrenochaeta glycines)
- Sclerophthora rayssiae
- Ralstonia solanacearum,
- Rathayibacter toxicus
- Synchytrium endobioticum
- Xanthomonas oryzae

APPENDIX IV - Key Word & Terms

(Adapted from OSP, CDC, and other sources)

- **Biological Hazards**: Any bacteria, viruses, rickettsia, parasites, prions, fungi, toxins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA), including any recombinant DNA or RNA, known to be, or suspected of being, hazardous to human, plants, and animals.

- **Institutional Biosafety Officer (IBO)**: An individual appointed by the University to oversee management of Biosafety risks and implementation of the Biosafety Program. The Institutional Biological Safety Officer, a full-time position within the University of South Florida, Research Integrity & Compliance, is a member of the IBC and serves as the Executive Secretary of the IBC.

- **Biosafety Level (BSL)**: A description of the degree of physical containment required to be employed to confine biohazardous organisms, including those containing recombinant DNA molecules, to reduce the potential for exposure of laboratory workers, persons outside of the laboratory, and the environment. Biosafety levels are graded from BSL-1 (the least stringent) to BSL-4 (the most stringent).

- **BMBL**: Common abbreviated term for CDC/NIH publication: *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2007*

- **CDC**: The Department of Health and Humans Services' Centers for Disease Control and Prevention.

- **DHHS**: U.S. Department of Health and Human Services

- **Institutional Biosafety Committee (IBC)**: A University committee created consistent with NIH Guidelines to review research involving recombinant DNA, Gene Therapy, and other research that entails biohazard risks (infectious agents, biological toxins, Select Agents).

- **NIH**: National Institutes of Health. The NIH is one of several health agencies within the Public Health Service, which is an agency within the U.S. Department of Health and Human Services (DHHS).

- ***NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)***: The *NIH Guidelines* outline principles for the safe conduct of research employing recombinant DNA technology. The *NIH Guidelines* detail practices and procedures for the containment of various forms of recombinant DNA research, for the proper conduct of research involving genetically modified plants and animals, and for the safe conduct of human gene transfer research. Originally created in 1976, the *NIH Guidelines* are a "living document" that is periodically revised to keep pace with the changing state of science**.** Although not regulatory by definition,

compliance with the *NIH Guidelines* is mandatory for investigators at USF due to NIH funding of research at USF. Failure to follow the *NIH Guidelines* by one investigator can lead to suspension or termination of NIH funding for all NIH sponsored programs at USF.

- **Office of Science Policy (OSP)**: The NIH office responsible for developing, implementing, and monitoring NIH policies and procedures for the safe conduct of recombinant DNA activities, including human gene transfer (gene therapy).

- **Recombinant DNA Advisory Committee (RAC)**: An NIH advisory committee whose principal role is to provide advice and recommendations to the NIH Director on (1) the conduct and oversight of research involving recombinant DNA, including the content and implementation of the *NIH Guidelines*, and (2) other NIH activities pertinent to recombinant DNA technology. A major element of this role is to examine the science, safety, and ethics of clinical trials that involve the transfer of recombinant DNA to humans.

- **Recombinant DNA guidelines**: See *NIH Guidelines for Research Involving Recombinant DNA Molecules*.

- **Recombinant DNA molecules**: Under the current *NIH Guidelines*, these are molecules constructed outside of living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or molecules that result from their replication. Refer to the *NIH Guidelines*, at the following website, for further details: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

- **Responsible Facility Official (RFO)**: The Responsible Facility Official (RFO) is the University position that is authorized to transfer and receive Select Agents on behalf of the University researchers. The RFO has been designated as the primary contact for compliance with the Select Agent Rule, including the registration of Select Agents with the CDC. The Vice President for Research is designated as the RFO at USF and the Institutional Biosafety Officer as the alternate RFO.

- **Restricted Person**: As defined in the USA PATRIOT Act, an individual that meets any one of the following criteria:
  1) A person who is under indictment for a crime punishable by imprisonment for a term exceeding 1 year;
  2) A person who has been convicted in any court of a crime punishable by imprisonment for a term exceeding 1 year;
  3) A person who is fugitive from justice;
  4) A person who is an unlawful user of any controlled substance;
  5) A person who is an alien illegally or unlawfully in the United States;
  6) A person who has been adjudicated as a mental defective or has been committed to any mental institution;
  7) A person who is an alien (other than an alien lawfully admitted for permanent residence) who is a national of a country as to which the Secretary of State has

made a determination (that remains in effect) that such country has repeatedly provided support for acts of international terrorism; currently these countries are:
   a. Cuba
   b. Iran
   c. Syria
   d. Sudan
8) A person who has been discharged from the Armed Services of the United States under dishonorable conditions.

- **Risk Groups (RG)**: Categories of biological agents based on their relative pathogenicity for healthy adult humans, as defined in the *NIH Guidelines*, that are used in making risk assessments, according to the following criteria:

  - Risk Group 1 (RG-1) agents are not associated with disease in healthy adult humans.
  - Risk Group 2 (RG-2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.
  - Risk Group 3 (RG-3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available.
  - Risk Group 4 (RG-4) agents are likely to cause serious lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Refer to *NIH Guidelines*, available at the following website, for further details: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html

- **Select Agent**: Any one of a number of microorganisms (bacterium, viruses, fungi, and rickettsia) or toxins listed in Appendix II of this document or at 42 CFR 73. The term also includes any genetically modified microorganisms or genetic elements from the listed organisms that are shown to produce or encode for a factor associated with a disease, and genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the listed toxins. Anticipated use of any Select Agents involving importation to the University of South Florida, or exportation from the University of South Florida requires registration with the CDC in advance.

- **Select Agent Rule**: Regulations 42 CFR 73 that became effective on February 7, 2003 as a result of federal legislation, delineating a number of Select Agents (see "Select Agents" above) that are of potential use to terrorists, and which must be registered with the CDC prior to importation to the University or exportation from the University. Noncompliance with the Select Agent Rule can result in loss of NIH funding, as well as civil penalties. *IMPORTANT! See the following website for important details that may be applicable to your research:* http://www.selectagents.gov/index.html

- **Vector**: An organism, such as an insect, that can carry a pathogen (disease-producing organism) from one host to another.

- **Vegetative form:** In bacteria, a stage of active growth, as opposed to a resting state or spore formation.
- **Viable**: Able to grow and multiply.
- **Virucide**: An agent that destroys or inactivates viruses.
- **Virus**: A microorganism, ranging in size from .01 to .25 microns (10 - 250 nanometers), that can reproduce only within living cells.
- **Virulence**: The disease-producing power of a microorganism.
- **Zoonosis**: A disease that can be transmitted from animals to humans.

APPENDIX V - Summary of Chemical Disinfectants

Disinfectant	Use Parameters	Effective Against*					Important Characteristics	Potential Application
		Vege-tative cells	Lipophilic viruses	Tubercle bacilli	Hydrophilic viruses	Bacterial Spores		
Alcohol (ethyl, isopropyl)	conc.: 70-85% contact time: 10-30 min.	+	+	+	±		eye irritant, toxic, flammable, inactivated by organic matter	surfaces - work & equipment
Chlorine Compounds	conc.: 0.05-0.5% (commercial bleach ≈ 5%) contact time: 10-30 min.	+	+	+	+	±	may leave residue; corrosive; skin, eye & respiratory irritant; inactivated by organic matter; makeup at least weekly	spills, equipment surfaces, instruments, glassware, water baths
Quaternary Ammonium Compounds	conc.: 0.1-2% contact time: 10-30 min.	+	+				Toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, floor maintenance, glassware, instruments
Phenolic Compounds	conc.: 0.2-3% contact time: 10-30 min.	+	+	+	±		leaves residue; corrosive; skin, eye & respiratory irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, floors, spills, glassware, instruments, water baths
Iodophor Compounds	conc.: 0.47% contact time: 10-30 min.	+	+	+	±		leaves residue; corrosive; skin & eye irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, glassware, water baths
Formaldehyde (Formalin)**	conc.: 4-8% contact time: 10-30 min.	+	+	+	+	±	leaves residue; skin, eye & respiratory irritant; toxic (carcinogen)	less effective than other disinfectants but can be used for equipment surfaces, glassware, instruments
Glutaraldehyde	conc.: 2% contact time: 10-600 min.	+	+	+	+	+	leaves residue; skin, eye & respiratory irritant; toxic	equipment surfaces, glassware, instruments

From: *Laboratory Safety: Principles and Practices*, second edition, Diane O. Fleming, John H. Richardson, Jerry J. Tulis, and Donald Vesley, eds., American Society for Microbiology, Washington, D. C.

* + = very positive response, ± = less positive response. A blank denotes a negative response or not applicable.

**due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

APPENDIX VI - Biological Toxin Inactivation

Consult the table below and call the Institutional Biosafety Officer at 974-0954 with questions.

Toxin	Autoclave	2.5% NaOCl	2.5% NaOCl + 0.25N NaOH
Abrin <100mg/PI*	Yes	No	No
Botulinum neurotoxins <0.5mg/PI*	Yes	Yes	Yes
Clostridium perfringenes epsilon toxin <100mg/PI*	Yes	Yes	Yes
Diacetoxyscirpenol (DAS), T-2 <1000mg/PI*	No	No	Yes (4 hour exposure required)
Palytoxin	No	Yes	Yes
Ricin <100mg/PI*	Yes	Yes	Yes
Saxitoxin <100mg/PI*	No	Yes	Yes
Shigatoxin & Shiga-like ribosome inactivating proteins <100mg/PI*	Yes	Yes	Yes
Staphylococcal enterotoxins <5mg/PI*	Yes	Yes	Yes
Tetrodotoxin 1000mg/PI*	No	No	Yes

*toxin amounts permissible per Principal Investigator

Chemical Destruction of Biological Toxins:
When using sodium hypochlorite or sodium hypochlorite + sodium hydroxide to destroy toxins, work in a fume hood or a biological safety cabinet and wear a long sleeved lab coat or gown, gloves and eye protection.

1. Place plastic backed absorbent paper (bench diaper) on the work surface
2. If not already in liquid form, put the toxin into solution.
3. Place the toxin container in a secondary container, such as a beaker or rack.
4. Add an equal volume of the sodium hypochlorite(or sodium hypochlorite + sodium hydroxide) to the primary container of toxin solution

5. Do not replace the cap on primary container.
6. Place a "WARNING / DO NOT USE" sign on the hood/cabinet.
7. Allow a minimum 60 minutes exposure time. (See table for additional exposure time recommendations.)
8. Secure the cap on the primary container. Double bag the material in zip-lock plastic bags and label it "Inactivated/denatured (TOXIN NAME)."
9. Complete a waste slip listing the toxin as "inactivated" and contact EH&S for disposal as hazardous waste.

Steam Sterilization (Autoclaving) of Toxins:

Work in a fume hood or a biological safety cabinet and wear a long sleeved lab coat or gown, gloves and eye protection.

1. Loosen the cap of the primary toxin container to allow steam penetration.
2. Place the primary container into a secondary disposable container (e.g.: small sharps container).
3. Place the sharps container in a loosely closed autoclave bag.
4 Place the bag in an autoclavable pan.
5. Autoclave at 121° C for 1 hour on liquid cycle (slow exhaust).
6. Discard the biohazard bag with its contents in biohazardous waste stream.

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APPENDIX VII - Standard Precautions

Excerpted from the OSHA Standard 29 CFR 1910.1030(d):
http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

Methods of compliance-
(1) General-Standard precautions shall be observed to prevent contact with blood or other potentially infectious materials. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

(2) Engineering and work practice controls.

(i) Engineering and work practice controls shall be used to eliminate or minimize employee exposure. Where occupational exposure remains after institution of these controls, personal protective equipment shall also be used.

(ii) Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness.

(iii) Employers shall provide handwashing facilities which are readily accessible to employees

(iv) When provision of handwashing facilities is not feasible, the employer shall provide either an appropriate antiseptic hand cleanser in conjunction with clean cloth/paper towels or antiseptic towelettes. When antiseptic hand cleansers or towelettes are used, hands shall be washed with soap and running water as soon as feasible.

(v) Employers shall ensure that employees wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment.

(vi) Employers shall ensure that employees wash hands and any other skin with soap and water, or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.

(vii) Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed except as noted in paragraphs (d)(2)(vii)(A) and (d)(2)(vii)(B) below. Shearing or breaking of contaminated needles is prohibited.

(A) Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical or dental procedure.

(B) Such bending, recapping, or needle removal must be accomplished through the use of a mechanical device or a one-handed technique.

(viii) Immediately or as soon as possible after use, contaminated reusable sharps shall be placed in appropriate containers until properly reprocessed. These containers shall be:
(A) Puncture resistant;
(B) Labeled or color-coded in accordance with this standard;

(C) Leak proof on the sides and bottom; and

(D) In accordance with the requirements set forth in paragraph (d)(4)(ii)(E) for reusable sharps.

(ix) Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in work areas where there is a reasonable likelihood of occupational exposure.

(x) Food and drink shall not be kept in refrigerators, freezers, shelves, cabinets or on countertops or benchtops where blood or other potentially infectious materials are present.

(xi) All procedures involving blood or other potentially infectious materials shall be performed in such a manner as to minimize splashing, spraying, spattering, and generation of droplets of these substances.

(xii) Mouth pipetting/suctioning of blood or other potentially infectious materials is prohibited.

(xiii) Specimens of blood or other potentially infectious materials shall be placed in a container which prevents leakage during collection, handling, processing, storage, transport, or shipping.

(A) The container for storage, transport, or shipping shall be labeled or color-coded according to paragraph (g)(1)(i) and closed prior to being stored, transported, or shipped. When a facility utilizes Universal Precautions in the handling of all specimens, the labeling/color-coding of specimens is not necessary provided containers are recognizable as containing specimens. This exemption only applies while such specimens/containers remain within the facility. Labeling or color-coding in accordance with paragraph (g)(1)(i) is required when such specimens/containers leave the facility.

(B) If outside contamination of the primary container occurs, the primary container shall be placed within a second container which prevents leakage during handling, processing, storage, transport, or shipping and is labeled or color-coded according to the requirements of this standard.

(C) If the specimen could puncture the primary container, the primary container shall be placed within a secondary container which is puncture-resistant in addition to the above characteristics.

(xiv) Equipment which may become contaminated with blood or other potentially infectious materials shall be examined prior to servicing or shipping and shall be decontaminated as necessary, unless the employer can demonstrate that decontamination of such equipment or portions of such equipment is not feasible.

(A) A readily observable label in accordance with paragraph (g)(1)(i)(H) shall be attached to the equipment stating which portions remain contaminated.

(B) The employer shall ensure that this information is conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, and prior to handling, servicing, or shipping so that appropriate precautions will be taken.

(3) Personal protective equipment-

(i) Provision. When there is occupational exposure, the employer shall provide, at no cost to the employee, appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection, and mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Personal protective equipment will be considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

(ii) Use. The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that in the specific instance its use would have prevented the delivery of health care or public safety services or would have posed an increased hazard to the safety of the worker or co-worker. When the employee makes this judgment, the circumstances shall be investigated and documented in order to determine whether changes can be instituted to prevent such occurrences in the future.

(iii) Accessibility. The employer shall ensure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the worksite or is issued to employees. Hypoallergenic gloves, glove liners, powderless gloves, or other similar alternatives shall be readily accessible to those employees who are allergic to the gloves normally provided.

(iv) Cleaning, Laundering, and Disposal. The employer shall clean, launder, and dispose of personal protective equipment required by paragraphs (d) and (e) of this standard, at no cost to the employee.

(v) Repair and Replacement. The employer shall repair or replace personal protective equipment as needed to maintain its effectiveness, at no cost to the employee.

(vi) If a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.

(vii) All personal protective equipment shall be removed prior to leaving the work area.

(viii) When personal protective equipment is removed it shall be placed in an appropriately designated area or container for storage, washing, decontamination, or disposal.

(ix) Gloves. Gloves shall be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin; when performing vascular access procedures except as specified in paragraph (d)(3)(ix)(D); and when handling or touching contaminated items or surfaces.

(A) Disposable (single use) gloves, such as surgical or examination gloves shall be replaced as soon as practical when contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

(B) Disposable (single use) gloves shall not be washed or decontaminated for re-use.

(C) Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibits other signs of deterioration or when their ability to function as a barrier is compromised.

(x) Masks, Eye Protection, and Face Shields. Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose, or mouth contamination can be reasonably anticipated.

(xi) Gowns, Aprons, and Other Protective Body Clothing. Appropriate protective clothing such as, but not limited to, gowns, aprons, lab coats, clinic jackets, or similar outer garments shall be worn in occupational exposure situations. The type and characteristics will depend upon the task and degree of exposure anticipated.

(xii) Surgical caps or hoods and/or shoe covers or boots shall be worn in instances when gross contamination can reasonably be anticipated (e.g., autopsies, orthopedic surgery).

(4) Housekeeping-

(i) General. Employers shall ensure that the worksite is maintained in a clean and sanitary condition. The employer shall determine and implement an appropriate written schedule for cleaning and method of decontamination based upon the location within the facility, type of surface to be cleaned, type of soil present, and tasks or procedures being performed in the area.

(ii) All equipment and environmental and working surfaces shall be cleaned and decontaminated after contact with blood or other potentially infectious materials.

(A) Contaminated work surfaces shall be decontaminated with an appropriate disinfectant after completion of procedures; immediately or as soon as feasible when surfaces are overtly contaminated or after any spill of blood or other potentially infectious materials; and at the end of the work shift if the surface may have become contaminated since the last cleaning.

(B) Protective coverings, such as plastic wrap, aluminum foil, or imperviously-backed absorbent paper used to cover equipment and environmental surfaces, shall be removed and replaced as soon as feasible when they become overtly contaminated or at the end of the work shift if they may have become contaminated during the shift.

(C) All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood for becoming contaminated with blood or other potentially infectious materials shall be inspected and decontaminated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feasible upon visible contamination.

(D) Broken glassware which may be contaminated shall not be picked up directly with the hands. It shall be cleaned up using mechanical means, such as a brush and dust pan, tongs, or forceps.

(E) Reusable sharps that are contaminated with blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed.

(iii) Regulated Waste.

(A) Contaminated Sharps Discarding and Containment. (1) Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are:

(i) Closable;

(ii) Puncture resistant;

(iii) Leak proof on sides and bottom; and

(iv) Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard.

(2) During use, containers for contaminated sharps shall be:

(i) Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., laundries);

(ii) Maintained upright throughout use; and

(iii) Replaced routinely and not be allowed to overfill.

(3) When moving containers of contaminated sharps from the area of use, the containers shall be:

(i) Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping;

(ii) Placed in a secondary container if leakage is possible. The second container shall be:

(A) Closable;

(B) Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and

(C) Labeled or color-coded according to paragraph (g)(1)(i) of this standard.

(4) Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.

(B) Other Regulated Waste Containment. (1) Regulated waste shall be placed in containers which are:

(i) Closable;

(ii) Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

(iii) Labeled or color-coded in accordance with paragraph (g) (1) (i) this standard; and

(iv) Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

(2) If outside contamination of the regulated waste container occurs, it shall be placed in a second container. The second container shall be:

(i) Closable;

(ii) Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

(iii) Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard; and

(iv) Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

(C) Disposal of all regulated waste shall be in accordance with applicable regulations of the United States and Territories, and political subdivisions of States and Territories.

(iv) Laundry.

(A) Contaminated laundry shall be handled as little as possible with a minimum of agitation.

(1) Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.

(2) Contaminated laundry shall be placed and transported in bags or containers labeled or color-coded in accordance with paragraph (g) (1) (i) of this standard. When a facility utilizes Universal Precautions in the handling of all soiled laundry, alternative labeling or color-coding is sufficient if it permits all employees to recognize the containers as requiring compliance with Universal Precautions.

(3) Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through of or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior.

(B) The employer shall ensure that employees who have contact with contaminated laundry wear protective gloves and other appropriate personal protective equipment.

(C) When a facility ships contaminated laundry off-site to a second facility which does not utilize Universal Precautions in the handling of all laundry, the facility generating the contaminated laundry must place such laundry in bags or containers which are labeled or color-coded in accordance with paragraph (g)(1)(i).

(e) HIV and HBV Research Laboratories and Production Facilities. (1) This paragraph applies to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. These requirements apply in addition to the other requirements of the standard.

(2) Research laboratories and production facilities shall meet the following criteria:

(i) Standard microbiological practices. All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

APPENDIX VIII - Regulations regarding shipping of biohazardous materials

The following are regulations that apply to the shipping and transportation of biohazardous materials:

- U.S. Department of Transportation, 49 CFR Parts 171-180 and amendments
- U.S. Public Health Service, 42 CFR Part 72, *Interstate Shipment of Etiologic Agents*
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, *Bloodborne Pathogens*
- U.S. Postal Service, 39 CFR Part 111, *Mailability of Etiologic Agents, Mailability of Sharps and Other Medical Devices, and Publication 52, Acceptance of Hazardous, Restricted or Perishable Matter*
- International Air Transport Association (IATA), *Dangerous Goods Regulations*
- International Civil Aviation Organization (ICAO), *Technical Instructions for the Safe Transport of Dangerous Goods by Air*
- United Nations (UN), *Recommendations of the Committee of Experts on the Transportation of Dangerous Goods*

APPENDIX IX - References

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