



Biosafety Manual

Laboratory Safety Policies and Procedures

Environment, Health and Safety Office
AdventHealth University
671 Winyah Drive
Orlando, FL 32803
Website: www.ahu.edu

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List of Abbreviations

- AHU = AdventHealth University
- APHIS = Animal and Plant Health Inspection Service, USDA
- BMBL = Biosafety in Microbiological and Biomedical Laboratories, 5th edition.
- BRS = Biotechnology Regulatory Service, USDA
- BSC = biological safety cabinet

- BSL = biosafety level
- CDC = Centers for Disease Control and Prevention
- CFU = colony forming units
- DOT = US Department of Transportation
- DPI = Division of Plant Industry of Florida Department of Agriculture and Consumer Services
- EPA = US Environmental Protection Agency
- FDA = US Food and Drug Administration
- FDACS = Florida Department of Agriculture & Consumer Services
- HBV = hepatitis B virus
- HCV = hepatitis C virus
- HEPA = high efficiency particulate air or high efficiency particulate arrestance
- HHS = US Department of Health and Human Services
- HIV = human immunodeficiency virus
- LD = Lethal Dose. LD50 is the amount of a material, given all at once, which causes the death of 50% of a group of test animals. The LD50 is one way to measure the short-term poisoning potential (acute toxicity) of a material.
- EHS = Environment, Health and Safety Office, AHU
- MSDS = material safety data sheets
- NIH = National Institutes of Health
- NIOSH = National Institute for Occupational Safety and Health, CDC
- NSF/ANSI = International standards created by NSF (National Sanitation Foundation) and ANSI (American National Standards Institute)
- OPIM = other potentially infectious materials
- OSHA = Occupational Safety and Health Administration – US Department of Labor
- PAPR = powered air purifying respirators
- PI = Principal Investigator
- PPE = personal protective equipment
- PPQ = Plant Protection and Quarantine Office, APHIS
- PSDS = pathogen safety data sheet
- RAC = Recombinant DNA Advisory Committee, NIH – Office of Science Policy
- RG = risk groups
- SOP = standard operating procedure
- USDA = US Department of Agriculture
- UV = ultraviolet
- WHO = World Health Organization

Biosafety: Fundamentals and Definitions

All research projects at the AdventHealth University (AHU) involving the following must be notified to the Environment, Health and Safety Office (EHS):

- Known human, animal, or plant pathogens or pathogenic material, BSL-2 or greater
- Suspected human or animal pathogens or pathogenic material
- Select agents
- Biological toxins having an LD50 of $\leq 100 \mu\text{g} / \text{kg}$ body weight

- Primary human tumor cells
- Cell lines transformed with a virus
- “Dual Use Research of Concern” experiments
- All projects involving recombinant or synthetic nucleic acids. Projects involving synthetic nucleic acids or organisms or cells that contain synthetic nucleic acids must be informed provided that the synthetic nucleic acid is either a) designed to integrate into DNA b) replication competent or able to replicate in a living cell or c) codes for a vertebrate toxin with an LD50 of <100 nanograms/kilogram.
- Materials that require state/federal permits or field releases of genetically modified organisms that require permits/notifications.

Biohazards

- Infectious or invasive organisms that are potentially harmful to humans, animals, plants or the environment including, but not limited to: bacteria, mycoplasma, viruses, parasites, fungi, algae, and human or non-human primate blood, cells, body fluids and tissues.
- Biological toxins and substances derived or excreted from organisms that are toxic or harmful to humans, animals, plants or the environment.
- Recombinant and synthetic nucleic acids, genetically modified micro-organisms, animals and plants which are not known to occur naturally or that express potentially harmful nucleic acids, such as DNA derived from pathogenic organisms or human oncogenes.

Regulatory Authority

The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (See Appendix A) apply to institutions that receive NIH funding for experiments involving recombinant or synthetic nucleic acid molecules. All recombinant or synthetic nucleic acid molecule projects must be registered with the EHS regardless of funding source. In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

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

The US Department of Health and Human Services (HHS) and/or the US Department of Agriculture (USDA) have designated certain infectious agents and biological toxins as severe threats to human, animal, or plant health. These “Select Agents” are subjected to federal oversight. The registrations of facilities possessing, using and transferring these agents or toxins is required under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 to improve the ability of the United States to prevent, prepare for, and respond to bioterrorism and other public health emergencies.

Federal guidelines are also formulated to advise institutions on Dual Use Research of Concern; biological research with legitimate scientific purpose that may be misused to pose a biologic threat to

public health and/or national security. The National Science Advisory Board for Biosecurity (NSABB) is responsible for developing guidelines and recommendations for research programs that may constitute dual use research of concern.

Biohazard Risk Assessment

Objective

To identify, manage and contain risks in the workplace associated with the biohazardous agent itself and how and where the agent will be used, in order to protect the health of workers, the public, and natural or managed environments. Risk management includes a combination of the following controls:

- Safe work practices
- Safety equipment
- Administrative policies
- Proper facilities

Risk Assessments

The PI is responsible for identifying the hazards associated with the agent and/or procedures, applying the appropriate risk management controls, and advising the staff of both the risks and controls. The following questions should be considered:

- What are the hazardous materials in the laboratory?
- What procedures are hazardous or increase the hazardous nature of the materials?
- What might happen if there was a problem?
- Who/What may be exposed and how?
- How serious are the consequences?
- How likely is it to happen?
- How can this be minimized?

Pre-existing diseases, medications, compromised immunity, pregnancy, or breast-feeding are some of the conditions that may increase the risk of an individual acquiring a laboratory acquired infection. Consultation with a physician knowledgeable in infectious diseases is advisable in these circumstances. The primary factors to consider in risk assessment and selection of precautions fall into the following two categories:

Laboratory-Specific Hazards

- Amount of biohazardous material to be used or stored.
- Concentration of the material.
- Use of equipment or procedures that impart energy to the material resulting in dissemination, aerosolization, splash, or splatter.
- Use of equipment or procedures that can cut, scratch, or puncture skin.
- Proximity of susceptible hosts or environment.
- Agent Specific Hazards
- Capability to infect/cause disease in a susceptible human, animal, or plant host

- Virulence as measured by disease severity.
- The availability of preventive measures and effective treatments for the disease.
- Probable routes of transmission of infection (respiratory, mucous membrane transmission higher risk than parenteral or ingestion routes).
- Infective dose or concentration needed to cause disease.
- Stability in the environment, resistance to disinfectants.
- Host range, species affected (e.g. ecotropic, amphotropic, zoonotic).
- Origin or endemic vs. exotic nature: Non-indigenous agents are of special concern because of their potential to introduce risk of transmission, or spread of human, animal, or plant diseases from foreign countries into the United States. The Centers for Disease Control and Prevention and US Department of Agriculture regulate the import of disease agents, clinical or environmental specimens, and other potentially infectious materials. Some agents are also regulated for interstate movement and for export.

Tools for Risk Assessments

Both the National Institutes of Health (NIH) and the World Health Organization (WHO) describe four general risk groups (RG) that address the risk to both the laboratory worker and the community/environment and are based on:

- An agent's capability to infect and/or cause disease in a susceptible human host
- An agent's virulence as measured by the severity of disease.
- Availability of preventive measures and effective treatments for the disease.
- Route of transmission of the natural disease.

Note that:

- The classification is intended for human pathogens but can also be applied to plant or animal pathogens.
- Risk groups correlate with, but do not equate to, biosafety levels; biosafety levels also take into account lab operations and procedures used.

Human Pathogens

Routes of Transmission

Possible routes of laboratory transmission of human or zoonotic disease agents are:

- Parenteral inoculations with needles or other contaminated sharp objects.
- Spills and splashes onto non-intact skin and mucous membranes.
- Ingestion
- Animal bites and scratches.
- Inhalation of infectious aerosols.

Sharps

Percutaneous injuries are a significant preventable route of infection. Injuries are commonly caused by medical sharps, (i.e. needles, scalpels, and lancets) that are intended to cut or puncture skin, but also occur from other sharp objects (i.e. Pasteur pipettes, broken glass, Cryostat blades, etc.).

Laboratories should substitute sharp items or glassware with less hazardous and/or plastic items whenever possible.

Aerosols

An agent capable of transmitting disease via infectious aerosols requires rigorous controls. Human pathogens of this type are a serious laboratory hazard, both for the person handling the agent and for other laboratory occupants. Note that pathogens not normally transmitted by the aerosol route can be an aerosol hazard when procedures that generate aerosols are used. The infective dose needed to cause disease and agent stability is particularly important in establishing the risk of airborne transmission of disease.

The following laboratory procedures can produce aerosols:

- Pipetting or pouring
- Electroporation
- Homogenization or blending
- Popping off tube caps
- Sonication
- Vortexing
- Loading or injecting syringes
- Intranasal/Intratracheal inoculation of animals
- Flame sterilizing tools
- Flow Cytometry
- Centrifugation

Note that in laboratories where large volumes or high concentrations of these biohazards are used, the risk of transmission increases.

Infectious Disease

Agents will vary in their infectious dose, i.e. the amount of agent needed to cause disease:

<u>Agent</u>	<u>Infectious Dose (CFU)</u>
Cryptosporidium	< 10
Mycobacterium tuberculosis	10
Listeria monocytogenes	<1,000
Salmonella typhi	100,000
Vibrio cholera	1,000,000
Rotavirus	10-100 i.u.
Botulinum neurotoxin	nanograms

A risk assessment, as described previously, will determine this information as well as an agent's stability in the environment, pathogenicity, virulence, routes of transmission, and availability of treatments or vaccines. Consider the impact that your work with these agents could have on the health of immunocompromised persons, especially if you work in a hospital environment or with patients.

Agent Origin

The origin of the agent is also important in risk assessment. Previously eradicated or foreign disease agents can be particularly hazardous to an immunologically naive population. Such agents will typically require a minimum of BSL-3 containment (not allowed in AHU laboratories). Agents ordered from colleagues/collaborators or even commercial sources may be contaminated with unexpected pathogens or have altered characteristics. Safety testing under more rigorous containment or with special practices may be prudent with uncharacterized material.

Host Factors

Host factors that can affect the degree of individual susceptibility to pathogens, or the severity of their disease are:

- Age
- Immune competence
- Medication
- Nutritional status
- Pregnancy
- Metabolic disorders
- Malignancy
- Vaccination status

Persons having an increased risk for exposure should discuss these risks with an Occupational Health Care provider. Some pathogens are especially hazardous to pregnant women (e.g. *Listeria monocytogenes*, *Toxoplasma gondii*, lymphocytic choriomeningitis virus) because they can cause miscarriage and birth defects. Pregnant women and women of childbearing age should discuss their exposure to infectious materials with an Occupational Health Care provider.

Containment

Four biosafety levels, BSL-1, 2, 3, 4, are described for activities involving biohazards. The levels are designated in ascending order by degree of protection provided to personnel, the environment and the community. These four levels describe the combinations of practices, safety equipment, administrative controls, and laboratory facility design/features required.

The risk assessment will also determine the appropriate biosafety level for the laboratory and any additional work-practices to be used. For example, a risk assessment may assign a project at BSL2+ indicating that the work is to be performed in a BSL2 laboratory with additional work-practices that are often utilized at a higher biosafety level (e.g. BSL3). See Control of Biohazards section for more detailed information on biological safety levels.

Plant Pathogens

State and Federal Regulations

The movement, use, possession, or release of exotic or potentially harmful plant-associated arthropods, biological control agents, plant pests, plant pathogens, noxious weeds, and invasive plants are regulated by the State of Florida as well as the USDA APHIS Plant Protection and Quarantine

Office (PPQ). The use of these regulated materials will require a Biological Agent registration with the Environment, Health and Safety Office in addition to the appropriate state or federal permits.

Note that some plant pathogens are controlled for export and regulated as select agents. The current list can be found at National Select Agency Registry Website. There are additional plant pathogens not on the select agent list that are also regulated for export.

Plant research involving noxious weeds, invasive plants, and certain plant pests, plant-associated microbes, and plant diseases (such as citrus canker) are regulated by the Florida Dept. of Agriculture & Consumer Services, especially when the import, export, or transfer of these materials is required. Contact the Division of Plant Industry (DPI of Florida Department of Agriculture and Consumer Services) for rules and regulations.

The USDA-APHIS also regulates plant pests, plants and plant products and the movement, importation, and field release of genetically-engineered plants and arthropods. See the detailed APHIS website for information, applications, permits, and notification documents. All field releases require EHS approval in addition to Federal and State approval. The EHS will request current versions of field release approvals as part of recombinant or synthetic nucleic acid molecules or field release project approval or continuation. In addition to the USDA APHIS, State of Florida Division of Plant Industry, USDA Biotechnology Regulatory Service (BRS), FDA (for genetically altered food crops), and EPA (for genetically modified organisms with pest control activity) may also regulate research with transgenic plants and plant-associated organisms.

Containment/Handling Practices for Regulated Experiments with Plants and Plant-Associated Organisms

Physical and biological containment requirements for laboratories, growth chambers and greenhouses are outlined in State (FDACS) or Federal (BRS or PPQ) transport/possession permits (see below) and in the NIH Guidelines Appendix P; these facilities will be inspected by the EHS as part of recombinant or synthetic nucleic acid molecules project approval and/or continuation. A risk assessment should be conducted to determine the level of containment/handling practices that are required. The risk assessment process considers:

- Specific organism(s) under study
- Geographic, ecological, and agricultural environment surrounding the study site
- Physical/mechanical barriers available, and
- Scientifically accepted culture techniques

All genetically-altered plants and plant-related organisms that will be grown or released outside need prior federal approval. Although it emphasizes containment principles for transgenic with Transgenic Information Systems for Biotechnology, is an excellent resource for plant biocontainment. Care must be taken to:

- Avoid the unintentional transfer of plant genes, recombinant or otherwise, to other plants
- Minimize unanticipated, harmful effects to organisms or the environment outside the experimental site/facility
- Avoid the inadvertent spread of pathogens or noxious weeds to crops or native vegetation
- Prevent the introduction of unwanted exotic organisms into a new habitat

Containment can come from physical or biological means. Examples of physical containment are the use of plant growth chambers or greenhouses, or catch trays under plants to prevent soil contamination. Examples of biological containment include the removal or inactivation of plant reproductive structures (pollen and seed), timing of experiments so that plant-associated microorganism(s) under study are not viable in the outside environment, and the exclusion of vectors or fomites that spread plant pathogens. As plant research usually does not pose a human health hazard, biosafety principles are designed instead to protect the natural and agricultural environment. Four biosafety level designations and associated safety practices for plant research exist: BL1-P, BL2-P, BL3-P, and BL4-P.

Plant-Related Recombinant or Synthetic Nucleic Acid Molecule Research

Research with genetically engineered plants, genetically engineered plant-associated microbes, and genetically engineered plant-associated macroorganisms (arthropods and nematodes) is covered by the NIH Guidelines Appendix P. Appendix P supersedes Appendix G (Physical Containment, laboratories) when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G for laboratory conditions. These guidelines are in place to prevent the accidental transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome (either nuclear or organelle genetic material) or the release of recombinant or synthetic nucleic acid-derived organisms associated with plants into the environment. All recombinant or synthetic nucleic acid research, including that with plants and plant-related organisms must be registered with the Environment, Health and Safety Office (see section on recombinant and synthetic nucleic acids)

Select Agents

In recent years, federal legislation regulating the possession, use, and transfer of agents with high adverse public health and/or agricultural consequences (HHS/CDC and USDA Select Agents), place much greater emphasis on the emerging field of biosecurity. In contrast with biosafety, a field dedicated to the protection of workers and the environment from exposures to infectious materials, the field of biosecurity prevents loss of valuable research materials and limits access to infectious materials by individuals who would use them for harmful purposes. Nevertheless, adequate containment of biological materials is a fundamental program component for both biosafety and biosecurity.

More information provided by the USDA and CDC on select agents is available on the Federal Select Agent Program at <http://www.selectagents.gov/>.

Recombinant and Synthetic Nucleic Acids

NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules:

Compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is mandatory for every institution receiving NIH funding for research involving recombinant or synthetic nucleic acids. In the context of the NIH Guidelines, recombinant and synthetic nucleic acids are defined as:

- Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids.

- Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
- Molecules that result from the replication of those described above.

It is the responsibility of the Principal Investigator (PI) to make sure that his/her laboratory is in compliance. All experiments involving recombinant and synthetic nucleic acids at AHU require approval of the EHS to determine the appropriate safety training, and safety compliance requirements based on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (See Appendix A). Example or project: Experiments that fall under Section III-A of NIH Guidelines requires approval by the Recombinant DNA Advisory Committee (RAC at NIH – Office of Science Policy), and NIH Director before initiation. Experiments on Section III-A-1-a are limited to studies that involve the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture (antibiotic resistance markers used for selecting and propagating plasmids in *E. coli* are not included). Examples: Cloning a gene for rifampin resistance into *Mycobacterium tuberculosis*, cloning a gene for tetracycline resistance into *Chlamydia trachomatis*.

Containment of Recombinant and Synthetic Nucleic Acids

The NIH Guidelines Appendix G. Physical Containment is the key reference in assessing risk and establishing an appropriate biosafety level (i.e. risk management or control). The Guidelines specify appropriate practices and training as well as the physical containment.

Similar to the Biosafety in Microbiological and Biomedical Laboratories (BMBL, 5th edition), the NIH Guidelines specify combinations of containment practices, safety equipment, and laboratory facilities. However, the NIH Guidelines stipulate an additional containment the application of highly specific biological barriers to limit either (1) the infectivity of a vector or vehicle for specific hosts, or (2) its dissemination and survival in the environment. Risks from the materials themselves, the vector (plasmid, organelle or virus), the host (bacterial, plant, animal cell), the procedures used, and proximity of susceptible hosts should be considered together.

Naked DNA

Work with naked DNA (i.e. not in an expression vector) is generally low risk except when the DNA contains oncogenic sequences (capable to cause cancer) or is a full length viral genome (in which case it is also important to consider naked RNA). Particular care must also be taken if the naked DNA/RNA is used with sharps or with solvents which have the ability to penetrate the skin (e.g. DMSO- dimethyl sulfoxide) or are in membrane fusing agents (e.g. Lipofectamine). If the DNA/RNA is likely to contain harmful sequences then consideration should be given to whether it may be possible to include a stage in the protocol whereby the DNA/RNA is denatured. A denaturing stage will eliminate any potential for expression and therefore, the hazard will be minimal. Denaturing techniques should not be confused with protein denaturing steps such as phenol chloroform treatment which do not affect the nucleic acid. RNA extracted from positive-strand RNA viruses, e.g. flaviviruses and alphaviruses, are infectious since the genomic RNA is the same sense as mRNA so can be translated immediately upon infection of a permissive cell.

Registration of Experiments Involving rDNA

Compliance with the NIH Guidelines is mandatory for investigators conducting recombinant or synthetic nucleic acid research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant or synthetic nucleic acid. The construction and use of recombinant/synthetic nucleic acids or recombinant organisms requires registration with the Environment, Health and Safety Office. The NIH Guidelines specifically address recombinant and synthetic nucleic acids and the AHU Environment, Health and Safety Office requires registration for all projects involving recombinant nucleic acids, e.g. RNA derived from rDNA, RNAi, etc.

Transport or environmental release (e.g. field testing) of recombinant organisms may be regulated by others (see previous section).

Biological Toxins

Biological Toxins:

- Are highly toxic in minute quantities.
- Have no established safe exposure limits.
- Exposure monitors are not readily available.
- Have limited toxicological data applicable to human exposures.
- Exposure risks are primarily from inhalation, ingestion, and accidental injection. Dried/powdered forms are particularly hazardous for their inhalation potential and tendency for electrostatic attachment to gloves, weighing spatulas, etc.
- Most are stable proteins (although trichothecene mycotoxins from fungi are carbohydrates) that require harsh “disinfectants” for inactivation.

Risk Factors to Consider for Working with Biological Toxins:

- Aerosol generating procedures (e.g. vortexing, grinding, centrifuging, intra-nasal inoculation of animals, sonication).
- Utilization of concentrated stocks or large quantities of toxins.
- Work with powdered or dried toxins.
- Work with highly lethal toxins or highly purified forms of less lethal toxins.

Use of Biological Toxins Having an LD₅₀ of ≤ 100 g/kg Body Weight Requires

- Project registration with the Environment, Health and Safety Office
- Inventory control log and signage.
- Standard Operating Procedures
- Security measures
- Proper disposal
- Minimum BSL-2 requirement for laboratory safety level.
- In general, BSL-2 containment, although a risk assessment may dictate other precautions.
- Some toxins are controlled for export and regulated as select agents requiring stringent regulations for possession, use, or transfer of non-exempt amounts.

Mammalian Cell Cultures

Laboratorians who handle or manipulate human, non-human primate, or other mammalian cell lines and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. The potential for human cell lines to harbor a blood borne-pathogen led OSHA to include human cell lines in the final rule unless they were specifically tested for, and documented to be free of, human blood-borne pathogens.

There also is evidence of accidental transplantation of human tumor cells to healthy recipients, indicating that these cells are potentially hazardous to the laboratory workers who handle them. Further, human and animal cell lines that are not well characterized or are obtained from secondary sources may introduce a biohazard to the laboratory. Note that cell lines purchased from commercial vendors historically were not routinely tested for viruses, including those that may be human or animal pathogens. However, ATCC now tests every lot of every human cell line manufactured after January 4th, 2010 for common human viral pathogens: HIV, HepB, HepC, HPV, EBV and CMV. For additional information please see the FAQ section of the ATCC website. Biosafety Level 2 is appropriate when work is performed with all human cell lines and any mammalian cell line that has not been well characterized or where the presence of an infectious agent may be unknown.

Human Blood and Other Potentially Infectious Material

Human blood and other potentially infectious material (OPIM) may contain infectious agents including, but not limited to, human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV).

Other potentially infectious materials (OPIM) are defined as:

- The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids.
- Any unfixed tissue or organ (other than intact skin) from a human (living or dead).
- HIV or HBV-containing cell or tissue cultures, organ cultures, culture medium or other solutions. Blood, organs or other tissues from experimental animals infected with HIV or HBV.

Containment

As with other biological hazards, combinations of safe work practices, safety equipment, administrative controls, and laboratory facility design/features are applied to minimize risks. Biosafety Level 2 is appropriate for work with human blood and OPIM. Should an exposure occur, thorough cleansing of the exposed area followed by immediate medical attention is required.

Biosecurity

The World Health Organization (WHO) Laboratory Biosafety Manual, 3rd Edition defines Biosecurity as institutional and personal security measures designed to prevent the loss, theft,

misuse, diversion or intentional release of pathogens and toxins (i.e. protect pathogens from dangerous people).

The risk assessment conducted as part of the biosafety program gathers information on the type of organisms handled, location of work, and personnel handling these agents. Based on this information, the potential for use of these agents for harmful purposes can be assessed. If such a threat is identified, a Biosecurity program must be implemented to protect against possible misuse of these agents. Such a program should involve participation from principal investigators, Environment, Health and Safety Office staff, laboratory staff, information technology staff, law enforcement agencies, and building security staff.

Various components of laboratory biosecurity measures are as follows:

- Threat identification.
- Accountability of pathogens and toxins in use by maintenance of accurate logs of the inventory, transfer of materials, and inactivation or disposal of the material.
- Limited access to the agents.
- Employee and visitor screening policy.
- Prompt reporting of any security breach.
- Information biosecurity to ensure security of sensitive electronic files.
- Restricted sharing of sensitive printed material/protocols.
- Guidelines for management of possible accidents or incidents involving these agents and prompt reporting of such occurrences.
- Periodic training
- Drills and exercises to evaluate and reinforce the biosecurity program; the components of which are updated and re-evaluated as necessary.

Background Screening

In support of efforts to maintain and foster safety and security of students, faculty, and staff, AHU requires pre-employment criminal background checks on new hires for faculty, staff and administration positions. Federal or state statutes or contracts may require criminal background checks be conducted on certain positions within the University, despite the classification. Thus, unless stated otherwise, hiring authorities may choose to complete pre-employment criminal background check on promotions, transfers, and hiring of temporary academic members and staff employees.

The Environment, Health and Safety Office evaluate projects for dual use concerns during the project registration process and require increased containment and security measures as appropriate.

All investigators have a responsibility to:

- Assess their research efforts for dual use potential and report as appropriate.
- Seek to stay informed of literature, guidance and requirements related to dual use research.
- Train others to identify dual use research of concern, manage it appropriately and communicate it responsibly.
- Serve as role models of responsible behavior, especially when involved in research that meets the criteria for dual use research of concern.
- Be alert to potential misuse of research.

Control of Biohazards

Containment or Biosafety Level (BSL) needed for safe work with a particular agent is based on combinations of:

- Laboratory practices (SOPs)
- Engineering Controls
- Personal Protective Equipment (PPE)
- Administrative policies

Four biosafety levels, BSL-1, 2, 3, 4, are described for activities involving biohazards. The levels are designated in ascending order by degree of protection provided to personnel, the environment and the community. These four levels describe the combinations of practices, safety equipment, administrative controls, and laboratory facility design/features required. See Appendix B for a summary of these Biosafety Levels and the *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition Section IV- Laboratory Safety Level Criteria for a more detailed description. Multiple controls provide sufficient redundancy to maximize safety. The factors considered when determining the BSL in which the work with that specific agent should be conducted include:

- Risk group of an agent
- Mode of transmission
- Procedural protocols
- Experience of staff
- Other factors
 - Classification of human etiologic agents on the basis of hazard can be found in Appendix B of the NIH Guidelines, and work with an agent is generally conducted at the BSL recommended for that agent in Section VIII of the BMBL, 5th Edition, December 2009.
 - More (or less) stringent practices may be specified by the EHS risk assessment review depending on information suggesting significant alteration in virulence, pathogenicity, antibiotic resistance patterns, vaccine/treatment availability or other factors.
 - Large volumes, high concentrations, or higher risk procedures will dictate an increase in BSL.
 - The EHS requirements must be adhered to unless new information to justify a change is provided to the EHS for review and approval.

Laboratory Practices

- Strict adherence to standard microbiological practices and techniques is emphasized.
- Persons working with infectious agents, potentially infectious material, or other biohazards must not only be aware of the potential hazards, but also must be trained and proficient in the practices and techniques required for handling such material safely.
- The principal investigator or coordinator of the laboratory is responsible for the safe conduct of work with any biohazardous agents or materials and providing, or arranging for, the appropriate training of personnel.
- It is recommended that each laboratory develops a laboratory-specific safety manual that coordinates with the institution-wide AHU Biosafety Manual which identifies the hazards

that exist or may be encountered, and specifies the practices and procedures that will be used to minimize or eliminate exposures or releases of these hazards. Consideration should be given not only to normal operations, but also to practices and procedures to follow in emergency situations.

- Wherever the scope of hazards is not adequately addressed by this general document, the principal investigator or laboratory coordinator is responsible to implement additional safety practices commensurate with the hazards associated with the agent or procedure. The practice should be written in a specific Laboratory Standard Operating Procedures. Examples include supplemental facility design and engineering features, safety equipment, or administrative controls.

Engineering Controls

These devices are used to contain or remove biohazards, monitor critical physical parameters or provide specific service. These include, but are not limited to, biological safety cabinets (BSCs), enclosed transport containers, directional airflow indicators, and safety centrifuge cups, micro-isolator tops on animal cages, self-sheathing needles and sharps containers.

The Biological Safety Cabinet (Primary Containment)

The Biological Safety Cabinet (BSC) is the principal device used to provide containment of infectious splashes, droplet, or aerosols generated by many microbiological procedures. All BSCs must be certified annually.

Three kinds of BSCs designated as Class I, II, and III, have been developed to meet varying research and clinical needs. Most BSCs use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. The various types of biosafety cabinets are described:

- The Class I BSC: This type of cabinet is not for aseptic or sterile technique. The Class I BSC provides personnel and environmental protection, but no product protection. It is usually hard ducted i.e. directly connected to the building exhaust system and is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. Those used for animal cage changing allow re-circulation of HEPA filtered air into the room. These require annual certification and more frequent filter changes.
- The Class II BSC: The Class II (Types A and B) biological safety cabinets provide personnel, environmental, and product protection. Air flow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through a certified HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory or ducted out of the building via thimble/canopy connection, which maintains a small opening around the cabinet exhaust filter housing (Type A1 and A2), or hard duct connection (Type B1 and B2). With a thimble connection the volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing. The thimble must be removable or be designed to allow for operational testing of the cabinet. The performance of a cabinet with this exhaust configuration is unaffected by fluctuations in the building exhaust system.

- The Class II, Type A1 BSC: Room air is drawn through the front grille via an internal blower to maintain an average inflow velocity of 75 feet per minute (fpm) (A2, B1, and B2 have 100 fpm) at the face opening of the BSC. HEPA filtered air splits over the work surface to the front and the rear grille. 30% of the air is exhausted while 70% recirculates through the HEPA filter back into the work area. This can cause build-up of toxic fumes-Type II, A1 BSC is not to be used to handle toxic, volatile chemicals.
- The Class II, Type A2 (formerly A/B3) BSC: This BSC has a minimum calculated measured inflow velocity of 100 fpm. Only when this BSC is ducted to the outdoors does it meet the requirements of the former class II type B3. All positive pressure biologically contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment.
- The Class II, Type B1 BSC: Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box. Carcinogens used in cell culture or microbial systems require both biological and chemical containment. Type B1 cabinets must be hard-ducted to their own dedicated exhaust system. Typically, 70% of the air is exhausted outside the building through HEPA filter; 30% is recirculated. Blowers on laboratory exhaust systems should be located at the terminal end of the ductwork. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor should be installed to sound an alarm and shut off the BSC supply fan, should failure in exhaust airflow occur. Since all cabinet manufacturers do not supply this feature, it is prudent to install a sensor in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.
- The Class II, Type B2 BSC: This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides simultaneous primary biological and chemical containment. Should the building or cabinet exhaust fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system installed by the manufacturers to prevent the supply blower from operating whenever the exhaust flow is insufficient. Presence of such an interlock system should be verified; systems can be retrofitted if necessary. A pressure-independent device should monitor exhaust air movement.
- The Class III BSC: The Class III BSC was designed for work with highly infectious microbiological agents, and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

Operations within a Class II BSC:

- BSC must be located:
 - Away from the entry to the laboratory and from the laboratory traffic.

- Adequate clearance must be provided around, and 12"-14" clearance above, the BSC for easy access and to provide for accurate air velocity measurement.
- Open windows, portable fans, laboratory equipment that create air movement, and chemical fume hoods must not be located close to a BSC.
- Good microbiological techniques should always be used when working in a BSC.
- Only materials and equipment required for the immediate work should be placed in a BSC so as not to disrupt the airflow.
- Frequent inward/outward movement needed to place objects in biohazard collection containers outside the BSC is disruptive to the integrity of the cabinet air barrier and can compromise both personal and product protection. Horizontal pipette discard trays containing a disinfectant (e.g. bleach) are recommended for use inside the BSC.
- Best practices recommend keeping clean materials at least 12 inches away from aerosol-generating activities will minimize the potential for cross-contamination.
- The general workflow should be from clean to contaminate (dirty). Materials and supplies should be placed in such a way as to limit the movement of dirty items over clean ones.
- Work at least 4" back from the front edge and never cover the front grill.
- When possible, open containers (tubes, bottles) should be held at an angle to prevent contamination. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impact of downward air. Items should be recapped or covered as soon as possible.
- Open flames:
 - Create turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface and are not permitted in the near microbe-free environment of a biological safety cabinet. Contact the Environment, Health and Safety Office regarding alternatives to the use of these devices.
 - Small electric furnaces are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops should be used to eliminate the need for heat or flame.
- Aspirator bottles or suction flasks:
 - Should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. This will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment.
 - Sufficient chemical decontamination solution (e.g. 100% bleach) must be placed in the flask to inactivate aspirated material as they are collected. Inactivated liquid material can be disposed of appropriately as noninfectious waste.
- Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. However, there is no treatment (e.g. autoclaving) of any biological waste at the University. All waste are treated by the Florida Hospital according to the guidelines of the Safety Department of the Florida Hospital.
- Ultraviolet (UV) lamps are not required or necessary in BSC.
- If installed, the lamps must be cleaned and checked periodically with a UV meter to confirm appropriate emission.
- UV lamps must be turned off when the room is occupied to protect eyes and reduce skin exposure.
- Close the sash in BSC when operating UV lamp.

- Spills inside the BSC must be handled immediately by the principle investigator. Large spills must be handled immediately by the EHS.
- The BSC must be professionally certified per NSF/ANSI49-2002 Standard when used to handle infectious and potentially infectious material:
 - After initial installation
 - At least annually thereafter
 - After the BSC is relocated or repaired

Horizontal Laminar Flow Clean Benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity. Horizontal clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical or veterinary laboratories and/or applications.

Vertical Laminar Flow Clean Benches also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous drugs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

Facility Design & Construction (Secondary Containment)

The design and construction of the facility contributes to laboratory worker protection, provides a barrier to protect persons outside the laboratory, and protects persons, animals, or plants in the community from the accidental release of biohazardous agents from the laboratory. Laboratory coordinators are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. Facility barriers are simplest for low hazard agents or activities; features are added as risk increases. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents.

In the typical biological laboratory, agents are transmitted or disseminated by direct or inadvertent contact with infectious items in the work environment. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, cleanable surfaces, availability of a decontamination method (e.g., autoclave), and hand washing facilities. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory.

Personal Protective Equipment

Under OSHA's primary Personal Protective Equipment (PPE) standards, PPE refers to "garments and devices designed to protect employees from serious workplace injuries or illnesses resulting

from contact with various workplace hazards". Examples of PPE include coveralls, lab coats, gloves, face shields, safety glasses, goggles, safety shoes, and respirators. OSHA mandates that employers:

- Determine the workplace hazards that require PPE.
- Provide workers with appropriate PPE.
- Ensure proper use and maintenance of PPE.
- Train employees to use PPE correctly, to know when and where PPE is necessary, understand its limitations, and to put on and take off PPE correctly.

Coveralls/Lab Coats

- Protective laboratory coat, gowns, or uniforms are recommended under BSL-1 guidelines and are required when working with hazardous material under BSL-2. Disposable gear should be used inside a BSL-3 facility and discarded before leaving the laboratory.
- PPE clothing should be removed before leaving the laboratory.
- Do not take laboratory clothing home. If contaminated, laboratory coats should be decontaminated with bleach before dispatching the coats to wash.
- Disposable lab coats are preferred. These are reusable and available from many suppliers.

Gloves

- Gloves should be worn to protect hands from hazardous materials. Disposable latex and nitrile gloves are commonly used. Some people develop latex allergy that can vary from mild to severe in intensity. Non-latex gloves should be available in such cases.
- Gloves come in various sizes. Wear gloves that fit properly.
- Integrity of gloves is very important. Before using, make sure there are no holes/tears in the gloves. Gloves should be changed immediately if torn or contaminated when work is in progress. Handling some material may require wearing two pairs of gloves.
- Gloves used to handle infectious or potentially infectious material should be discarded in a biohazard container lined with a red, autoclavable biohazard bag and not in regular trash. If you have questions regarding proper disposal, contact the EHS. Gloves contaminated with hazardous chemicals must be discarded as dry chemical waste.
- Wash hands with soap and water as soon as possible after removing gloves. If a handwashing sink is not immediately accessible, hand sanitizer should be kept handy for immediate use, which should be followed by hand washing as soon as possible.
- Do not re-use or wash disposable gloves.
- Gloves should not be worn outside the laboratory in public areas (e.g. in elevators or cafeteria), or when opening door handles.

Eye and Face Protection

PPE to protect eyes to infectious agents in the form of splashes, sprays, or respiratory droplets. The EHS will determine and recommend the use of these based on risk assessment of the type of material handled. Examples of eye and face protection include:

- Safety glasses (with side shields) are recommended when working with infectious material to prevent potential splashes entering the eyes. Safety goggles, on the other hand, are recommended when working with harmful chemicals.

- Face shields alone do not provide adequate protection against splashes and should always be used along with safety glasses or goggles.
- A surgical mask provides minimal personal protection against splashes or sprays of hazardous material; the mask mainly prevents the wearer from spreading infected droplets. It is not a respirator.
- Respirators. N95 or higher filtering respirators provide partial face but not eye protection. The NIOSH N95 (N99 & N100 have higher filtration capacity) respirators are designed to filter very small particles. These respirators should fit snugly on the face and require annual 'fit testing'. Powered Air Purifying Respirators (PAPRs) used in some high aerosol generating procedures are full face respirators and provide eye as well as face protection. Before using a PAPR, medical clearance and training is required. The Environment, Health and Safety Office will advise regarding the use and type of respirators required based on the type of hazard anticipated.

Eye and face protection devices should be decontaminated after use (e.g. spraying safety glasses with 70% ethanol) or discarded in biohazard container (e.g. surgical masks, N95). Persons wearing contact lenses should always wear eye-protection when handling hazardous material.

Safety Shoes

Full coverage shoes must be worn in the laboratory. Disposable shoe covers are sometimes required in animal housing and procedure areas, and while attempting to clean up biological spills. The shoe covers should be discarded as biohazardous waste.

Laboratory Assessment and Improvement

Biosafety facility reviews/surveys/inspections are conducted in laboratories on a periodic basis. The purpose of these surveys/audits is several fold: 1) as part of the risk assessment process to identify and correct potential hazards, 2) to facilitate compliance with local, state, and federal requirements, and 3) to provide guidance and information on relevant biosafety issues to Principal Investigators, staff and students. Regular self-audits are also recommended. Inspections are typically scheduled, but may occur as unscheduled events in certain instances. Laboratories working with select agents and those funded by certain agencies (e.g. Dept. of Defense) need frequent inspections. The checklist used for the inspection depends on the type of research and/or the biosafety level(s) assigned to the project(s).

- Inspections of facilities working with pathogenic or potentially pathogenic materials are based on the requirements specified in BMBL 5th edition.
- Facilities working with recombinant or synthetic nucleic acids are inspected for compliance with the appropriate appendices of the NIH Guidelines:
 - For lab-scale recombinant or synthetic nucleic acid molecules work, Appendix G
 - For Large Scale (>10L) recombinant or synthetic nucleic acid molecules work, Appendix K
 - For recombinant or synthetic nucleic acid molecules work involving plants, Appendix P (note that the biosafety levels described by the NIH in Appendix P are referred to as BSL1P- BSL4P).

Decontamination and Sterilization of Biologicals

This section describes basic strategies for decontaminating surfaces, items and areas in laboratories to eliminate the possibility of transmission of infectious agents to laboratory workers, the general public and the environment. Decontamination refers to the removal of debris, blood, and proteins, and most microorganisms which usually renders the area, item, material or device safe to handle (i.e. reasonably free from a risk of disease transmission). Disinfection refers to physical or chemical means of eliminating most if not all pathogenic microorganisms, excluding spores. Sterilization renders items free of all microorganisms, including spores. Antisepsis is the process of disinfecting living tissue or skin and reduction or removal of transient microbial flora.

Disinfectants

Disinfectants are chemicals or mixture of chemicals used in the laboratory to 1) treat a surface or an item before or after routine use, or 2) to treat a surface or an item after a spill or "contaminating event." Because disinfectants are antimicrobial, they may also be toxic to the user. Therefore, Safety Data Sheets (SDS) and other manufacturer's product information should be available and thoroughly reviewed before using these products.

There are many different types and formulations of disinfectants. The principal investigator should ensure that the proper product is effective against the specific microorganism being studied and that manufacturer's instructions are followed.

- The FDA (Food and Drug Administration) regulates those products that are marketed as sterilants or sanitizing agents used on critical and semi-critical medical devices, e.g. surgical instruments and flexible endoscopes. A list of products currently on the market that are labeled as sterilants is located on the EPA website.
- The EPA also regulates moderate and low level disinfectants as "chemical germicides" used for environmental surfaces under their pesticide regulations. A list of registered antimicrobial products effective against certain bloodborne/body fluid pathogens, Mycobacterium tuberculosis (tubercle bacteria), HIV-1, Hepatitis B, Hepatitis C viruses, as well as products classified as sterilizers is also published by the EPA.

It is important to note that most disinfectants assume pre-cleaning to remove gross organic material/protein prior to use. In addition, whenever a disinfectant or a sterilant is used, proper safety precautions must be followed as per manufacturer's instructions. Appropriate safety equipment (gloves, apron and safety goggles) must be utilized and procedures performed in well-ventilated areas.

The following is a list of general categories of disinfectants. Please note that there are several different products and different formulations in each category.

Alcohols

The most commonly used alcohols, ethanol (ethyl alcohol) and isopropanol (isopropyl alcohol or 2-propanol), are most effective at concentrations of 70% (v/v) in water. Higher and lower concentrations are less effective. Alcohols are active against vegetative bacteria, fungi, and lipid containing viruses but not against spores. Their action on lipid-containing viruses is variable.

Alcohols are difficult to use as contact disinfectants because they evaporate rapidly and do not penetrate organic matter well. Alcohol-based rubs may be used to decontaminate lightly soiled hands when proper hand washing is not available. However, it is important to wash hands with soap and water as soon as possible. When using alcohols, it is best to clean an object, then submerge it in alcohol for the appropriate time. Alcohols are often used in concert with other disinfectants such as formaldehyde (see toxicity warning below) or chlorine (2000 ppm chlorine in alcohol). Alcohol is not an EPA-registered tuberculocidal or HIV listed disinfectant. Alcohols are flammable and must not be used near open flames.

Chlorine Compounds

The most commonly used and generally effective disinfectant is sodium hypochlorite (common household bleach). It is active against a wide variety of microorganisms including bacterial spores and *Mycobacterium tuberculosis*. A 1:50 dilution, supplying approximately 1000 ppm available chlorine, of the common household bleach (5.25% sodium hypochlorite) is very effective as a general laboratory disinfectant, and a 1:10 dilution supplying approximately 5000 ppm available chlorine is effective against spills involving blood or other organic material. Higher concentrations should be utilized for work with bacterial spores. Note that the presence of high concentrations of protein can inactivate the action of chlorine products. Dilute hypochlorite solution must be prepared daily to be maximally effective. There are more concentrated sodium hypochlorite solutions available, therefore it is critical to read the product information carefully to determine the proper dilution. It is a strong oxidizing agent and therefore can be corrosive to metal.

Formaldehyde

Formaldehyde is a gas that is available either dissolved in water and methanol as a 37% formaldehyde solution (formalin), or as a solid (paraformaldehyde) that may be melted to release the gas.

Formaldehyde gas kills all microorganisms and spores but not prions. It is used for space decontamination and to decontaminate biological safety cabinets. This method of decontamination is an extremely dangerous process requiring properly trained, experienced personnel. Formaldehyde dissolved in water is active at 1-8% solutions and can be used to decontaminate hard surfaces. However, because formaldehyde is an irritant at low concentrations (0.1 to 5 ppm) and a probable carcinogen, its use as a hard surface disinfectant is limited to situations in which it is particularly needed. Due to its toxic effects, there are no EPA-registered disinfectants that contain formaldehyde.

Glutaraldehyde

Most commonly used for high level disinfection of medical equipment, e.g. endoscopes. Glutaraldehyde is usually supplied as a 2% solution and requires activation by the addition of an alkaline agent prior to use. The activated product may be stored for about 1-4 weeks and should be discarded when turbid. Glutaraldehyde is active against all microorganisms, but is toxic, an irritant, and mutagenic and should be used only when necessary. The manufacturer's guidance must be followed when using glutaraldehyde-based products as there are many different formulations that have been designed for specific uses.

Hydrogen Peroxide

Hydrogen peroxide is usually available as a ready-to-use 3% solution or as a 30% solution to be diluted 1:5-1:10 to decontaminate work surfaces of laboratory benches and biosafety cabinets. It is active against a wide array of microorganisms. However, it is a strong oxidizing agent and should not be used on aluminum, copper, zinc, or brass. Hydrogen peroxide is unstable at high temperatures and in light.

Iodine and Iodophors

Iodine and iodophors are compounds in which the iodine is combined with a solubilizing or carrier agent and are general all-purpose disinfectants with an action similar to that of chlorine products. The appropriate concentration for iodine-containing products is 75 ppm available iodine for disinfecting work surfaces. Concentrations may be much higher for other purposes. Like chlorine compounds, the effectiveness of iodine compounds may be diminished in the presence of protein/organic material. Iodophor compounds that are used for antiseptics (germicide applied to tissue or skin) are not appropriate for use as hard surface disinfectants and vice versa. Read the product material for appropriate dilutions and applications.

Phenolic Compounds

Phenolic compounds are active at 0.2 - 3% concentrations against all forms of vegetative microorganisms but not against spores. They have limited effectiveness against non-lipid viruses and when properly formulated are anti-mycobacterial. There are many common disinfectants based on phenol and they should be used according to the manufacturer's recommendations.

Quaternary Ammonium Compounds

Compounds in this class are active at concentrations of 0.1 - 2%. They are active against vegetative bacteria and lipid viruses, but not against bacterial spores, non-lipid viruses, or tubercle bacilli. These compounds should be used only when a low-level disinfectant is required.

Vapor Phase Hydrogen Peroxide

- Requires specialized equipment
- Temperature: 4°C-60°C
- Concentration: 30%, less than 10 mg/liter
- Non-toxic end products of water and oxygen
- Limited to surfaces, no penetration
- Corrosive to some materials
- Degrades natural rubber and nylon

Chlorine Dioxide Gas

- Dilute chlorine gas and sodium chlorite, less than 25 mg/liter
- Temp 25°C-30°C, pre-humidification required
- Limited to surfaces, no penetration
- Corrosive to some materials
- Mucous membrane irritant

Formaldehyde Gas (from heating paraformaldehyde)

- Kills all microorganisms and spores at temperatures >20°C; not active against prions
- Temp 20°C-22°C, humidity 70%
- Conc. 0.3 gm./cu ft. of volume
- Time 6-8 hours
- Toxic irritant and suspected carcinogen
- Limited penetration, primarily surface action
- Requires aeration and time for formaldehyde to off-gas, usually 8 hours

Equipment Decontamination

The following procedure must be followed before surveying, disposing of, moving, or repair of University equipment (e.g. refrigerators, freezers, incubators, biosafety cabinets etc.) that may be contaminated/potentially contaminated with biohazardous material.

- Wipe the equipment with an appropriate disinfectant (e.g. 10% bleach solution followed by 70% ethanol to remove any residual bleach).
- Biosafety cabinets must be professionally decontaminated.
- Contact the EHS to schedule the disposal of the equipment.

Laboratory Decontamination

Space decontamination is a specialized activity and must be performed by specialists with proper training and PPE as this typically requires gaseous decontamination (formaldehyde) or hydrogen peroxide vapor. Please contact the EHS for additional information regarding decontamination of large spaces. Liquid chemical germicides formulated as disinfectants may be used for decontamination of surfaces. The EHS recommends 10% chlorine bleach (approximately 5,250 ppm chlorine) as an inexpensive effective disinfectant for routine use (please confirm effectiveness on the specific biohazard in-use).

Biomedical and Biological Waste

Training

All employees who generate biological waste or use a sharps box shall be trained regarding the proper segregation, handling, packaging, labeling, storage, and treatment (if any) of biological waste. Refresher training is required annually.

According to Florida Statute (Ch. 64E-16 F.A.C.), records of the training session shall be maintained for each employee, along with an outline of the training program.

Training records shall be retained for a period of three (3) years. The training records will be maintained by Environment, Health and Safety Office.

All individuals that generate biological waste must segregate biological waste from other types of waste at the point of origin into the following categories:

Potentially Infectious and Infectious Biological Waste

Waste items that are contaminated with:

- Human, animal, or plant pathogens
- Recombinant or synthetic nucleic acids and recombinant organisms
- Laboratory wastes containing human or primate blood, blood products, tissue, cell cultures, and other potentially infectious material (OPIM) including used, absorbent materials contaminated with blood, blood products, or OPIM or non-absorbent, disposable devices that have been contaminated with blood, body fluids or OPIM
- Cultures

Place this waste in the red biohazardous waste bag or red bag-lined cardboard biological waste box for disposal. There is no treatment (e.g. autoclaving) of any biological waste at the University. All waste are treated by the Florida Hospital according to the guidelines of the Safety Department of the Florida Hospital.

Biological waste containers and bags for material that is infectious/potentially infectious to humans must be labeled with the biohazard symbol. Filled or partially filled biological waste containers and boxes should not be held for more than 30 days.

Non-infectious Waste

Additionally, waste items that are:

- Used labware (tissue culture dishes and flasks, petri dishes, centrifuge tubes, test tubes, pipettes, vials, etc.) from labs that is NOT contaminated with any of the biological wastes listed as potentially infectious and infectious biological waste.
- Unused medical devices. This material does NOT qualify for disposal as Clean Lab Ware.
- Gloves or other disposable personal protective equipment from labs that are NOT contaminated with any of the biological wastes listed above, and not contaminated with hazardous chemicals.

This type of waste can go disposed in the regular trash.

Sharps Waste

Instruments are those that are intended to cut or penetrate skin (e.g., metal lancets, scalpel blades, needles, or syringe/needle combinations) must be placed in red, hard plastic sharps boxes, even if unused. The sharps box should be closed when it is $\frac{3}{4}$ full and discarded within 30 days after closure. Sharps boxes are placed into the red bag-lined cardboard biological waste box for disposal.

Instruments that can cut skin, but are not intended to do so (fragile glass, glass slides and cover slips, razor blades, pipettes and pipette tips), should be disposed of in a manner that prevents harm. This instruments should be placed in sharps box, small rigid box that is then placed in a biohazard bag, plastic sleeve (to hold the pipettes together in a bundle) that is then placed in a biohazard bag.

Biohazardous Waste Boxes

- Sturdy, pre-printed cardboard biowaste boxes displaying the biohazard sign are used as the terminal receptacle. Line the box with a red bag.
- Do not overfill; boxes must weigh less than 45 lb.

- Tape all seams. Label with date, PI name, room number, and telephone number.
- Do not keep biowaste boxes for more than 30 days.

Biohazardous Waste Bags

Refer to the Biomedical and Biological Waste section of the manual to determine if your waste must be placed in a red bag.

- Do not put liquid waste into the red bags.
- Items that can poke a hole in the bag must be packaged or contained in a way that minimizes the chance of a puncture.
- State regulations require that red bags must be disposed of within 30 days of when the first item was placed into the bag.
- Red biohazardous bag or red bag-lined cardboard biowaste box is used to collect biowaste (e.g. biohazardous waste bags, and closed sharps containers) for disposal by a commercial waste transport company.
- Each bag, including the liner bag, must be securely closed before sealing the biowaste box. Per federal DOT regulations, "The bag must be capable of being held in an inverted position with the closed end at the bottom for a period of 5 minutes without leakage".
- Label with date, PI name, room number, and telephone number.
- The lab must order/supply these red bags specific for biohazardous material waste.

Sharp Containers

- Label with date, PI name, room number, and telephone number.
- Close when $\frac{3}{4}$ full and place in the red bag-lined cardboard biowaste box for disposal.

Biological Waste Packing, Labeling, & Transport

- All biohazardous waste box, bags and sharp waste needs to be properly sealed as indicated above.
- Label the waste container with the word "TRASH".
- Laboratory PI and/or Laboratory Coordinator need to contact the environmental personnel within 24 hours to schedule the pick-up.
- Environmental personnel needs to transport all the biohazardous waste in a red biohazardous bin to its final destination. Environmental personnel are instructed to use gloves when initially picking up the waste and then disposing of gloves before leaving the laboratory. Upon delivery to final destination, gloves should be worn to transfer biohazardous waste bags/boxes from the biohazardous waste bin to the final destination.

Exposures and Incidents

Exposures

An exposure can be known immediately, such as a percutaneous injury from a contaminated item or animal, or splash to the face and eyes when adequate PPE was not worn. In other instances,

an exposure can be suspected, e.g. a culture flask of high titer virus dropped and broke on the floor when the worker was wearing no respiratory protection. Another possibility is that the worker may become ill and will have no knowledge of an exposure; for example if there has been a failure of engineering controls or personal protective equipment, or a bite from an insect vector.

Prompt reporting and medical treatment are important. Report exposures to the PI and Laboratory Coordinator as soon as possible and to the EHS within 24 hours. EHS will inform the AHU Security Office for further records to provide medical surveillance. The affected individual and/or PI should be prepared to provide the physician with specifics on the type of biohazardous materials present in the workplace. A good practice is to have on hand a copy of the Pathogen Safety Data Sheet (PSDS) for that you can take with you to the medical provider. If genetically engineered pathogens are a possible source of exposure, consider any drug resistance traits (natural or engineered), and any alterations to host range, tissue tropism, pathogenicity, virulence or stability in the environment.

Biohazard Exposure, Cuts or Non-Intact Skin Biohazard Exposure

- Remove protective clothing or PPE as needed to gain access to the affected area.
- Wash hands.
- Wash the affected part while allowing the wound to bleed freely (if applicable). Use soap if available, but avoid strong chemical disinfectants that can damage skin, e.g. bleach.
- Apply an appropriate disinfectant from the first aid kit (e.g. antibiotic ointment).
- Notify the PI or lab coordinator and inform them of the circumstances of the injury, including what was being handled at the time.

Splash to Face, Eyes or Mucous Membranes

- Proceed to the nearest eyewash station and activate it.
- Rinse face/mouth/nose/eyes.
- Eyes should be flushed for at least 15 minutes.
- Forcibly hold eyelid open to ensure effective rinsing behind eyelids.
- Move eye side-to-side and up-down during rinsing. Remove contact lenses.
- Place contaminated clothing in a red bag or biohazard bag for decontamination.
- Obtain medical treatment.
- Watch for symptoms of exposure or delayed onset effects.

Accidental Ingestion

- Seek medical treatment.
- Call AHU Security Phone 407-353-4002 to report exposure.

Inhalation Exposure

- Take off PPE normally and exit lab
- Seek medical treatment.
- Call AHU Security Phone 407-353-4002 to report exposure.

Illness Develops in the Absence of Any Known Exposure Event

If you develop fever with or without other symptoms consistent with the agent you work with:

- Seek medical treatment.
- Tell the health care provider about the agents that you work with in the lab.
- The laboratory or facility and work practices will be evaluated by the PI and the EHS for hazards that may have led to the exposure.

Incidents: Spills and Injuries

Handling Biological Spills

Advance preparation for management of a spill is essential. Work quickly to contain and inactivate the spill. A "bio spill kit" should be available and contain the following:

- Tongs/Forceps/scoop and dust pan for broken glass/sharps.
- Paper towels or absorbent material.
- Appropriate disinfectant (remember to check the expiration date and replace as needed).
- Respirators, if necessary, as determined by Environment, Health and Safety Office risk assessment.
- Latex or nitrile gloves.
- Autoclave or biohazard bag.
- Safety glasses with side shields.

Spill in the Biosafety Cabinet

- Leave the cabinet on/running to prevent escape of contaminants from the cabinet.
- Cover the area with paper towels or other absorbent material.
- Pour appropriate disinfectant (e.g. a fresh 1:10 dilution of household bleach, 0.5% sodium hypochlorite) over the spill. If necessary, sufficient disinfectant solution shall be used to ensure that the drain pans and catch basins below the work surface contain disinfectant. Disinfect under the front exhaust grill if needed. Walls and equipment in the biological safety cabinet that may have been splashed shall be wiped with disinfectant.
- Let disinfectant solution sit for 30 minutes.
- Use tongs to pick up absorbent materials and place in a biohazard bag or sharps container as appropriate.
- Wipe up excess disinfectant solution.
- Place material in biohazard bag.
- Rinse all disinfected areas with 70% ethanol and allow to dry.

Spill Inside and Outside the Laboratory, Outside of Containment Device

- Clear area of all personnel and stay there to keep them out of the spill.
- If in a room, notify room occupants of the spill so they don't enter the area.
- If infectious aerosols are a concern, all persons should leave the laboratory immediately. Close the door and post a sign on it to prevent entry for 30 minutes while aerosols settle and/or are cleared by the ventilation system.
- If clothing is known (or suspected) to be contaminated, remove the clothing with care, folding the contaminated area inward. Place the clothing into a biohazard bag for autoclaving.
- Wash all potentially contaminated body areas as well as the arms, face and hands. Shower if necessary.
- Any exposed persons should seek medical advice or treatment.

- Notify the PI or lab coordinator.
- Notify the EHS for:
 - Spills or accidents in BSL2 laboratories resulting in an overt exposure of susceptible hosts/individuals to toxins, recombinant or synthetic nucleic acid, or pathogens
 - Spills you are uncomfortable handling
 - Spills involving select agents
 - Spills of high consequence agents
- Protective clothing should be worn to clean the spill area. Latex or nitrile gloves, autoclavable, or disposable footwear, safety glasses, and an outer garment. If you have been issued an N95 respirator to work with this agent, put that on.
- Take the "bio spill kit" and place paper towels, spill pillows, or other absorbent materials around and on the spill. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down.
- Carefully pour a fresh 1:10 dilution of household bleach (0.5% sodium hypochlorite) or other appropriate disinfectant over the absorbent materials; avoid splashing and work from the outside towards the center.
- Let disinfectant solution sit for 30 minutes.
- Pick up the absorbent materials. Use tongs, scoop, or dustpan if sharps may be present. Discard all towels and other clean up materials into a bucket or biohazard bag (or sharps container if appropriate) as they are used.
- Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant.
- Re-apply disinfectant to the area and wipe.
- Place the discard container and other materials in a biohazard bag.
- Remove shoes or shoe covers, outer clothing, respirator, and gloves and disinfect or preferably autoclave.
- Wash hands, arms and face; shower if necessary.
- If gaseous, decontamination of the whole room is required (major spill, spill entered hard to reach areas, etc.), contact the EHS.

Safe transport of biohazardous material outside the laboratory is essential. Materials should be packaged securely (double-contained in unbreakable container with lid and biohazard signage) to avoid such spills. In addition, the person transporting the material should be knowledgeable about the hazards of the material and how to respond to a spill.

Blood Spills

- Put on gloves
- Cover the contaminated area with paper towels
- Flood the towels with a freshly prepared 1:10 dilution of household chlorine bleach or other properly-prepared, EPA registered tuberculocidal disinfectant solution.
- Leave disinfectant solution on spill area for at least 30 minutes.
- Pick up absorbent material with tongs, scoop, or dustpan if sharps may be present. Discard all clean up material into a discard container or sharps container if appropriate, or directly into a biohazard bag

- Reapply disinfectant to the area and wipe.
- Place the discard container and other materials in a biohazard bag.

Since chlorine bleach can corrode some items and surfaces, items treated with chlorine should be rinsed thoroughly with water (or 70% ethanol) to remove chlorine residue. Other high-level disinfectants (i.e. 2% glutaraldehyde) may be used after consultation with the EHS.

Biological Spill on Body

- Remove contaminated clothing.
- Wash exposed area with soap and water for 5 minutes.
- Place contaminated clothing in a red biohazard bag for decontamination.
- Obtain medical attention as required.
- Call AHU Security Phone 407-353-4002 to report exposure.

Immediate Life threatening or Serious Injuries

These injuries include burns, eye injuries, chemical exposures, head injuries, and similar traumatic injuries. Remain calm.

- Call for EMERGENCY RESPONSE - 9-1-1.
- Call AHU Emergency Security Phone 407-353-4002.
- Initiate lifesaving measures, as required.
- If the person is alert and responsive, but cannot move, leave the person there; medical responders will put on PPE and treat the person in the lab.
- If the person is alert and able to move, assist the person to the lab door and help them take off their PPE. Meet emergency responders outside the lab in the corridor.
- If the person is unconscious, drag the person into the clean area and wait for emergency responders.
- Have another lab member meet responders at the entrance to escort them to the lab.

Appendix A: Important Literature

- NIH Guidelines for research involving recombinant or synthetic nucleic acid molecules (NIH Guidelines):
- http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html#_Toc351276449
- Biosafety in Microbiological and Biomedical Laboratories (BMBL), CDC, 5th Edition:
- <http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf>

Appendix B: Summary of Biosafety Levels

Biosafety Level 1 (BSL-1)

- Agents: defined and characterized strains of microorganisms not known to consistently cause disease in healthy adults e.g., *B. subtilis*, *S. cerevisiae*, non-pathogenic *E. coli*. Includes recombinant DNA activities using such non-pathogenic organisms as hosts for the expression of genes incorporated into bacterial plasmids or low risk viral vectors such as baculovirus or Adeno Associated Virus.
- Work practices: standard microbiological practices/aseptic technique.
- Safety equipment: gloves and lab coats are required; eye protection recommended.
- Facilities: bench top sink available for hand washing.

Biosafety Level 2 (BSL-2)

- Agents: associated with human diseases of varying severity, e.g., Hepatitis B and C, HIV, *S. typhi*, human retroviruses, *S. aureus*. Includes recombinant DNA activities using viral vector systems such as Adenoviruses and some Retroviral vectors, particularly Lentiviral vectors, and expression of recombinant DNA in BSL-2 organisms.
- Transmission: inoculation and other percutaneous injuries, ingestion, mucous membrane exposure
- Work practices: BSL-1 practices, with the addition of: limited access, ‘Biohazard’ signs, ‘sharps’ precautions, defined procedures for Regulated Medical Waste (RMW) disposal and medical surveillance (as needed).
- Safety equipment: Class I or II Biological Safety Cabinet (BSC) or equivalent containment for manipulations with potential for aerosolization or splashing; lab coats, gloves, eye/face protection.
- Facilities: BSL-1 facilities, with the addition of: available autoclave, directional airflow, no air recirculation, disinfection/decontamination procedures in place.

Biosafety Level 3 (BSL-3)

- Agents: serious or lethal diseases transmissible via aerosols, e.g., *M. tuberculosis*, SARS. Recombinant DNA activities using genetic material from BSL-3 organisms or such organisms as host cells. There is no BSL-3 facilities at this University.

Biosafety Level 4 (BSL-4)

- Organisms in this category are of such extremely high risk that only a handful of laboratories nationwide work at this level. There is no BSL-4 facilities at this University.

Table: Summary of Biosafety Levels 1 and 2 for Activities Performed with Infectious Agents at AHU.

	BSL-1	BSL-2
Agents	<p>Well-characterized agents not known to consistently cause disease in healthy adults, and of minimal potential hazard to lab personnel and environment.</p> <p>Appropriate for undergraduate and secondary educational training & teaching laboratories.</p> <p><i>*It is the responsibility of the lab instructor to determine the biosafety level classification of the microorganism in use. If question, contact EHS. The EHS will do routine audit on this regards.</i></p>	<p>Agents associated with human disease.</p> <p>BSL2 recommendations and OSHA requirements focus on the prevention of percutaneous, ingestion and mucous membrane exposure(s).</p> <p>AHU does not support any biohazard with classification greater than BSL-2.</p> <p><i>*It is the responsibility of the lab instructor to determine the biosafety level classification of the microorganism in use. If question, contact EHS. The EHS will do routine audit on this regards.</i></p>
Practices & Techniques	<ul style="list-style-type: none"> • Lab personnel have specific training in those procedures conducted in the laboratory • Supervised by a scientist with general training with the agent in use. • Control access to the laboratory. • Hand washing after handling cultures and before exiting lab • Eating, drinking, applying contact lens or cosmetics, the storage of food is prohibited • Mouth pipetting prohibited • “Sharps” policy instituted • All procedures minimize the creation of aerosols • Work surfaces decontaminated after spills and end of day • Waste disposal policy instituted • Biohazard sign posted at entrance when infectious agents are present , with name of agent(s) and name/phone # of supervisor • Insect/rodent control program is in effect. • Biohazard warning signs 	<p>BSL-1 plus:</p> <ul style="list-style-type: none"> • Lab personnel have specific training in handling pathogenic agents and are directed by competent scientists. • Policy/procedures whereby only persons meeting specific entry/training requirements may enter laboratory. Access to the laboratory is restricted when work is being conducted • Individuals at increased risk of acquiring infection are limited/restricted from the laboratory area • Each institution should consider the need for collection and storage of serum samples from at-risk personnel. • Biohazard sign (as BSL-1, plus): biosafety level, required immunization, required personal protective equipment, & any procedures required for exiting lab • Immunizations or tests provided for agents in laboratory (hepatitis B vaccine/TB skin testing)

		<ul style="list-style-type: none"> • Personnel receive appropriate training in safety precautions, exposure prevention, “sharps” precautions, and annual updates for procedure/policy changes • Biosafety manual defining infectious waste handling/decontamination and medical surveillance policies • Decontamination policy for work surfaces, spills, and contaminated equipment. • An accident policy involving an accidental/overt exposure to infectious materials that requires immediate reporting to lab director for documentation/medical evaluation/surveillance/and necessary treatment.
<p>Safety Equipment (primary barriers)</p>	<ul style="list-style-type: none"> • Work performed on open bench top • Lab coats, gowns, or uniforms to be worn to protect street clothes • Gloves are worn when hands may contact potentially infectious materials, surfaces or equipment. Disposable gloves are not to be re-used, washed or used to touch “clean” surfaces (telephones, etc.). • Hands are washed following glove removal. • Protective eyewear should be worn for procedures in which splashes is anticipated. • Persons wearing contact lens should also wear goggles or a face shield 	<p>BLS-1 plus:</p> <ul style="list-style-type: none"> • Properly maintained certified Biological Safety Cabinet (BSC) Class II for all manipulations involving splashes or aerosols of infectious materials. • Personal protective equipment (PPE’s): • Protective laboratory clothing. This clothing is removed and left in the lab area before leaving for non-laboratory areas. It is either disposable or laundered by the institution; it should never be taken home. • Face protection (goggles, mask, face shield or splatter guard) is used for anticipated splashes or sprays of hazardous materials for manipulations outside the BSC
<p>Facilities (secondary barriers)</p>	<ul style="list-style-type: none"> • Lockable doors for access control • Sink for handwashing • The laboratory is not necessarily separated from the general traffic patterns in the building 	<p>BLS-1 plus:</p> <ul style="list-style-type: none"> • Autoclave available • Recommended that sinks for handwashing be equipped with foot, knee, or automatic faucet operation

	<ul style="list-style-type: none"> • Lab designed to be cleaned easily. Carpets and rugs are not permitted • Bench tops impervious to water and resistant to moderate heat, organic solvents, acids, alkalis or chemicals used to decontaminate the work surfaces. • Furniture can support anticipated loading and uses, with spacing between cabinets, benches, and equipment accessible to cleaning • Windows fitted with screens • No recirculation of exhaust air 	<ul style="list-style-type: none"> • Locate BSC for optimal operation to maintain parameters for containment • Eyewash station is readily available • Negative airflow in lab • Impermeable work surface • Sealed/locked windows • Self-closing access controlled doors. • Bench tops impervious to water and resistant to heat, organic solvents, acids, alkalis or chemicals used to decontaminate the work surfaces. • Chairs with non-porous material that can be easily cleaned and decontaminated with disinfectant.
<p>Supplies</p>	<ul style="list-style-type: none"> • Biohazard container/bags • Dust pan and broom • Disinfectant (e.g. bleach and ethanol) • Sharps container • Disposable gloves • Lab coats • Goggles or face shield if needed. 	<p>BSL-1 plus:</p> <ul style="list-style-type: none"> • Autoclave tray • Secondary container for transporting biohazards (sealed, leak proof and labeled as biohazard). • Face and respiratory protection based on procedure.

Appendix C: Recommended Teaching Laboratory Safety Instructions

Instructions to the Faculty:

Recommended general laboratory safety procedures and rules to provide to students taking courses using biological hazards in a teaching BSL-1 laboratory. Biological substances (biohazardous) include medical waste, microorganism, tissues, cell-lines, nucleic acids and plant.

It is required that all instructor keep a log with the students' signature affirming that he/she/they have being trained and are ready to work in the laboratory.

The primary objective of this document is to provide a general guide for training students to work in laboratories. Wherever the scope of hazards is not adequately addressed by this general document, the principal investigator or laboratory coordinator must develop specific Standard Operating Procedures and address them with the students.

Instructions to the Students:

General Laboratory Safety Procedures and Rules for _____ (*course name*) _____ Laboratory Course (*indicate course code*)

Location: Indicate laboratory location (e.g. Campus Center 222)

Primary Contact: Professor Full name
(Principle Investigator) Office: Location (e.g. Campus Center 222)
Office: phone number

Secondary Contact: Lab Coordinator name
(Laboratory Coordinator) Office: Location (e.g. Campus Center 222)
Office: phone number

Emergency Department: Campus Security
407-353-4002

EHS Office: Environment, Health and Safety Office
Office: 407-303-7747 ext. 1103936

Fire/Police/Ambulance: **911**

General Laboratory Safety Procedures and Rules

Laboratory Safety

All students must read and understand the information in this document with regard to laboratory safety and emergency procedures prior to work in the laboratory. Your personal laboratory safety depends mostly on you. Effort has been made to address situations that may pose a hazard in the lab but the information and instructions provided cannot be considered all-inclusive.

With good judgment, the chance of an accident in the laboratory is very small. Nevertheless, research and teaching workplaces are full of potential hazards that can cause serious injury and or damage to the equipment. Working alone and unsupervised in laboratories is prohibited.

Safety training and/or information should be provided by a faculty member, teaching assistant, or staff member knowledgeable on this duty to all new students that will have access to this laboratory.

Emergency Response

- It is your responsibility to read safety and fire alarm posters and follow the instructions during an emergency.
- Know the location of the fire extinguisher, eye wash, and safety shower in your lab and know how to use them.
- Notify your instructor immediately after any injury, fire or explosion, or spill.
- Know the building evacuation procedures.

Common Sense

Good common sense is needed for safety in a laboratory. It is expected that each faculty, staff and student will work in a responsible manner and exercise good judgment and common sense. If at any time you are not sure how to handle a particular situation, ask the Laboratory Instructor for advice. **DO NOT TOUCH ANYTHING WITH WHICH YOU ARE NOT COMPLETELY FAMILIAR!** It is always better to ask questions than to risk harm to yourself or damage to the equipment.

Personal and General Laboratory Safety

- Never eat, drink, or smoke while working in the laboratory.
- Prior to using any reagent, read labels carefully.
- Do not use any equipment unless you are trained and approved as a user by the laboratory supervisor.
- Wear safety glasses or face shields when working with hazardous materials and/or equipment if instructed by lab supervisor.
- Wear gloves when using any biohazard or toxic material.
- Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals are not be worn in the lab at any time.
- If you have long hair or loose clothes, make sure it is tied back or confined.
- Keep your lab space clean and organized. Keep the work area clear of all materials except those needed for your work. Extra books, purses, etc. should be kept away from equipment that requires air flow or ventilation to prevent overheating.

- Disposal – You are responsible for the proper disposal of used material if any in appropriate containers provided by your laboratory supervisor. Dispose of sharps in the sharps containers provided in the lab.
- Equipment Failure - If a piece of equipment fails while being used, report it immediately to your laboratory supervisor. Never try to fix the problem yourself because you could harm yourself and others.
- Never pipette anything by mouth. Never taste anything.
- Clean up your work area before leaving.
- Wash hands before leaving the lab.
- Turn off heat block when not in use.
- Keep all pieces of equipment together (e.g. gel apparatus)
- Do not take anything out of the lab, unless authorized by your instructor.
- If a reagent bottle is empty, return it to the instructor. Do not put in the trash.
- If you are missing an item from your lab station, ask for a replacement – do not just take one from another lab station or laboratory storage.
- If you break something, let the lab supervisor know immediately.
- Never do unauthorized experiments.
- Never work alone in laboratory.
- Do not leave an on-going experiment unattended.
- Always inform your instructor if you break a thermometer. Do not clean mercury yourself!!
- Never use open flames in laboratory unless instructed by your laboratory instructor.
- Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates.
- Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes.
- Clean your lab bench and equipment, and lock the door before you leave the laboratory.
- Discuss with your instructor the location and use of the SDS. Discuss potential hazardous chemicals that will be used in lab.
- Discuss the location and appropriate use of biohazard bins, sharp boxes, and broken glass boxes.
- Discuss the location and appropriate use of fire extinguisher(s), fire blanket(s), flammables cabinet, first aid kit, and spill kit.
- Discuss the location of appropriate storage of your belongings including backpacks and books while taking the lab.
- Discuss with your instructor about all the additional policies pertaining to this laboratory discussed in the “Laboratory Safety Manual, Hazardous Waste Management Manual, and Biological Safety Manual” provided by the Environment, Health and Safety office.