UNIVERSITY OF FLORIDA

2014

Biosafety Manual

Business Affairs

Division of
Environmental Health & Safety

BioSafety Manual

University of Florida
Business Affairs
Environmental Health and Safety

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2014

Emergency Contacts

General

Police Emergency: University Police Department	**392-1111**
Fire Emergency: Gainesville Fire Rescue	**911**
Medical Emergency: Gainesville Fire Rescue	**911**
Gas leak: Gainesville Fire Rescue	**911**

Spills/Releases/Accidents: Notify the Principal Investigator, Lab Director/Supervisor and as appropriate for the following items:

	Business Hours	**After Hours**
Asbestos	**392-3392**	**392-1111**
Biological or Recombinant Materials	**392-1591**	**392-1111**
Select Agents	**392-1591**	**392-1111**
Chemicals (laboratory)	**392-1591**	**392-1111**
Mercury	**392-8400**	**392-1111**
Pesticides	**392-1904**	**392-1111**
Radioactive Materials: Campus	**392-7359**	**392-1111**
Radioactive Materials: Health Science Center	**392-1589**	**392-1111**

Needle Stick Hotline: 1-866-477-6824 (OUCH) Notify the Principal Investigator, or Lab Director / Supervisor and during working hours employees must call the UF Worker's Compensation Office (UFWC) at (352) 392-4940 prior to seeking treatment for work related injuries that are not immediate medical emergencies. See chapter on exposures and incidents for additional information.

Student Health Care Center Clinic, D2-49: (352) 294-5700

Table of Contents

Authority & Responsibilities

By authority delegated from the University President, the Vice President for Business Affairs is responsible for the safety of all University facilities. Under this authority, policies are developed to provide a safe teaching, research, service, housing and recreational environment.

The Division of Environmental Health and Safety was established in 1974 and given the responsibility for the management of all safety practices and the administration of the program.

The mission of the Environmental Health and Safety Division (EHS) is to support and advance the teaching, learning and research activities of the University through promotion of a safe and healthy campus environment. This is accomplished providing and coordinating programs and services that minimize safety, health, environmental and regulatory risks to the University of Florida community in a manner consistent with responsible fiscal and environmental stewardship. Inherent in this mission is the charge to provide a safe and healthy environment in which the University's activities can be pursued.

The University adopts all applicable federal and state safety laws, rules and regulations in order to carry out its duties and responsibilities. In additions, EH&S will reference standards or codes related to safety, which have been adopted and promulgated by nationally recognized standards-setting organizations. The interpretation of safety codes and standards is the responsibility of the Division of Environmental Health and Safety.

In order to assure an effective Environmental Health & Safety program for the University of Florida, it is imperative that all individuals associated with the University comply fully with the policies and procedures set forth in this manual.

Curtis Reynolds
Vice President
Business Affairs

Policy Statement

It is the policy of the University of Florida (UF) to provide a safe working and learning environment. The Biosafety Office has developed this manual as a guidance document to familiarize UF faculty, staff, students, volunteers, and visitors with the institution-wide policies and procedures for the safe use of biohazardous material at the University and its affiliates. When these policies and procedures are followed, the risk of occupational exposures to biohazards as well as the risk of accidental environmental release of biohazardous materials is minimized.

The primary responsibility for insuring safe conduct and conditions in the laboratory or research area resides with the principal investigator (PI). The PI should be familiar with the contents of this manual, make certain all his or her staff are familiar with it, and ensure all work with biohazardous materials is conducted in compliance with University policies and procedures. This Biosafety Manual should be used in conjunction with the UF Laboratory Safety Manual which provides additional general safety information. These manuals, produced by EHS, describe policies and procedures that are required for the safe conduct of research at the University of Florida.

Responsibilities

- **All individuals** working with or handling biohazardous materials should be committed to safety, and must demonstrate the ability to understand and follow:
  1. Safe work practices
  2. Applicable local, state, and federal requirements for work with these materials.

- **The Principal Investigator (PI)** is responsible for ensuring that all members of the laboratory are familiar with and adhere to safe research practices. In the clinical laboratory setting, the faculty member who supervises the laboratory is responsible for safety practices. The PI agrees to:
  1. Follow all local, state, and federal requirements applicable to his or her research.
  2. Register (and update) his or her work with the Biosafety Office and/or Institutional Biosafety Committee
  3. Inform laboratory staff and visitors of the hazards they may encounter and provide information on how to minimize the hazard exposure.
  4. Report exposures to or releases of biohazardous material to the Biosafety Office.
  5. Provide a safe work or learning environment.

- **Lab managers, supervisors, technicians** and others who provide supervisory roles in laboratories and clinical settings are responsible for overseeing the safety practices in laboratories.

- **Employees** who work with biological materials are responsible for:
  1. Reading this manual and understanding the contents.
  2. Carrying out the safety practices outlined in this manual.
  3. Reporting any problems, accidents, and spills to the PI or lab manager.

- **Environmental Health & Safety** will provide guidance, information, review, monitoring, and training regarding biosafety programs, when appropriate. This includes:
  1. Registering and tracking of projects utilizing biohazards.
  2. Evaluating work practices, safety and personal protective equipment, and facilities used for research or clinical activities with biohazards.
  3. Developing and implementing administrative controls (along with the Institutional Biosafety Committee) for biohazards.
  4. Serving as a liaison between the PI and local, state, and federal regulators.

- **Institutional Biosafety Committee** (IBC)
  1. Reviews and approves all experiments involving recombinant or synthetic nucleic acids.
  2. Works in conjunction with the EH&S Biosafety Office to establish, monitor, and enforce policies or procedures for work with biohazardous materials, including BSL3 and select agents.

Biosafety: Fundamentals and Definitions

All research projects at UF involving the following must be registered with the Biosafety Office by submitting the appropriate registration forms:

- 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 LD_{50} of ≤100 μg /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 registered 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 LD_{50} of <100 nanograms/kilogram.
- Materials that require state/federal permits or field releases of genetically modified organisms that require permits/notifications. A current copy of the permit or notification must be filed with the Biosafety Office.

Consult the Biosafety Office at 352-392-1591 or bso@ehs.ufl.edu for any questions on biohazards.

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 NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules apply to institutions, including the University of Florida (UF), that receive NIH funding for experiments involving recombinant or synthetic nucleic acid molecules. All recombinant or synthetic nucleic acid molecule projects must be registered 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” and are subject to federal oversight. The registration 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. A number of authorities regulate or provide best practice guidelines for the transport, storage, use and disposal of biohazards, ranging from local policies to international regulations. A list of these biosafety and biosecurity authorities and regulations, along with their websites, can be found in Appendix B. In addition, many are described throughout this document.

Biohazards Risk Assessment

Objective

To identify and manage/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/laboratory director 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 an occupational 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.
- Animal experiments.

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 Assessment

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 (see **Table 1**):

- 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.

Important guidance documents for risk assessment include Section VIII, CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, 2009 and Appendix B of the NIH Guidelines.

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. Safer versions of medical sharps may be found at the University of Virginia website Safety Device List.

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
- Changing bedding of infected animals
- 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 Dose
Agents will vary in their infectious dose, i.e. the amount of agent needed to cause disease:

Agent	Infectious Dose
Cryptosporidium	< 10
Mycobacterium tuberculosis	10
Listeria monocytogenes	<1,000
Salmonella typhi	100,000
Vibrio cholerae	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 naïve population. Such agents will typically require a minimum of BSL-3 containment. 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. The UF Student Health Care Center (SHCC) can provide such guidance: SHCC Clinic D2-49, 294-5700.

Containment
Four biosafety levels, BSL-1, 2, 3, 4, are described for activities involving biohazards (BSL4 projects are not permitted at UF). 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 Table 2 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.

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. **No BSL-4 experiments are permitted at UF.**

Animal Pathogens

When research work involves the use of laboratory animals, the risk assessment should include not only the agent and procedure risks, but also a consideration of the animal species to be used. Certain species are associated with:

- Physical risks to the handler, e.g. bites and scratches that may provide a route of exposure.
- Allergens
- Zoonotic agents, animal disease agents capable of infecting humans.

Principal investigators utilizing animals in their experiments must prepare Standard Operating Procedures (SOPs) that must document, but are not limited to, 1) the skills and training required for the safe handling of animals, 2) appropriate caging for containment of allergens or pathogens (zoonotic and/or experimental), and 3) documented methods to prevent the release of, or exposure to, pathogens during a) cage changing, b) delivery of the test material, c) animal transport, and d) necropsy.

Zoonoses

Sixty one percent of human disease agents are zoonotic or of zoonotic origin (*Risk factors for human disease emergence*, LH Taylor, SM Latham, MEJ Woolhouse, Phil. Trans. R. Soc. Lond. B (2001) 356, 983-989). Experimental animals can shed these zoonotic agents or infectious agents under study in saliva, urine, or feces. Experiments that demonstrate transmission of disease from an infected animal to a normal animal housed in the same cage are reliable indicators of hazard. However, experiments that show no such transmission do not rule out hazard. For example, experimental animals infected with *Francisella tularensis*, *Coxiella burnetii*, *Coccidioides immitis*, or *Chlamydia psittaci*, have caused many laboratory acquired infections (LAI), but rarely infect cage mates. See table below for examples of zoonotic diseases.

Examples of Zoonotic Diseases

Agent	Diseases
Bacillus anthracis	Anthrax
Borrelia burgdorferi	Lyme Disease
Brucella species	Brucellosis
Bartonella species	Cat scratch disease and related infections
Trypanosoma species	Trypanosomiasis (Chagas Disease)
Ringworm parasites	Dermatophytosis, dermatophilosis, sporotrichosis
H1N1 influenza	Swine flu
Eastern Equine Encephalitis Virus	Eastern Equine Encephalitis
E. coli O157:H7	Hemolytic Uremic Syndrome
Yersinia pestis	Plague
Francisella tularensis	Tularemia, Rabbit Fever
Coxiella burnetii	Q Fever
Rickettsia	Rocky Mountain spotted fever
Hantavirus-Sin Nombre Virus	Hantavirus pulmonary syndrome in North & South America

Routes of Transmission

Transmission of an agent from animal to human or animal to animal may occur by:

- Direct contact from the animal or contaminated items to mucous membranes, the GI tract (i.e. ingestion), or onto non-intact skin.
- The percutaneous route, via cuts, bites, scratches, needle sticks.

- Aerosols or droplets expelled by the animal from coughing, sneezing, barking, etc.,
- Vector-borne transmission of disease from fleas, ticks, and other arthropods that may be on the animal.

Non-human Primates (NHPs)

Disease transmission to and from non-human primates is of particular concern. The Animal Contact Medical Monitoring Program addresses the health risks of work with these animals, primarily tuberculosis and B-Virus (Herpesvirus simiae) infection. Please familiarize yourself with this information as well as that provided by Animal Care Services if you will work with NHPs. See table below for examples of human and NHP pathogens.

Examples of Human and Non-human Primate Pathogens

Agent	Diseases
Variola virus	Smallpox in humans
Polio virus	Paralytic poliomyelitis in humans
Vibrio parahemolyticus	Shellfish poisoning in humans
Non-zoonotic Influenza	Flu in humans. Humans have native influenza viruses in addition to potentially being infected by influenza viruses from other species.
Human Immunodeficiency Virus (HIV)	AIDS in humans
Simian Immunodeficiency Virus (SIV)	AIDS in NHPs. HIV and SIV are very closely related genetically.
Herpes Simplex-1 Virus	Cold sores in humans; rapidly fatal brain and central nervous system disease in some NHPs
Cerocopithicene Herpes simiae B virus	Cold sores in NHPs; rapidly fatal brain and central nervous system disease in humans

Containment: Animal Biosafety Levels

Four biosafety levels are described for activities involving infectious disease work with commonly used experimental animals. These four levels of combined practices, safety equipment, administrative controls, and laboratory facility design and features are designated Animal Biosafety Levels (ABSL) 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment. See **Table 3** for a summary of these Animal Biosafety Levels and the BMBL, 5th edition Section V—Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities for a more detailed description**. No ABSL4 experiments are permitted at UF.**

One additional biosafety level designated BSL3-Agriculture (or BSL3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL3-Ag facilities and USDA High Consequence Pathogens is provided in Appendix D of the BMBL.

NIH Guidelines for rDNA Experiments Involving Whole Animals

The NIH also devotes a section of the NIH Guidelines for experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. Appendix Q of the NIH Guidelines specifically addresses the physical and biological containment of large-sized animals, or those that have growth requirements (e.g. cattle, swine, sheep, goats, horses, and poultry) that preclude the use of typical containment for laboratory animals.

Animal Contact Medical Monitoring Program

Individuals who will be working with animals or in proximity to animals are required to participate in the Animal Contact Medical Monitoring Program, an educational and medical monitoring program designed to protect UF employees, students, and volunteers from animal-related illnesses. Medical monitoring is based on the type and frequency of exposure to animals and consists of a risk assessment, follow-up

assessments and tests/immunizations as needed. It is part of the UF Occupational Medicine Program. The educational section provides individuals with health information specific to animal contact and promotes safe work practices. The requirements of the program are based upon those outlined in the Public Health Services document, Guide for the Care and Use of Laboratory Animals and Occupational Health and Safety in the Care and Use of Research Animals published by the National Research Council. For more information on the Animal Contact Medical Monitoring Program, please refer to the Occupational Medicine Program section of this manual.

Plant Pathogens and Greenhouses

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 Biosafety 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. Please see the Bioagent Export Control List.

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) 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 IBC approval in addition to Federal and State approval. The Biosafety Office and/or IBC 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.

The following websites provide an overview of the regulatory process and related links:

- Florida Dept. of Ag. & Consumer Services, Dept. of Plant Industry (FDACS–DPI)
- USDA Biotechnology Regulatory Service (BRS) – 7CFR Part 340
- USDA Plant Protection & Quarantine (PPQ) – 7 CFR Part 330
- NIH Guidelines Appendix P (recombinant or synthetic nucleic acid molecules , transgenics, GMOs)
- APHIS PPQ Containment Guidelines
- FDA, for plant made pharmaceuticals or biotech foods and feeds
- EPA, for plant incorporated protectants

Video links from the “Regulatory Biosafety for Plant Research” symposium held on April 2010 at UF are available below for reference

- Regulation of Agricultural Biotechnology Products in the U.S
- PPQ Plant Pest and Biocontrol Permitting Policy
- State of Florida Perspective on Plant Pest Permitting

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 Biosafety Office 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 (see regulations section). Although it emphasizes containment principles for transgenic plants and associated organisms, the book "A Practical Guide to Containment, Greenhouse Research with Transgenic Plants and Microbes", available online at 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. **No BL4-P experiments are permitted at the University of Florida.**

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 Biosafety 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. Select Agent issues are covered in detail in the UF Select Agent Policy and Procedures Manual (contact the Biosafety Office for additional information) and on the Select Agent Program website. 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.

Recombinant and Synthetic Nucleic Acids

NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules: Summary of Experiments Covered

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. The following is a summary of experiments covered by the NIH Guidelines and is intended to assist in determining which category/categories experiments fall under:

Section III-A
Experiments Requiring IBC Approval, Recombinant DNA Advisory Committee (RAC) Review, and NIH Director Approval before Initiation.

III-A-1-a. This category is 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.*

Section III-B
Experiments Requiring IBC and NIH/OBA Approval before Initiation.

III-B-1. This category is limited to experiments involving the cloning of toxin molecules with LD_{50} of < 100 ng/kg body weight.
Examples: Botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin

Section III-C
Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review before Research Participant Enrollment.

III-C-1. This category pertains to experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participants.

Sections III-D, III-E, and III-F
The majority of the recombinant nucleic acid research at UF falls into one of these sections. The information regarding the various applications in these categories is presented in table format for quick reference.

Section III-D (see table 6)

Experiments Requiring IBC Approval before Initiation (note that BSL-4 agents cannot be used at UF).

Section III-E (see table 6)
Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation.

Section III-F (see table 6)
Experiments Exempt from IBC Review but Require Registration.

Note: The NIH has determined that rDNA from infectious agents of BSL-2 or higher is ***not*** exempt and must be approved by the IBC. Additionally, certain cloning vectors, such as Adeno or Sindbis based vectors, or amphotrophic MMLV-based vectors, are some examples of rDNA that are ***not*** exempt.

When conducting the Risk Assessment for work with genetically modified organisms (GMOs)

- Consider the same factors used for the wild-type organism.
- Consider the possibility that the genetic modification could alter the organism's pathogenicity, invasiveness, survivability, or susceptibility to effective treatments/controls.
- Note that important information may not be available for a newly engineered agent.
- Consider risks for human infection as well as risks to the environment.

Examples:

- Expression system (vector and host combination used to express a gene) potential risks:
    1. Ability to express gene in mammalian or human cells
    2. Replication competent or defective vector
        a. If replication defective, consider the ability to acquire replication competence through reversion, recombination/reassortment, complementation.
    3. Stability in the environment
    4. Immunogenicity in intended or non-intended host
    5. Tropism (target tissue or cell type) of gene expression
        a. Disruption of normal gene expression/function possible
    6. Modifications regarding pathogenicity, stability, host range, or tissue tropism.

- Non-coding/regulatory elements of the expression system (promoters, terminators, activators) potential risks:
    1. Level of gene expression from the promoter/enhancer.
    2. Promoter/enhancer driven tissue or cell type specificity.
    3. Well-characterized endogenous vs. uncharacterized artificial/novel promoters.
    4. Interactions with flanking sequences or competing promoters at or near the insertion site.

- Insert or transgene potential risk (i.e. impact of expression in intended or unintended host):
    1. Oncogenes or oncogenic potential (transcription factors, GTP-binding proteins, protein kinases)
    2. Toxins/cytotoxins
    3. Cytokines, growth factors, immunomodulatory proteins.
    4. RNA interference; siRNA, knockdown beneficial gene, knock down unintended gene(s).
    5. If from a pathogen, has the material been fully denatured (e.g. heat treatment), or could there be infectious organism still present?

- Risks associated with the recipient host:
    1. Pathogenic host
    2. Invasive host
    3. Transfer of altered pathogenicity, stability, resistance to treatments/controls to host.

- Scale of work:

Experiments involving more than 10 liters of material are subject to special requirements from the NIH. Appendix K. Physical Containment for Large Scale Uses of Organisms Containing Recombinant or Synthetic Nucleic Acid Molecules.

- Location of work:

The proximity of susceptible hosts is an important consideration for biological containment of the transgene. Such environmental protection measures are covered not only by the NIH Guidelines but also by the State of Florida Division of Plant Industry, the USDA Biotechnology Regulatory Service, the FDA (for genetically engineered foods), and the EPA (for genetically modified organisms with pest control activity).

Containment

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 BMBL 5th edition, the NIH Guidelines specify combinations of containment practices, safety equipment, and laboratory facilities. However, the NIH Guidelines stipulate an additional containment mechanism, "biological 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 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) 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 research (i.e. University of Florida). The construction and use of recombinant/synthetic nucleic acids or recombinant organisms requires registration with the Biosafety Office and/or Institutional Biosafety Committee. The NIH Guidelines specifically address recombinant and synthetic nucleic acids and the UF Biosafety Office requires registration for all projects involving recombinant nucleic acids, e.g. RNA derived from rDNA, RNAi, etc. Registration documents and instructions are provided on the Biosafety Website, see Registration Forms.

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 needles or sharps in experiments with toxins.

The use of biological toxins having an LD50 of ≤ 100 g/kg body weight (see tables 4, 5) requires:

- Project registration with the Biosafety Office (See Registration Forms)
- Inventory control log and signage.
- Standard Operating Procedures
- Security measures
- Proper disposal
- In general, BSL-2 containment, although a risk assessment may dictate other precautions.
- Some toxins are controlled for export and regulated as select agents (Table 6) requiring stringent regulations for possession, use, or transfer of non-exempt amounts (Table 7).

Resources:

- "Safety and health Considerations for Conducting Work with Biological Toxins", Johnson, Mastnjak, and Resnick; Applied Biosafety, 6(3) pp. 117-135, 2001
- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition

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 bloodborne 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 bloodborne 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 in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids.
- 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. UF has the Needle Stick Hotline (1-866-477-6824) in place for all BBP exposures and evaluates affected employees for treatment and post-exposure prophylaxis at the Occupational Medicine Clinic located in room D2-49.

UF Bloodborne Pathogen (BBP) Program

To curb the spread of these diseases in occupational settings, the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030) was implemented in 1992. The University of Florida follows this standard to protect staff, students, volunteers, and affiliates with potential exposure to human blood or OPIM as part of their work or participation in a University program; these individuals are required to participate in the UF Bloodborne Pathogen Program. The University of Florida has a university-wide Exposure Control Plan and individual departments have site-specific exposure control plans in place. Laboratory or clinic-specific Standard Operating Procedures are required for work with human blood or OPIM. To facilitate compliance a template is available (see link above).

Compliance with the UF Bloodborne Pathogen (BBP) program requires initial and annual BBP training, documented hepatitis B vaccination (or documented declination of the vaccine) as well as annual training on the proper segregation, handling, and disposal of biomedical waste. The training must be site-specific. To facilitate this, departments have a designated BBP trainer. Contact the Biosafety Office to identify your departmental trainer. For more information on the UF BBP Program, see the Occupational Medicine Programs section of this manual

Clinical Laboratories

Clinical laboratories typically receive clinical specimens with requests for a variety of diagnostic and clinical support services. Generally, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens.

Containment

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be performed safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA BBP standard. This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute http://www.clsi.org/. The CDC has also published Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories; Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel.

BSL-2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the

nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC is indicated to protect the integrity/sterility of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

UF Environmental Health & Safety has a Clinic Safety Specialist who can be contacted for more information; please call 392-1591.

Viral Vectors

The IBC (Institutional Biosafety Committee) reviews all projects involving use of recombinant viral vectors in vitro and in animals. The Recombinant or Synthetic Nucleic Acid Project Registration form is used to register the projects with the Biosafety Office. The most commonly used vectors are derived from adeno-associated virus (AAV), adenovirus (Ad), retrovirus (γ-retrovirus and lentivirus), poxvirus (Vaccinia), herpes simplex virus (HSV), Baculovirus, and Rabies virus. The vectors are engineered so that they no longer have the infectious replicative properties of the parent virus. Their purpose is to infect cells and deliver the gene or nucleic acid of interest with a goal to infect only the intended target and not the laboratorian or unintended hosts. A brief description of each of the vectors listed above is provided below.

Adeno-associated Virus (AAV):

AAV are small, non-enveloped, single-stranded DNA viruses belonging to Parvovirus family. They are non-pathogenic, defective viruses, requiring a helper virus e.g. adenovirus (Ad) or herpes virus (HSV) for productive replication of its genome. Under the NIH guidelines, AAV is a Risk Group 1 (RG1) agent. This virus, a type of parvovirus, is popular for gene transfer applications because it is non-pathogenic yet able to infect dividing and non-dividing cells, and can integrate into the host genome. The biosafety level for research with these vectors is determined on a case-by-case basis, depending on the associated genes. BSL-1/ABSL-1 is generally recommended with non-consequential inserts, BSL-2 or higher if expressing genes with oncogenic/toxin producing capacity or if a helper virus is used to produce recombinant AAV vector.

Structure: The viral genome is flanked by two inverted terminal repeats (ITRs) and consists of 2 open reading frames (*orfs*) encoding 4 regulatory proteins: Rep, involved in viral replication, and 3 capsid (Cap) structural proteins that are serotype-specific. Cap proteins have distinct tissue-binding affinity. Recombinant AAV (rAAV) generally lacks one or both of these genes which are supplied by the helper. Wild type (WT) AAV can stably integrate into specific location (AAVS1) on long arm of chromosome 19 and thus establish latent infection.

Design and production: pAAV expression plasmid devoid of the Rep and Cap genes and carrying the gene of interest, and either the WT helper viruses Ad/Herpes or one or two helper plasmids supplying Rep, Cap, and other essential adenoviral genes are transfected into the packaging cell line e.g. Human Embryonic Kidney 293 (HEK293). rAAV vector is purified from the culture supernatant.

Risk Assessment: AAV is a Risk Group 1 (RG1) agent. Biosafety level is decided on case-by-case basis, depending on the expressed genes. BSL-1/ABSL-1 is generally recommended with non-consequential inserts, BSL-2/ABSL-1+ (+ denotes additional safety recommendations e.g. use of specific PPE, safe sharps etc.) or higher if expressing oncogenic/toxigenic genes or if helper virus is used for production. Use of BSL-2 as a good microbiological practice when handling rAAV in vitro is always recommended.

Advantages of using AAV vector:

- It is safe and efficacious, devoid of viral genes.
- It may generate minimal immune response.
- It has wide range of tissue targets.

- Integration of rAAV genomic sequences in the absence of the AAV Rep proteins is insufficient and is not targeted to Chr 19.

Disadvantages of using AAV vector:

- It has a limited cloning capacity (5Kb); not suitable for large genes.
- May contain helper virus contamination.
- Large scale production is difficult.

References:

- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.
- R.H.Smith. Adeno-associated virus integration: virus versus vector. Gene Therapy (2008) 15: 817-822
- D. McCarty, S. Young Jr., & R. Samulski. Integration of adeno-associated virus (AAV) and recombinant AAV vectors. Annu. Rev. Genet. (2004) 38: 819-45
- Bec CL, & Douar, AM. Gene therapy progress and prospects-vectorology: design and production of expression in AAV vectors. Gene Therapy (2006) 13:805-813.

Adenovirus (Ad):

Ads are non-enveloped, double stranded DNA viruses that replicate in the nucleus. The viruses are grouped into six species, A-F, and currently include 51 serotypes. They are associated with gastrointestinal, ocular and respiratory infections and are transmitted by fecal-oral route.

Structure: Ad consists of a capsid which covers the genome that has inverted terminal repeats (ITRs) at each end. The genome consists of an early transcription region constituted by E1 (A & B), E2, E3, and E4 units, and a late transcription region. E1A is the first transcription unit to be expressed; the transcribed proteins can immortalize cells. E1A deletion renders the virus replication-deficient and eliminates its oncogenic potential. E1B proteins are responsible for viral mRNA transport from cell cytoplasm to the nucleus. E1A and E1B together form the transforming gene of the virus. E2, E3 and E4 function to suppress host immune responses and are not essential for viral replication.

Design & Production**:** The life cycle of the virus begins with its attachment to host cell surface receptors and entry inside the cells by endocytosis. In the cytoplasm, the capsid is released and the genome enters the nucleus where first the early genes are transcribed followed by the late phase in which viral replication takes place. Three generations of Ad vectors are commonly used: First generation, in which E1 and/or E3 are deleted, second generation in which additional to E1 and/or E3 deletion, E3 and E4 may be deleted, and the 'gutless/helper dependent vectors in which all genes are removed and structural proteins are provided by a helper virus.

Risk Assessment: Adenovirus is classified as a RG2 agent. IBC recommends a minimum of BSL-2 for in vitro handling of the vector and ABSL-1+ for animal injections, with + denoting use of "safe sharps", substituting plasticware for glass, and use of certain appropriate personal protective equipment. Post-injection animal housing is recommended at ABSL-1. The biosafety level maybe enhanced based on the nature of transgenes e.g. those with oncogenic potential or involving toxin production.

Advantages of using rAd vectors:

- Can infect dividing and non-dividing cells
- Ability to obtain high titers
- Capacity to clone large gene inserts
- Low particle-infectivity ratio
- Non-integrating; non-oncogenic

Disadvantages of using rAd vectors:

- Can elicit severe immune response

- Non-integrating-so cannot obtain stable, long-term expression
- Broad tropism so can infect non-target tissue
- Can cause common cold if replication competent virus is generated. This hazard is minimized by using replication defective vectors.

References:

- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.
- Shenk, T.E. Adenoviridae: the viruses and their replication. In *Fields Virology,* Fields, B.N., & Knipe, D. (Eds.). Philadelphia: Lippincott, Williams, and Wilkins, 2001; 2265-2300.
- Raper, S.E., Chirmule, N., Lee, F.S., Wivel, N.A., Bagg, A., Gao, G.P., et al. Fatal systemic inflammatory response syndrome in an ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 80: 148-158 (2003)
- Hutchins, B., Sajjadi, N., Seaver S., Shephard, A., Bauer, S.R., Simek, S., et al. Working toward an adenoviral vector testing standard. *Mol Ther* 2:532-534 (2000)

Baculovirus:

Baculoviruses are a group of enveloped, double-stranded DNA viruses capable of infecting certain invertebrate species but incapable of infecting or replicating in mammals or plants. *Autographica californica multiple nucleopolyhedrovirus* (AcMNPV) is most commonly used. The vector is generated using established insect cell lines. Experimental work with Baculovirus vectors is generally recommended at BSL-1. Based on the risk assessment, biosafety levels maybe higher if the vector is cloned with oncogenic/toxic inserts.

Advantages of using Baculovirus Vectors:

- Non-infectious to human cell lines
- Do not replicate in mammalian cells
- Do not induce antibody production in humans
- Can accommodate very large inserts (up to 38Kb)
- Promising human gene therapy tool

Disadvantages of using Baculovirus Vectors:

- Rapidly inactivated by complement

Reference:

- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.

Herpes simplex virus (HSV):

HSV is a linear double stranded, enveloped DNA virus belonging to Family Herpesviridae. The large and complex genome is covered by a capsid which, in turn is surrounded by an envelope. HSV-1 is most commonly used as a vector.

Viral Replication: Initially, the viral envelope attaches to host cell receptors. Fusion of the receptor with the envelope creates pores through which the virus enters the host cell. Early, then late proteins are transcribed. The genome enters the nucleus where the virus is packaged. HSV-1 can either go to a lytic phase in which the cell is lysed to release the virus or a latent phase in which it can remain dormant in a host cell for a long time.

Three types of HSV-1 based vectors have been developed:

- Recombinant replication competent vectors which have deleted accessory genes. These are generally used as oncolytic viruses.

- Recombinant replication defective vectors. In which early genes (α genes) are replaced by genes of interest; the deleted replication genes are provided by helper cell lines.
- Amplicon vectors. These are defective helper-dependent vectors that consist of plasmids carrying genes of interest, HSV-1 origin of replication and all packaging signals co-transfected with a helper virus into a cell line which permits the growth of the helper virus. To reduce cytotoxicity, helper viruses maybe replaced by a temperature sensitive (*ts*) mutants, by HSV-1 replication-defective mutants, or by using a helper-virus free packaging system.

Risk Assessment: HSV-1 is a Risk Group 2 (RG2) agent which can cause human infections. BSL-2 for in vitro work and ABSL-2 for animal experiments with the vectors is generally recommended. The recommendation may be decided on case-by-case basis depending upon the nature of inserts carried by these vectors.

Advantages of using Herpes Viral Vectors:

- Ability to package large DNA fragments (up to 30kb)
- Wide host range; Can achieve persistent infection in neuronal cells
- Suitable for non-dividing cells
- Non-integrating

Disadvantages of using Herpes Viral Vectors:

- Cytopathogenic
- Latency may switch off gene expression
- Capable of inducing immune response
- Transient transgene expression

References:

- Gene Therapy Herpesvirus Vectors Explained.
- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.
- Roberrto Manservigi, Rafaela Argnani, and Peggy Marconi. HSV Recombinant Vector for Gene Therapy. The Open Virology Journal (2010) 4: 123-156

Poxvirus (Vaccinia):

Poxviruses are double-stranded, enveloped DNA viruses belonging to poxvirus family. The viruses provide an excellent tool for recombinant DNA technology due to their large genome size capable of accommodating large inserts, up to 25Kb, and their ability for autonomous replication. The viruses are also very stable. Following are poxviruses of particular interest:

Genus *Orthopoxvirus*	e.g. vaccinia, variola, cowpox, monkeypox, and (now eradicated) small pox viruses
Genus *Leporipoxvirus*	e.g. myxoma virus
Genus *Molluscipoxvirus*	e.g. molluscum contagiosum virus (MCV),
Genus *Avipoxvirus*	e.g. canarypox and fowlpox viruses.

Viral replication: Poxviruses replicate in the cytoplasm of infected host cells by binding to host cell receptors, uncoating, expressing early genes that encode non-structural proteins, and then expressing late genes encoding structural proteins to make the virus.

Vector production: Highly attenuated strains of vaccinia virus e.g., MVA (Modified Vaccinia Ankara, cannot replicate in human cells), and NYVAC (derived from Copenhagen vaccine strain, replicates poorly in mammalian cells) and Avipoxviruses ALVAC (derived from canarypox virus) and TROVAC (derived

from fowlpox virus) are most widely used as vectors to express recombinant genes. The vectors are generated in infected cells by homologous recombination in which cells infected with vaccinia virus are simultaneously transfected with transfer plasmid carrying the gene of interest. The recombinant virus is isolated by plaque purification.

Risk Assessment: Safety issues arise since these recombinant viruses can replicate in mammalian cells and can cause severe infection in immunocompromised individuals. Laboratory acquired infections have been reported as a result of accidental inoculation.

- All projects involving poxviruses are reviewed by the IBC (Institutional Biosafety Committee)
- Working with vaccinia is usually recommended at BSL-2 or higher and animal work at ABSL-2 or higher. Final assessment of the biosafety level is decided by the IBC on case-by-case basis.
- Monkeypox is a Risk Group 3 (RG3) virus and a select agent. Work with this virus has to be conducted at BSL-3 or above
- Researchers working with vaccinia and other poxviruses can potentially get exposed to the virus by ingestion, parenteral inoculation, aerosols, or through broken skin
- Researchers who directly handle vaccinia vectors (other than highly attenuated MVA, NYVAC, ALVAC and TROVAC) in culture or work with animals infected with these, are required to receive vaccinia vaccination before they start working with these agents and to get re-vaccinated at least every 10years. Persons working with monkeypox virus must be vaccinated every 3 years.
- Immunocompromised individuals must not handle these viruses

Advantages of using Vaccinia Viral Vectors:

- Lyophilized poxvirus vectors are stable, cheap and easy to manufacture
- Accomodates large inserts
- Highly attenuated strains are very safe to use
- Capable of autonomous replication

Disadvantages of using Vaccinia Viral Vectors:

- Non-highly attenuated strains are infectious
- Vaccinia vaccination is required for persons working with non-highly attenuated strains.
- Immunocompromised individuals must not handle these viruses

References:

- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.
- Melanie Kremer, Asisa Volz, Joost H.C.M. Kreijtz, Robert Fux, Michael H. Lehmann, and Gerd Sutter. Easy and Efficient Protocols for Working with Recombinant Vaccinia Virus MVA. Methods in Molecular Biology (2012); 890: 59-92
- Gene Therapy Vaccinia Virus Explained
- Laboratory-Acquired Vaccinia Exposures and Infections-United States, 2005-2007. MMWR April 18, 2008: 57(15); 401-404

Rabies virus:

Rabies virus is a member of Rhabdoviridae family. These are bullet shaped enveloped, single-stranded, negative-sense RNA viruses. The viruses have affinity for neurons and spread within and between neurons in a retrograde fashion. Because of this unique property, the recombinant vectors are often used to track neuronal circuits.

Replication: Rabies glycoprotein (G) is essential for its packaging and trans-synaptic spread. The viral genome encodes for 5 proteins. Initially, the viral coat is removed in the cytoplasm of infected cells. Individual viral proteins are transcribed with the help of viral RNA-dependent RNA polymerase. Replication produces a positive strand RNA which, in turn makes the negative strand RNA. The complete life cycle occurs in the cytoplasm.

Production: Use of recombinant rabies virus with deleted G-glycoprotein (RV∆G) limits its trans- synaptic neuronal spread. RV∆G can be pseudotyped with VSV-G (Vesicular Stomatitis Virus Glycoprotein), EnvA and EnvB from avian sarcoma leucosis virus or HIV to incorporate non-native envelope proteins and change its tropism. The virus can be labelled with reporter genes (e.g. GFP, mCherry etc.) to allow tracking of the virus in infected cells.

Risk Assessment: Rabies is a Risk Group 2 (RG2) virus. Experiments with rabies virus vectors are generally recommended at BSL-2 for in vitro work and ABSL-2 for its use in animls. Biosafety level maybe higher depending upon the nature of the inserts.

Advantages of using Rabies Viral Vectors:

- Useful in retrograde neuronal tracking
- G-deleted vector cannot spread to surrounding cells
- Non-integrating

Disadvantages of using Rabies Viral Vectors:

- Can be cytotoxic on long-term use
- Cannot use for promoter-specific expression of transgenes (since it is a RNA virus)

References:

- Fumitaka Osakada & Edward M. Callaway. Design and generation of recombinant rabies virus vectors. Nature Protocols (2013) 8(8): 1583-1601

Retrovirus:

Retroviruses are enveloped, single-stranded RNA (ssRNA) viruses belonging to Family Retroviridae. The family consist of simple oncogenic retroviruses e.g. Murine Leukemia Virus (MLV) and complex lentiviruses. The virus has an outer protein capsid (envelope) surrounding the genome. The genome consists of 3 genes: *gag* which encodes the viral core, *pol* which encodes reverse transcriptase (RT) and integrase and *env* encoding the viral envelope protein, flanked by two incomplete long terminal repeats (5' and 3' LTR) comprising U3, R, and U5. Retroviruses attach to the host cell receptors. The core is released into the cytoplasm where, with the help of RT, the ssRNA is converted to dsDNA. In the host cell nucleus of a dividing cell, the dsDNA integrates into the genome with the help of viral integrase, is transcribed and translated into viral proteins. The cycle is completed by protein assembly and viral budding from the host plasma membrane. For vector production, the virus is made replication defective by replacing the *gag*, *pol*, and *env* genes with the genes of interest (GOI). A packaging cell line e.g. human embryonic kidney derived HEK293T is transfected with this expression plasmid; the eliminated genes are supplied on separate plasmids to prevent recombination. Simple retroviruses can also be made by transfecting the expression plasmid into packaging cell lines carrying the *gag*, *pol* and *env* genes. The host range of recombinant retroviral vectors is determined by its envelope. Deletion within the 3' U3, the enhancer/ promoter region renders the vector self-inactivating (SIN), reducing the risk of generation of Replication Competent Retrovirus (RCR) and insertional mutagenesis and increasing the safety of the vector.

Gammaretrovirus:

Based on the nature of the envelope, retroviruses can be ecotropic (can only infect mouse or rat cells) or amphotropic or xenotropic (capable of infecting a variety of cells including human cells). *NIH Guidelines for Research involving Recombinant or Synthetic Nucleic Acid Molecules*, March 2013, recommends containment levels used for Risk Group 1 (RG1) agent for ecotropic strains (e.g. MLV) requiring BSL-1 containment and practices. BSL-2 or higher containment, depending on the nature of the transgene, is recommended for amphotropic (e.g. VSV-G pseudotyped) and xenotropic strains. RCR data is required to decide the Animal Biosafety Level (ABSL) with a recommendation of ABSL-2 or higher if the data is not available. RCR test for MLV is well established and should be done for vectors other than ecotropic vectors. The following biological assays that are generally used include:

- S+/L- assay: Vector stocks are amplified by passaging in permissive cell lines (e.g. *Mus dunni*; CRL-2017). Culture supernatant is used to transduce naïve cell line (PG4; CRL-2032) which expresses murine sarcoma virus genome (S+) but not MLV (L-). Foci of transformed cells indicate presence of RCR in the supernatant. Cell lines and positive control virus (4070A VR-1450) are available from the ATCC.
- Marker Rescue assay: Permissive cell line used in amplification process contains a retroviral vector with marker gene e.g. for drug resistance. A positive RCR is indicated by the development of resistant clones.

Advantages of using Gammaretroviral Vectors:

- Can be used in actively dividing cells
- Used in clinical trials-though 4 cases of leukemia were reported

Disadvantages of using Gammaretroviral Vectors:

- Can only transduce actively dividing cells
- Can stably integrate into host genome
- Insertional mutagenesis is a point of concern.
- RCR can occur by combination between vector and either the viral genes, or endogenous viral sequences especially from murine-based cell lines.

References:

- Lakshmi Sastry and Kenneth Cornetta. (2009). Detection of Replication Competent Retrovirus and Lentivirus. *Methods in Molecular Biology, Methods and Protocols,* 506, 243-263
- Tobias Maetzig, Melanie Galla, Christopher Baum and axel Schambach (2011). Gammaretroviral Vectors: Biology, Technology and Application. Viruses3, 677-713
- L.-J. Chang and E.E. Gay. (2001). The Molecular Genetics of Lentiviral Vectors-Current and Future Perspectives. *Current Gene Therapy* 1, 237-251
- Keith Bupp and Monica Roth. (2000). Strategies and mechanisms for retrovirus retargeting. In *Viral Vectors: Basic Science and Gene Therapy.* Edited by A. Cid-Arregui and A. Garcia-Carrancà

Lentivirus:

Lentiviruses are complex retroviruses that include Human Immunodeficiency virus (HIV-1), Simian Immunodeficiency virus (SIV), and Feline Immunodeficiency virus (FIV-1). HIV-1 -derived vectors are extensively used in research as viral vectors. The vectors are particularly useful due to their ability to infect both the dividing and non-dividing cells. Aside from retroviral genes *gag*, *pol*, and *env*, lentiviruses contain other regulatory and accessory genes e.g. *rev, tat, nef, vpr, vpu and vif* that have a role in viral replication and infection. Lentiviral vectors (LVV) are typically made by transient transfection of 3-4 plasmids into HEK293 or 293T cell line. The vector has evolved over time to address the safety concerns. The first generation LVV was manufactured using all HIV-1 genes except the envelope gene. In the second generation packaging system, made with 3 plasmids, all except 4 HIV-1 genes *viz. gag, pol, rev* and *tat* are eliminated. In the third generation vector which utilizes 4 plasmids, *tat* is removed, *gag/pol* and *rev* are placed on separate expression cassettes and there is a chimeric 5'LTR (long terminal repeat).

Other safety features of the vector include a pseudotyped envelope (e.g. VSV-G), which enhances the vector tropism and 3'LTR deletions which eliminates the viral LTR enhancer/ promoter activity making it transcriptionally inactive (SIN or self-inactivating LVV).

Replication Competent Lentivirus (RCL) testing. Even if the vector is non-replicating, there is a theoretical possibility of homologous recombination event between different vector components rendering it replication competent (RCL). Several assays are used to test the vector stocks for presence of RCL, notably a cell culture-based assay in which RCL in the vector stock, if present is amplified by culturing onto a permissive cell line (various lines are available). Supernatant containing viral particles is collected

and inoculated on to an indicator cell line. Cells/supernatants are analyzed for viral markers using various tests e.g.:

- $p24^{gag}$ antigen ELISA. Decrease in protein expression with successive passages indicates absence of RCL. A kit is available (Perkin Elmer) to detect protein expression. Positive control standard (purified p24 *gag* protein) is included in the kit. The test is easy, rapid and reproducible but the kits are expensive.
- Product-enhanced reverse transcriptase (PERT) assay to detect reverse transcriptase associated with the virus. This is reported to be a very sensitive assay.
- *psi-gag* and *VSV-G* envelope PCR assays using genomic DNA extracted from indicator cell line. Plasmid DNA containing the respective sequences is used as positive control.
- Marker Rescue assay: GFP expressing cell line (e.g. HeLa4G) is transduced with LVV vector stock. The cells are passaged a few times after which the media is inoculated onto a GFP-negative cell line (e.g. 293T). GFP expression is studied by FACS/ immunofluorescence.

Positive controls are required and must be included with the tests. At least 2 consecutive assays on independently generated LVV preparations are usually recommended. Use of at least two separate assays that detect different viral genes or functions is advised.

Risk Assessment:

- BSL-2+ is recommended for in vitro use of LVV; + emphasizes appropriate personal protective equipment, safety sharps and substitution of plastic for glass. Caution is always advised to take measures to avoid self-inoculation with LVV to prevent insertional dysregulation of cellular genes that can occur at a frequent rate
- ABSL-2 is recommended for animal injections and housing for use of second generation or lower LVV where RCL data is not available, lowered to ABSL-1+ with instructions for using safe sharps, safety needles, protection of mucous membranes, skin, eyes, and conducting work in certified biosafety cabinets to minimize the risk to researchers in case of accidental exposure to the vector, with ABSL-1 for subsequent housing if the vector stock is tested for RCL and results of 3 consecutive tests are reviewed by the Institutional Biosafety Committee (IBC).
- All LVV made using 3^{rd} and 4^{th} generation systems are considered replication incompetent and RCL testing is not required. These are generally recommended at ABSL1+ for animal handling and ABSL-1 for housing
- ABSL-1+/ABSL-1 is also recommended if the vector backbone has tested negative for RCL in the past (by UF PI) or if the documentation of RCL testing for vectors obtained from PIs outside of UF, or from a commercial source is available and is reviewed by the IBC.
- Based on the risk assessment, biosafety levels maybe higher if LVV is cloned with oncogenic/toxic inserts.
- Large scale LVV preparation (>10L) is recommended at BSL-3 containment level

Advantages of using Lentiviral Vectors:

- Can be used in non-dividing cells
- Used in clinical trials

Disadvantages of using Lentiviral Vectors:

- Can stably integrate into host genome
- Insertional mutagenesis is a point of concern.
- RCL can occur by combination between vector and the viral genes

References:

- Stéphanie Durand and Andrea Cimarelli (2011). The inside out of Lentiviral vectors. Viruses 3, 132-159
- James N. Warnock, Claire Daigre, & Mohamed Al-Rubeai. Introduction to viral vectors. Methods in Molecular Biology (2011) 737: 1-25.

- Kenneth Cornetta, Jing Yao, Aparna Jasti, Sue Koop, Mokhaila Douglas, David Hsu, Larry A. Couture, Troy Hawkins and Lisa Duffy. Replication-competent lentivirus analysis of clinical grade vector products. Molecular Therapy (2011); 19(3): 557-566.
- L-J Chang, V. Urlacher, T. Iwakuma and J. Zucali. Efficacy and safety analyses of a recombinant human immunodeficiency virus type 1 derived vector system. Gene Therapy (1999); 6: 715-728.
- Lakshmi Sastry and Kenneth Cornetta. (2009). Detection of Replication Competent Retrovirus and Lentivirus. *Methods in Molecular Biology, Methods and Protocols,* 506, 243-263
- Philip W. Hargrove, Steven Kepes, Hideki Hanawa, John C. Obenauer, Deiqing Pei, Cheng Cheng, John T. Gray, Geoffrey Neale and Derek A. Persons. Globin Lentiviral Vector Insertions can perturb the Expression of Endogenous Genes in β-thalassemic Hematopoietic Cells. Molecular Therapy (2008); 16(3): 525-533
- Adam S. Cockrell & Tal Kafri. Gene delivery by lentivirus vectors. Molecular Biotechnology (2007) 36: 184-204

Sindbis Virus

Sindbis Virus is a positive strand, enveloped alphavirus belonging to family *Togaviridae.* The virus is blood-borne and replication defective vectors has been used to treat mouse tumors since the vector can be effectively administered systemically. Packaged vector, however, is self-replicating and can amplify in host cells to express high levels of transgenes.

References:

- Lisa Venticinque and Daniel Meruelo. Sindbis viral vector induced apoptosis requires translational inhibition and signaling through Mcl-1 and Bak. Molecular Cancer (2010), 9:37
- K-W Peng and E. Galanis. Sindbis vectors: illuminating the path to ovarian cancer therapy. Gene Therapy (2005) 12, 381-382.

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, biosafety officers, 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.

The following resources should be reviewed for best practices and regulations for laboratories possessing restricted agents. It should be noted that these regulations are not limited to the individual laboratories possessing restricted agents.

- Select Agent Requirements (42 C.F.R. Parts 72 and 73, 7 C.F.R. Part 331, 9 C.F.R. Part 121) addressing Select Agents and Toxins, Registered Entities, Restricted Access, Training and SOPs
- Army Regulations Regarding Biological Surety for DOD facilities, institutes receiving DOD furnished select agents or research funding involving select agents
- Biosecurity recommendations - BMBL 5th Ed. Section VI - Laboratory Biosecurity
- National Science Advisory Board for Biosecurity
- The Select Agent Program Laboratory Security Guidance Document

Background Screening

In support of efforts to maintain and foster safety and security of students, faculty, and staff, the University of Florida requires pre-employment criminal background checks on new hires for TEAMS and Faculty 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.

All criminal background checks will be coordinated through Recruitment and Staffing, Human Resources. Determination of the type of criminal background checks to be conducted will be made by Human Resource Services in conjunction with the hiring department. For more detailed information regarding background checks and their costs or to request a background check, simply complete the Request for Background Screening form and fax it to Recruitment and Staffing at (352) 846-0668. The available background checks are:

- Alachua On-Line (AOL)
- Florida Department of Law Enforcement (FDLE)
- FBI Livescan
- FBI Manual
- 435 Livescan
- 435 Manual
- HireRight

The University of Florida will ensure that all background checks are held in compliance with federal and state statutes, such as Fair Credit Reporting Act, when applicable.

In addition, all staff that may be associated with work involving select agents will require a Security Risk Assessment (SRA). A security risk assessment is the method used by the Criminal Justice Information Service (CJIS) to evaluate an individual's suitability to access Select Agents. This risk assessment is intended to identify those individuals who are prohibited from access to Select Agents and based on the restrictions identified in the USA PATRIOT Act. Additionally, individuals that will work with Tier 1 select agents (i.e. new hires, graduate students, etc.,) must clear the UF pre-access suitability assessment. All individuals with access to Tier 1 agents must enroll in the UF on-going suitability assessment program. The UF Select Agent Program Responsible Official coordinates all activities regarding applications for security risk assessment with CJIS as well as the UF suitability assessments. For additional information regarding access to select agents, suitability assessment clearance and program enrollment, and security risk assessments, please contact the Biosafety Office at (352) 392-1591.

Dual Use Research

The National Science Advisory Board for Biosecurity (NSABB), a federal advisory group formed in 2004, is developing guidelines to advise federal agencies (e.g. NIH, HHS, etc.) on the identification, oversight, and possible regulation of “dual use” research. The NSABB charter was renewed in April 2012. Dual use research of concern (DURC) is that which, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment or

material. The catalyst for increased oversight of such research was the release of the 2004 National Academies Publication Biotechnology in an Age of Terrorism (a.k.a. the "Fink Report"). The report urged increased biosecurity for dual use research and identified seven categories of research that are of particular concern:

- Enhance the harmful consequences of a biological agent or toxin.
- Disrupt immunity or the effectiveness of an immunization without clinical and/or agricultural justification
- Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies.
- Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin.
- Alter the host range or tropism of a biological agent or toxin.
- Enhance the susceptibility of a host population.
- Generate a novel pathogenic agent or toxin, or reconstitute an eradicated or extinct biological agent.

In March 2012 The United States Government published a Policy for Oversight of Life Sciences Dual Use Research of Concern that is focused on a specific set of agents (see section III of this policy) along with the categories of research experiments of particular concern regarding these agents similar to the above:

- Enhances the harmful consequences of the agent or toxin
- Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification
- Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
- Increases the stability, transmissibility, or the ability to disseminate the agent or toxin
- Alters the host range or tropism of the agent or toxin
- Enhances the susceptibility of a host population to the agent or toxin.
- Generates or reconstitutes an eradicated or extinct agent or toxin listed in Section III.1 of the Policy.

The IBC and Biosafety 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.

The Executive Orders that address these issues:

- Executive Order 13486 (Jan. 2009) 'Strengthening Laboratory Biosecurity in the United States': Work Group to evaluate and recommend physical and personal security regulations and practices for SA facilities.
- Executive Order 13546 (July 2010) 'Optimizing the Security of Biological Select Agents and Toxins in the United States'. The Federal Experts Security Advisory Panel (FESAP) issued a report in November 2010 with revisions in December 2010 and January 2011 and recommended:
  1. Agents Comprising Tier 1 BSAT
  2. Removal of BSAT on the SA list
  3. Practices to ensure personnel reliability with access to BSAT and Tier 1 BSAT
  4. Practices for physical and cyber security for facilities with BSAT and Tier 1 BSAT

The amended regulations addressing the above revisions became effective December 4, 2012 and include 7 CFR part 331, 9 CFR part 121 and 42 CFR part 73.

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 (**BSL-4 projects are not permitted at UF**). 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 Table 2 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
    1. 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
    2. More (or less) stringent practices may be specified by the Biosafety Office and/or the Institutional Biosafety Committee (IBC) risk assessment review depending on information suggesting significant alteration in virulence, pathogenicity, antibiotic resistance patterns, vaccine/treatment availability or other factors,
    3. Large volumes, high concentrations, or higher risk procedures will dictate an increase in BSL.
    4. The Biosafety Office or IBC requirements must be adhered to unless new information to justify a change is provided to the IBC and/or Biosafety Office 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 director, principal investigator or supervisor 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.
- Each laboratory should develop or adopt a laboratory-specific safety manual that coordinates with the institution-wide UF 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.
- The laboratory director is responsible for implementing additional safety practices commensurate with the hazards associated with the agent or procedure. 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, 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, droplets, or aerosols generated by many microbiological procedures. All BSCs must be certified annually. Precision Air, 352-332-4653, holds the current contract to provide this service.

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 types of BSCs commonly used in laboratories are described in detail in the BMBL chapter: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets. 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 lfpm (A2, B1, and B2 have 100 lpfm) 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 lpm. 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 manufacturer 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:
  1. Away from the entry to the laboratory and from the laboratory traffic.
  2. Adequate clearance must be provided around, and 12”-14” clearance above, the BSC for easy access and to provide for accurate air velocity measurement
  3. 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 (see Principles of Good Microbiological Practice handout) 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 contaminated (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:
  1. 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 Biosafety Office regarding alternatives to the use of these devices.
  2. 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:

1. 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.
2. 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. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag or discard tray inside the BSC. 200 ml of water should be added to the bag or tray prior to autoclaving.
- When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing 200 ml of water to ensure steam generation during the autoclave cycle. The bag should be vented or the discard pan should be covered in the BSC prior to removal to the autoclave. The bag should be transported and autoclaved in a leak-proof tray.
- Ultraviolet (UV) lamps are not required or necessary in BSC.
  1. If installed, the lamps must be cleaned and checked periodically with a UV meter to confirm appropriate emission.
  2. UV lamps must be turned off when the room is occupied to protect eyes and reduce skin exposure.
  3. Close the sash in BSC when operating UV lamp
- Spills inside the BSC must be handled immediately; see the Exposures and Incidents section of the manual and the Biosafety Office poster Handling Biological Spills
- The BSC must be professionally certified per NSF/ANSI49-2002 Standard when used to handle infectious and potentially infectious material:
  1. After initial installation
  2. At least annually thereafter
  3. 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 directors 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 don and doff 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. Employer should ascertain that in-house laundry service is in place (if not, contact S & S Cleaners, 503 SW 3rd Street; 372-4184). If contaminated, laboratory coats should be decontaminated with bleach before dispatching the coats to the laundromat.
- Disposable lab coats are preferred. These are reusable and available from Fisher Scientific (state contract).

Gloves

- Gloves should be worn to protect hands from hazardous materials. Disposable latex and nitrile gloves are commonly used. Some people develop latex allergy (see Latex Allergy A Prevention Guide) 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. In several UF buildings, e.g. in Health Science center, gloves are not permitted in regular trash (perceived as medical/biomedical waste). If you have questions regarding proper disposal, contact the Biosafety Office (392-1591).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 and face is useful in preventing potential mucous membrane (e.g. ocular) exposure to infectious agents in the form of splashes, sprays, or respiratory droplets (see Eye Protection for Infection Control). The Biosafety Office 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' under UF's Respiratory Protection Program. 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 Biosafety 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, while attempting to clean up biological spills, and in BSL-3 laboratories. The shoe covers should be discarded as biohazardous waste, and autoclaved if contaminated.

Administrative Controls

Institutional policies have been established and are enforced by UF to ensure the safety of laboratory workers, the public, the environment and the institution.

These programs are discussed in greater detail in this manual and include, but are not limited to occupational medical surveillance, training and compliance, incident reporting, and laboratory signage. The Biosafety Office and IBC are responsible for developing and ensuring compliance with administrative controls.

Laboratory Assessment and Improvement (Biosafety Facility Reviews/Surveys/Inspections)

Biosafety facility reviews/surveys/inspections are conducted in laboratories, green houses and animal facilities 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, BSL3 agents, and those funded by certain agencies (e.g. Dept. of Defense) are inspected frequently. The checklist used for the inspection depends on the type of research and/or the biosafety level(s) assigned to the project(s).

- Facilities working with pathogenic or potentially pathogenic materials are generally inspected based on the requirements specified in BMBL, 5th edition. Specific requirements for BSL1 - BSL4 are outlined in Section IV, Laboratory Biosafety Level Criteria. Checklists based on these criteria

for BSL1, BSL2, and BSL3 facilities are available from the Biosafety Office (BSL4 projects are not permitted at UF).

- Facilities with pathogenic or potentially pathogenic materials in animals is inspected based on the requirements specified in BMBL, 5th edition. Specific requirements for ABSL1 - ABSL4 are outlined in Section V, Vertebrate Animal Biosafety Level Criteria. Checklists based on these criteria for ABSL1, ABSL2, and ABSL3 facilities are available from the Biosafety Office (ABSL4 projects are not permitted at UF).
- Laboratories that work with biological toxins are inspected for compliance with UF policies, as described in the Standard Operating Procedures template**,** and federal guidelines detailed in Appendix I of the BMBL, 5th edition.
- Facilities working with recombinant or synthetic nucleic acids are inspected for compliance with the appropriate appendices of the NIH Guidelines:
    1. For lab-scale recombinant or synthetic nucleic acid molecules work, Appendix G
    2. For Large Scale (>10L) recombinant or synthetic nucleic acid molecules work, Appendix K
    3. 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).
    4. For recombinant or synthetic nucleic acid molecules work involving large animals Appendix Q (note that the biosafety levels described by the NIH in Appendix Q are referred to as BSL1-N-BSL4-N)

Areas where biological agents regulated by state or federal permits are used, stored or planted/released are inspected based upon the conditions specified by the permit itself, as well as best practices listed by the agency issuing the permit (State of Florida Division of Plant Industry, USDA Biotechnology Regulatory Service, FDA and EPA (for genetically modified organisms with pest control activity), USDA/APHIS Import/Export Services, and CDC Etiologic Agent Import Permit Program).

Institutional Biosafety Committee (IBC)

An Institutional Biosafety Committee (IBC) is required at institutions that receive funding from the National Institutes of Health (NIH) for research involving recombinant or synthetic nucleic acid molecules. The UF IBC is a Presidential-level committee with appointments made by UF's Vice President for Research. All recombinant or synthetic nucleic acid research at UF regardless of funding source, must be conducted in accordance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules; most of which requires registration with the UF IBC. The UF IBC also reviews and approves registrations for research performed in BSL3/ABSL-3 laboratories and USDA/HHS Select Agents.

The IBC works in conjunction with the UF Biosafety Office to establish, monitor, and enforce policies or procedures for work with biohazardous materials. The IBC is authorized to inspect research facilities, register, review, and approve research protocols; and to take actions to enforce safe research practices or halt research activities in the event of unsafe practices that endanger worker, community, or environmental health.

The IBC meets every month for review and approval of protocols and to discuss other business relevant to the charter of the IBC.

Committee Charter

- To ensure that all recombinant or synthetic nucleic acid research conducted at UF or sponsored by UF is conducted in compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, and to review and approve research that is in conformity with the Guidelines.
- To ensure that research protocols involving BSL3 agents and USDA/HHS Select Agents, including but not limited to recombinant or synthetic nucleic acids, are reviewed and found to comply with all national, state, and local requirements.
- To interpret guidelines and/or regulations pertaining to biological materials, and provide technical assistance to the UF Biosafety Office and University Community on these matters, such as the safe handling, transport, use, and disposal of biological materials, including recombinant or synthetic nucleic acid molecules.

Membership

- Membership consists of two community members (non-UF), a non-doctoral member, the University Biosafety Officer, and other members with expertise in biosafety, plant science, animal science, virology, bacteriology, pathology, and (human) gene therapy.

Procedure for IBC Protocol Registration and Review

- Principal Investigators must submit a registration form for all protocols requiring IBC review to the Biosafety Office in accordance with this schedule.
- After initial review of the protocols by the Biosafety Office staff, investigators may be asked to modify or correct protocols. All modifications and corrections must be submitted to the Biosafety Office prior to the IBC meeting.
- Experiments involving deliberate transfer of recombinant or synthetic nucleic acid molecules into human subjects must also be reviewed by the NIH OBA (Appendix M of the NIH Guidelines) and University of Florida IRB.
    1. If UF has an Institutional conflict of interest, such as owning the technology, the Western Institutional Review Board (WIRB) can be the IRB of record, but the UF IRB must be allowed to comment prior to submission to the WIRB; the WIRB will take those comments into consideration.
    2. Transfer of recombinant or synthetic nucleic acid molecules into human subjects studies also require the submission of: a study protocol, informed consent, investigator brochure and copies of relevant RAC correspondence
- Experiments involving any animal work must also be approved by the University of Florida IACUC. IACUC protocols will not receive final approval until Biosafety Office and/or IBC approval is obtained. The Biosafety Office staff advise the IACUC staff of approved, denied, or tabled projects.
- Experiments involving any USDA/HHS Select Agents must also be registered with the CDC or USDA. These protocols must be submitted to the Biosafety Office who will coordinate with the CDC/USDA for registration.

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, Material Safety Data Sheets (MSDS) 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 researcher or clinician 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 antisepsis (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

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 of biological waste. Refresher training is required annually. Training may be accomplished through the on-line UF Biomedical Waste Training program. For assistance, please see the training section of this manual or call the Biosafety Office at 352-392-1591.

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 EH&S and the department.

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

Infectious, Potentially Infectious, Recombinant or Synthetic Nucleic Acid Biological Waste

Waste items that are, contain, or are contaminated with:

- Human, animal, or plant pathogens
- Recombinant or synthetic nucleic acids and recombinant organisms
- Laboratory and clinical 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

Laboratory waste containing infectious, potentially infectious, or recombinant or synthetic nucleic acid molecules must be inactivated prior to leaving the facility. The preferred method is steam sterilization (autoclaving), although incineration or chemical inactivation (e.g. treatment with household bleach) may be appropriate in some cases. Storage of all non-inactivated waste in this category is restricted to within the generating laboratory. Infectious or pathogenic waste must be held in a closed/covered biowaste container and may not be stored longer than 24 hours prior to inactivation. 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 Biological Waste

Waste items that are:

- Used labware (tissue culture dishes and flasks, petri dishes, centrifuge tubes, test tubes, pipettes, vials, etc.) from clinical or biomedical labs that is NOT contaminated with any of the biological wastes listed in Infectious, Potentially Infectious or Recombinant or Synthetic Nucleic Acid Biological Waste category above. This material does NOT qualify for disposal as Clean Lab Ware.
- Unused medical devices. This material does NOT qualify for disposal as Clean Lab Ware.

- Gloves or other disposable personal protective equipment from clinical or biomedical labs that are NOT contaminated with any of the biological wastes listed in Infectious, Potentially Infectious or Recombinant or Synthetic Nucleic Acid Biological Waste category above and not contaminated with hazardous chemicals.
- Blood, blood products, tissues, or items contaminated with these, from animals not known to, or expected to, contain pathogens.

This waste does not require inactivation before it leaves the facility. Place this waste in the red bag-lined cardboard biological waste box for disposal.

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 ¾ full and discarded within 30 days after closure. Sharps boxes are placed into the red bag-lined cardboard biological waste box for disposal. If contaminated with infectious, potentially infectious, or recombinant or synthetic nucleic acid, the sharps box must be autoclaved before 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. Use:

- Sharps Box (date when first placed into use and dispose within 30 days of first use).
- 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.
- If contaminated with infectious, potentially infectious, or recombinant or synthetic nucleic acid, the material must be inactivated before disposal.

Mixed Radioactive/Biological Waste

The infectious, potentially infectious, or recombinant or synthetic nucleic acid component(s) of mixed radioactive/biohazardous waste shall be inactivated (if possible) prior to its release to Radiation Safety Services for disposal as radioactive waste. Please contact the Radiation Safety Office (392-7359) regarding the best method of inactivation.

Mixed chemical/biological waste

The infectious, potentially infectious, or recombinant or synthetic nucleic acid component(s) of mixed chemical/biohazardous waste shall be inactivated (if possible) prior to turning it over to EH&S Hazardous Materials Management for chemical disposal. Precautions should be taken to prevent the generation and release of toxic chemicals during the inactivation process. In general, autoclaving is not recommended. Note that the chemical component of the waste may inactivate the biohazard (e.g. as in the case of fixative solutions). Please contact the Hazardous Materials Management Facility at 352-392-8400 or the Biosafety Office (392-1591) for guidance regarding particular chemicals. Chemical waste must be segregated, stored, labeled, and handled per the requirements outlined in the Chemical Waste Management Guide.

Human Remains/Tissues

Contact the Biosafety Office (352) 392-1591 or the State Anatomical Review Board (352) 392-3588 for information. Off campus facilities should contact the Biosafety Office at (352) 392-1591 for guidance.

Biological Waste Packing, Labeling, & Transport

The transport of biohazardous waste outside of the lab (i.e. to an autoclave or incinerator) must be in a closed, leak-proof bag or container; bags must be contained in a leak proof tray and transported on a cart to and from the autoclave. Do not leave non-inactivated waste unattended. Laboratory staff needing to transport properly packaged and labeled biowaste boxes to a secure storage/pick up area must protect the boxes from the weather and not leave the boxes unattended. For those laboratories that do not have an established routine pickup or an established secure storage/pick up area in the facility, the following is required for transporting Biomedical Waste to the loading dock of the Health Science Center for disposal:

- A state vehicle is required for transport, not a personal vehicle.
- Must move less than 25 lbs. at a time (I box).
- The locked silver Semi Trailer at the loading dock of the Health Center is the disposal site.

- Call Building Services, 294-5500, to arrange to meet someone with the keys.
- The usual meeting site is AG-129, ground floor of the Dental Sciences Bldg.
- Leave a voice mail message if need be, and someone will return your call.
- Be prepared to show your photo ID, if requested.

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.
- Biowaste box availability: In the Health Science Center (HSC) call 294-5500 for routine scheduled biowaste box delivery, pickup or problems. Labs not on a routine delivery should call 392-4414 to obtain occasionally needed biowaste boxes. Personnel from outside of the HSC call 392-5775 to arrange to pick them up in room AG133 of the HSC.

Biohazardous Waste Bags

Refer to the biohazardous 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.
- A red bag–lined cardboard biowaste box is used to collect biowaste (e.g. bags of autoclaved waste, closed sharps containers, other materials requiring red bag disposal per guidance in the biowaste handling section of this manual) for disposal by a commercial waste transport company (currently Stericycle). A very small percentage of UF biowaste is collected into red bags for bulk disposal (no box); this applies only to select animal facilities. Red liner bags or red bulk disposal bags **must be certified and stamped** by the manufacturer with *state and federal* regulations for bag composition, tear resistance, and impact resistance.
- Two types of red liner bag (or red bulk disposal bag) are available that meet the requirement:
  1. Red, certified and stamped, 4 mil thick, autoclavable bags that fit the 30 gallon cardboard biowaste box as a liner are available from VWR (catalog # 14220-098, VWR BAG BIO R 2M 38 X 48 IN, CS 100) at UF contract pricing.
  2. Non-autoclavable, red, certified and stamped, 1 mil thick liner bags will be available from Stericycle (via HSC custodians or the truck driver).
- 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”. Note: for successful autoclaving, do not tightly seal the bag *before* autoclaving – steam must be allowed to enter the bag; *tie tightly after autoclaving* when the bag has cooled.
- Label with date, PI name, room number, and telephone number.
- The lab must order/supply these red bags e.g. VWR catalog # 14220-098, VWR BAG BIO R 2M 38 X 48 IN, CS 100.

Sharp Containers

- Label with date, PI name, room number, and telephone number.
- Close when ¾ full and place in the red bag-lined cardboard biowaste box for disposal.
- Sharps boxes are free and are delivered to HSC personnel (call 294-5500). Personnel from outside the HSC should call 392-5775 to arrange to pick them up in room AG133 at the HSC.

Animal Carcasses

The disposal of animal carcasses and other animal materials and tissue shall be through Animal Care Services or Veterinary Medicine disposal devices only. These devices are for animal materials only. Please contact Animal Care Services (273-9230) for further information. Material obtained from the

Animal Science slaughter facility may be returned there for disposal if not contaminated with infectious, potentially infectious, or recombinant or synthetic nucleic acid material.
No animal carcasses or tissue pieces shall be disposed of as regular trash or through the biomedical/biological waste box. Animal carcasses and other animal material that may contain infectious animal or human pathogens require containment (red bags, sealed containers labeled with the biohazard symbol) before moving to Animal Care Services or the Veterinary Medicine disposal facilities.

Disposal of Biological Toxins

Because they can be extremely hazardous, even in minute quantities, biological toxins require strict safeguards against their inhalation, absorption through skin or mucous membranes (typically due to a splash), ingestion, or percutaneous injury. Information on the safe use of biological toxins can be found at Safety and Health Considerations For Conducting Work With Biological Toxins and Regulation of Select Agents and toxins, and appendix I of the BMBL. The UF Toxin SOP template, the University of Virginia Biotoxin Inactivation Table and Table 9 in this manual also provide general guidelines.

- Specific inactivation and disposal requirements are in place for acute biological toxins. Some toxins are quite resistant to conventional methods of inactivation. These agents cannot be simply placed in the biomedical waste or picked up by EH&S Hazardous Waste Services. Each lab working with acute toxins must complete the Acute Toxin SOP which will detail inactivation/disposal requirements for the specific toxin being handled.
- All liquid waste from toxins must be inactivated and collected by EH&S for disposal as hazardous waste. Contact the EH&S Hazardous Materials Management Office (352) 392-8400 for toxin waste pickup.

Autoclave Testing and Use

The rules governing the use and testing of autoclaves are based on Chapter 64E-16.007 of the Florida Administrative Code. Autoclaves shall be tested before being placed into service and then periodically for effectiveness:

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

A commercially available test indicator kit that uses bacterial spores *(Geobacillus stearothermophilus)* is the approved method of testing autoclave efficiency. Most spore vial test kits require 56^{0} to 60^{0} C incubation of the autoclaved test vial along with a non-autoclaved control vial. Incubation causes surviving spores to grow. Please read the product information sheet for appropriate storage information. Spore vials should not be frozen. Each batch of vials has an expiration date; vials should not be used after the expiration date.

New autoclaves

Before placing an autoclave into service, a test load approximating the weight and density of the type of waste generated shall be autoclaved with test spore vials. The spore vials should be placed at the bottom, top, front, rear, and center of the autoclave chamber. This can be achieved by either:

- Placing vials at those positions within one large test load
- Assembling several smaller test packs with 1 vial at the center of each and placing the packs at those locations within the chamber.

The appropriate parameters for sterilization including temperature, pressure, and treatment time shall be determined in this way.

Autoclaves In-Use

For periodic testing, place a spore vial in the very center of a test load prior to autoclaving.

Record Keeping

The following records regarding autoclave use must be maintained:

- Maintenance records

- Autoclave use log must be available near the autoclave; each load of material inactivated shall be logged as follows:
    1. The date, time, and operator name.
    2. The type and approximate amount of waste treated.
    3. The post-treatment confirmation results by either, a. recording the temperature, pressure, and length of time the waste was treated, or b. the temperature and pressure monitoring indicator
- Confirmation of sterilization:
    1. Record the temperature, pressure, and length of time the load is sterilized. Please note that temperature sensitive autoclave tape is not sufficient to indicate that the load achieved sterilization conditions as the tape will change color at lower temperatures.
    2. Save the autoclave print-out (if the autoclave has a working printer).

Autoclave Operating Procedures

A written sterilization procedure shall be in place for each workplace. This shall include the following:

- Parameters: Appropriate parameters for sterilization shall be determined from the testing with spore vials. The time it takes to sterilize a load will change depending upon the load density and the sterilization cycle one chooses. Tests have been performed which imitate these various situations. Please follow the established guidelines.
- Protocol: Identification of standard treatment containers and proper load placement shall be made.
- Cleaning: The autoclave and work areas shall be cleaned after every use and the work area shall be disinfected, as needed.

Autoclave Operation and Safety Training

Autoclave training is provided by the EH&S Biosafety Office upon request. The training is conducted at the autoclave and is geared toward the research staff. The proper use and maintenance of autoclaves as well as safety training is covered. Please contact the Biosafety Office (352-392-1591) to schedule a training session.

Equipment Decontamination

The following procedure must be followed before surveying, disposing of, moving, or repair of UF 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.
- Once the equipment is decontaminated, complete the form (see Handouts section) and fax to the Biosafety Office at 352-392-3647.
- The Biosafety Office will review the form to ensure that the decontamination method used is appropriate and then sign the form. The form will then be returned to the laboratory.
- Affix the form to the equipment and remove the biohazard sticker on the equipment if present.
- Note that biosafety cabinets must be re-certified before operating at the new location.

The following references were consulted:

- Disinfection, Sterilization, and Preservation. Fifth edition. 2001. Seymour S. Block ed., Lippincott Williams & Wilkins, Philadelphia
- Laboratory Biosafety Manual. Third edition. 2004. World Health Organization, Geneva.
- Biological Safety: Principles and practices. Third edition. 2000. Diane O. Fleming et al. Eds. ASM Press, Washington DC
- Manual of Clinical Microbiology, Volume 1. 9th edition. 2007. Patrick Murray, Ellen Baron, James Jorgensen, Marie Landry, & Michael Pfaller eds., ASM press, Washington DC
- Prudent Practices in the Laboratory: Handling and disposal of chemicals. 1995 National Research Council. National Academy Press, Washington.
- Laboratory Decontamination of HHS-listed and HHS/USDA Overlap Select Agents and Toxins. Applied Biosafety 2013, Volume 18:2, p.59-72

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 Biosafety Office for additional information regarding decontamination of large spaces.
Liquid chemical germicides formulated as disinfectants may be used for decontamination of surfaces. The Biosafety Office 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). Additional information regarding the activity levels of select liquid germicides may be located in the BMBL appendix B.

Emergencies

All personnel handling biological agents must have the proper training and experience to work with these materials safely. The laboratory-specific SOPs must also include procedures to ensure a safe work environment during an emergency, as well as protocols for specific incidents including spills, releases, exposures, laboratory accidents or theft/loss of an agent. Further, emergencies and incidents might also have far reaching effects beyond the individual or laboratory involved, therefore detailed notification and communication protocols should also be developed to ensure the incident is properly managed. The following summarizes the major elements to be addressed in the laboratory-specific SOP and staff training programs.

- In the event of a major disaster affecting the campus, the UF Homepage is the official source of UF emergency related information.
- Personnel should know what constitutes a potential exposure or release and report these to the PI or lab supervisor immediately. The PI or lab supervisor must then report this to the Biosafety Office within 24 hours and, as appropriate, to medical care providers.
- All personnel must be familiar with the hazards/risks of the materials they handle and the symptoms of disease or illness associated with these materials. This allows the discovery of a previously undetected exposure or release and countermeasures taken to prevent further harm.
- Time and situation permitting, contain and secure all biological agents during any and all emergencies. Agent storage areas shall remain locked to ensure security is maintained as well as protecting first responders from a potential exposure.
- Familiarize oneself with the location and operation of emergency equipment such as the eyewash station, safety shower, first aid kit, chemical and biological spill kits, fire extinguishers and emergency exits.
- PIs and lab supervisors should periodically review emergency procedures and information with lab staff and students. Quick reference protocols and emergency contact information should be posted in the laboratory. See Handouts section on the Biosafety website.
- When possible, lab staff, PI/lab supervisor should meet and escort emergency personnel on site.
- All emergencies are better handled by two persons. The buddy system is required when working with hazardous materials, particularly after normal business hours on weekends or holidays.
- Emergency Contact Telephone Numbers are located on the EH&S website emergencies page.

Personnel Responsibilities/Assignments During a Disaster

All laboratories and/or research areas should pre-assign an individual(s) to perform the following tasks in the event of a disaster:

- Determine the whereabouts of all employees and reiterate emergency assignments.
- Notify departmental administration during normal business hours, or after hours call the University Police Department Communications Center at (352) 392-1111.
- Secure buildings and properties. Report status of employees and unit to appropriate administrators.

- After the disaster has occurred, determine the status of all employees, the security of biological agents, implement a plan of action and provide services as needed, and begin to make arrangements for repairs of facilities.

Emergency Evacuation Procedures

In situations requiring immediate action, public safety responders (University Police, Fire, and Environmental Health & Safety) may order an evacuation. When evaluating a possible evacuation, consideration will be given to the specific threat (bomb, fire, gas leak, explosion, severe weather incident, etc.), its context (time of day, likelihood, etc.) and the recommendation of first responders.

- Identify at least two routes of emergency egress from your workplace.
- In general, during an evacuation:
    1. Stay calm; do not rush and do not panic.
    2. Safely stop your work.
    3. Secure all agents if threat to life is not immediate.
    4. Gather personal belongings if it is safe to do so.
    5. Close lab doors.
    6. Use the nearest safe exit. Do not use the elevator.
    7. Move at least 300 ft. from the building.
    8. Account for all of your co-workers.
    9. Wait for instructions from emergency responders.
    10. Do not re-enter the building or work area until you have been instructed to do so by the emergency responders.
    11. Occupants are required by law to evacuate the building when the fire alarm sounds.

Fire Safety

DO NOT USE THE ELEVATOR!!!

Pre-determine two means of egress from your normal workplace. Learn the location of the nearest fire alarm pull station and portable fire extinguisher. If you discover a fire in a University of Florida building, perform the following:

- Pull the fire alarm and call 911.
- Fire alarm pull stations are normally located near each exit. If the building is not equipped with a fire alarm system, notify other occupants as you exit the building.
- Do not attempt to fight the fire with portable fire extinguishers or fire hoses unless the fire is small and you have been trained in their proper use. DO NOT PUT YOUR LIFE IN DANGER WHILE ATTEMPTING TO CONTROL A FIRE. When in doubt, evacuate.
- Remain calm while talking to the operator. Be prepared to answer several questions as to location, size of fire, your name, number of persons in building (if known) and any injuries. Remain on the line until the operator is finished.
- Meet fire or police personnel when they arrive at the building. Stand by to answer any questions they may have concerning the fire.
- Once out of the building DO NOT RE-ENTER THE BUILDING FOR ANY REASON, unless emergency personnel have given the "ALL CLEAR" signal.

If the fire is inside your room:

- Leave your room and close the door.
- Pull the fire alarm and call 911.
- If the fire is small and you have been trained to use a fire extinguisher, attempt to put it out. (Again, DO NOT PUT YOUR LIFE IN DANGER WHILE ATTEMPTING TO CONTROL A FIRE. When in doubt, evacuate.). Remember the acronym “PASS”; P (pull the pin), A (aim at the base of the fire), S (squeeze the trigger), S (sweep the nozzle from side to side).

If the fire is not in your room, but in a room you must pass through to get out or exit:

- With your hands, test the door for heat before opening.
- If the door is HOT:
  1. Stay in your room or lab.
  2. Phone for help.
  3. Stay calm.
  4. Seal cracks with wet towels.
  5. Wait for help.
- If the door is COOL:
  1. Take your room key.
  2. Open the door slowly.
  3. WALK to the nearest exit and leave the building.
  4. If the exit is unsafe, return to the room and remain there.
  5. If the hall is smoky, stay low or crawl out on your hands and knees.

Break-In/Security Breach

- Call 352-392-1111 for University police department.
- Notify PI/lab supervisor and the Biosafety Office 392-1591 of the break in.
- If the break in has resulted in a spill or other release of agent, contain the material as per laboratory spill procedures.
- Escort University Police personnel at the scene.
- The PI and Biosafety Office will conduct an inventory check and report results to appropriate authorities as needed.
- If loss or theft of agent is discovered at any time, immediately call the PI and the Biosafety Office to report the occurrence. An investigation will be initiated.

Workplace Violence

All threats and other inappropriate behavior that create an immediate concern for safety should be reported immediately to the University Police Department (UPD) at (352) 392-1111 or local law enforcement if off campus. You may also dial 911, but remember, you must first dial 9 to get an outside line. Examples of behavior requiring a call to authorities include:

- Direct or veiled threats
- Written sexual or violent notes – intimidation verbally or physically
- Carries a weapon (Florida Statutes and University Policy prohibit firearms and certain other articles that could be weapons on state property)
- Makes suicidal comments or threats
- Involved in fights or assaults
- Stalks co-workers or their family

For additional information, see the UF Workplace violence policy.

Hurricane Watch

- Secure and contain all biological agents in preparation for a hurricane.
- Autoclave cultures that are not essential.
- Check for adequate CO2 or LN2 backup systems for freezers if so equipped.
- Make sure freezers or other critical equipment are plugged into emergency power; plug in only essential equipment so as not to overload the system.
- See the checklist to prepare lab areas for an impending hurricane:
- The UF homepage is the official source of UF Information. Official emergency information for Alachua County is broadcast on:
  1. Radio: WUFT-FM / WJUF-FM / WLUF-LP / WRUF / AM850 / Rock 104
  2. Television: WUFT-TV
- Additional information regarding safety and preparedness may be found on the UF emergency management webpage
- After the storm, project personnel should check the laboratory and verify all is in order. Any problems should be reported to the PI and Biosafety Office.

Tornadoes and Other Natural Disasters

There is generally little or no warning given at the approach of a tornado. In the event of a tornado:

- Be familiar with the terminology:
  1. Tornado Watch - conditions are favorable for the formation of tornados.
  2. Tornado Warning - indicates that a tornado has been sighted or is indicated on radar.
- In a tornado warning IMMEDIATELY seek shelter, preferably in the hallway of a main office building, an interior bathroom or an interior closet.
- AVOID windows; flying debris can kill. Protect yourself by getting under a heavy desk or table. Also, remember to protect your head.
- After the storm, project personnel must check on other personnel and on the storage area and verify all is in order. Any problems should be reported to the PI and Biosafety Office.

Bomb Threats

A bomb threat may be received in the form of an actual threat such as those communicated by telephone, or observing a suspicious package or material. While most threats are false or misleading, there is always the potential that a threat is real, and therefore life threatening. Correct and consistent procedures allow for the best decisional options based on the information known.

If you receive a telephoned bomb threat, use the checklist from the University Police Department (UPD) to gather as much information as possible. A suspicious-looking box, package, object, or container in or near your work area may be a bomb or explosive material. Do not handle or touch the object. Move to a safe area and call the University Police (392-1111) immediately. Use a telephone in a safe area. Do not operate any power switch, and do not activate the fire alarm.

UPD will decide if evacuation is necessary. Evacuation is determined by the UPD only after a thorough evaluation of all available information e.g. results of the bomb threat checklist, information from support agencies, etc. UPD also considers if just disrupting operations serves the purpose of the bomber. If the bomber describes in detail the type of device, its location, and/or the placing of the device, then the bomb scene officer may have more reason to believe that the device has, indeed, been planted.

If you are told to evacuate, follow these procedures:

- Stop work; secure all agents you are working with (including select agents).
- All persons will make a preliminary search around their immediate areas for suspicious items.
- All persons, as they leave, will remove those items that they brought in (briefcases, water bottles, lunch bags), turn off radios, and unplug office machines.
- Use the stairs, not the elevator.
- When evacuation of a building is accomplished, only authorized personnel are permitted entry until the threat is resolved.

If a device is actually discovered, either as a result of a bomb threat or during routine operations, evacuation procedures should be carried out immediately. All persons will evacuate at least 300 ft. from the building. This distance takes into account items like propane bottles or natural gas lines that could contribute to the explosive force of a bomb within a facility. Personnel should account for their coworkers to determine if anyone is still in the building.

Natural/LP Gas Leak

If you smell gas:

- Call 911 right away. This will summon both UPD & Gainesville Fire Rescue (GFR) HazMat crew.
- Extinguish all sources of flame.
- Secure all agents.
- Do not switch lights on or off.
- Stay calm; do not rush and do not panic.
- Gather your personal belongings if it is safe to do so.
- Use the nearest safe stairs and proceed to the nearest exit. **Do not use the elevator**.
- Exit the building and go at least 300 ft. away.
- Account for coworkers.
- Wait for any instructions from emergency responders.
- Do not re-enter the building or work area until you have been instructed to do so by the UPD & GFR.

Suspicious Package

All suspicious packages should be reported immediately to the University Police Department (UPD) at (352) 392-1111. For information on what constitutes a suspicious package, see the CDC Emergency Preparedness and Response Site regarding the guidance document: Guidance on Initial Responses to a Suspicious Letter/Container With a Potential Biological Threat.

Explosions

- Take cover under sturdy furniture, or leave the building if directed to do so by emergency responders.
- Stay away from windows.
- Do not light matches.
- Stay calm; do not rush and do not panic.
- Gather your personal belongings if it is safe to do so.
- Use the nearest safe stairs and proceed to the nearest exit. Do not use the elevator.
- Exit the building and go at least 300 ft. away

- Account for coworkers
- Wait for any instructions from emergency responders.

Power Outage

Because the heating/air conditioning/ventilation system may not maintain directional airflow into the lab or provide for normal ventilation, the laboratory is not safe to work in when there is a power outage. If the lab has no natural light, check to make sure emergency lighting is available, supplied as part of the building facilities or furnished by the lab (e.g. plug in battery operated flashlight that comes on automatically if there is a power failure). If the power fails while persons are working in the lab, they should immediately:

- Stop work.
- Cover, contain, and secure the materials.
- Wipe down work surfaces with disinfectant.
- Doff PPE as normal.
- Exit the lab and secure the door.
- Report the power outage.

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/Supervisor as soon as possible and to the Biosafety Office within 24 hours. The affected individual and/or supervisor should be prepared to provide the Occupational Health Service, and/or attending 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) from the Public Health Agency of Canada for that agent 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.

The Biosafety Office will conduct an investigation of the event and determine what, if any, safety lapses lead to the exposure and what should be done to prevent future exposures. For employee exposures/lab-related illness or injuries, contact the UF Workers' Compensation Office (UFWC) at (352) 392-4940 to report the situation, receive instructions and provide information needed to complete the First Report of Injury or Illness form. Please do this prior to actually going to an authorized medical provider except as noted above for major medical emergencies. An Injury and Incident Report form is used to document the employee exposure; this form is sent within seven days via campus mail to the Office of Risk Management at EH&S, PO Box 112190.

Needlestick or BBP exposure

- For all Needlestick or BBP exposures exposures in the Gainesville area call the Needlestick Hotline at 1-866-477-6824 (OUCH) 24 hours a day, 7 days a week and follow their instructions. Online assistance instructions are also found on the SHCC website.
- Jacksonville personnel - proceed to the Employee Health Office (904 244-9576) in Suite 505, Tower 1, 5th and Jefferson between the hours of 7 – 4. Go to the Emergency Room after hours.
- Off-site further than one-hour travel time from UF; go to the nearest medical facility.

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 supervisor and inform them of the circumstances of the injury, including what was being handled at the time.
- For known exposures during normal business hours, contact the SHCC at (352) 294-5700. For known exposures after normal business hours, or if the SHCC is not available, contact your personal physician or proceed to the immediate care facilities listed below. For unknown exposures, contact your personal physician for the immediate care facilities listed below.
- Employees enrolled in the BioPath Program should proceed the Shands ER for known exposures. For unknown exposures, the Biosafety office recommends proceeding to the ER or contacting your personal physician.
- Jacksonville UF employees should report to the Employee Health Office in Suite 505, Tower 1, 5th and Jefferson from 7 AM to 4 PM. After hours proceed to the Emergency Room. If you are on an off-site rotation further than one-hour travel time from UF campus, seek care at the nearest medical facility.

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.
- Report the incident to PI/Supervisor and Biosafety Office (352) 392-1591.

Accidental Ingestion

- Seek medical treatment.
- Report incident to PI/Supervisor and Biosafety Office (352-392-1591).

Animal Bites and Scratches

- For small wounds – allow to bleed freely. If necessary, control bleeding by applying direct pressure with a sterile gauze or bandage.
- Immediately wash with copious quantities of soap and water. If eyes or mucous membranes are exposed, irrigate the area for at least 15 minutes with water.
- Seek medical treatment.
- Report incident to PI/supervisor, Animal Care Services Director and Biosafety Office (352) 392-1591.
- If the bite or scratch is from a non-human primate, contact the following physician regarding Monkey B virus (Herpesvirus Simiae, CHV-1) exposure: Dr. Kenneth Rand, Work: (352) 265-0111 x44875, Pager: (888) 543-1806, Cell 352-222-4613
    1. The physician will evaluate the injury and may decide to culture the wound for B-virus (*Herpesvirus Simiae*) or collect blood for a baseline titer against B-virus, or use prescription drugs for preventative therapy.
    2. The physician directing the care of the patient will contact the Director of Animal Care Services for instructions regarding the need for cultures or serology from the monkey inflicting the injury upon the patient.
    3. Symptoms suggestive of B virus infection, (see Special Issues section on B Virus and the Health Canada pathogen safety data sheet), should be reported immediately to the medical consultant. When the possibility of B virus illness is seriously entertained, appropriate diagnostic studies should be performed and specific antiviral therapy should be instituted.

4. The physician may wish to consult Dr. Scott Schmid at (404) 639-0066; cell: (404) 725-5652 at Measles, Mumps, Rubella, and Herpes (MMRH) Branch, Division of Viral Diseases, CDC, and Dr. Julia Hilliard at (404) 413-6550; cell: (404) 358-8168, at National B Virus Resource Center at Georgia State University, Atlanta, GA for laboratory assistance. For additional information see the GSU website on the B Virus Exposure Protocol.

- Following a bite or scratch, the animal handler should be instructed to report immediately any skin lesions or neurologic symptoms (such as itching, pain, or numbness) near the site of the wound or any other unusual illness. It is the responsibility of the supervisor, when no illness is reported, to determine the clinical status of the handler at weekly intervals for 1 month after the exposure.
- As soon as possible after the incident contact UF Workers' Compensation (352) 392-4940 to report the incident. Workers' Compensation will complete the state required First Report of Injury form for the employee.

Inhalation Exposure

- Doff PPE normally and exit lab
- Seek medical treatment.
- Report incident to PI/supervisor and Biosafety Office (392-1591).

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.
- Report incident to PI/Supervisor and Biosafety Office (392-1591).
- The laboratory or facility and work practices will be evaluated by the PI and the Biosafety Office 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 Biosafety Office/IBC 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 in the Centrifuge

- Allow aerosols to settle for 30 minutes before attempting to clean up the spill. Keep the centrifuge closed during this time. Post a sign on the centrifuge so others don't try to open it.
- Gently open the centrifuge to prevent re-aerosolization.
- Place absorbent materials in the chamber and pour a fresh 1:10 dilution of household bleach (0.5% sodium hypochlorite) over them. Let sit 30 minutes.
- Use tweezers, forceps, or tongs to pick up broken sharps – place in a sharps container.
- Carefully remove carriers to a tub containing a fresh 1:10 dilution of household bleach (0.5% sodium hypochlorite). Soak 30 minutes. Rinse with water.
- Wipe the interior and lid of the centrifuge with a fresh 1:10 dilution of household bleach (0.5% sodium hypochlorite).
- Wipe all areas with plenty of water to prevent corrosion. Dry and follow with 70% ethanol.

Spill Inside the Laboratory, Outside of Containment Device

- 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 supervisor.
- Notify the Biosafety Office for:
    1. Spills or accidents in BSL2 laboratories resulting in an overt exposure of susceptible hosts/individuals to toxins, recombinant or synthetic nucleic acid, or pathogens
    2. Spills in BSL3 laboratories resulting in an overt *or* potential exposure of susceptible hosts/individuals to toxins, recombinant or synthetic nucleic acid, or pathogens
    3. Spills you are uncomfortable handling
    4. Spills involving select agents
    5. 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 and autoclave.
- 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 Biosafety Office.

Spill Outside the Laboratory

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. In the event of a spill outside the lab:

- Clear area of all personnel and stay there to keep them out of the spill.
- Have someone call your lab or PI/Supervisor or the Biosafety Office 352-392-1591 for help; spills of high consequence agents require that the Biosafety Office and University Police Department (352-392-1111) be called.
- 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 (when it becomes available) 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 supervisor.
- Notify the Biosafety Office for:
    1. Spills or accidents of BSL2 material resulting in an overt exposure of susceptible hosts/individuals to toxins, recombinant or synthetic nucleic acid, or pathogens.
    2. Spills of BSL3 materials resulting in an overt or potential exposure of susceptible hosts/individuals to toxins, recombinant or synthetic nucleic acid, or pathogens
    3. Spills you are uncomfortable handling
    4. Spills involving select agents
    5. 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/forceps, scoop, or dustpan if sharps may be present. Discard all towels and other clean up materials into a bucket, 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 a biohazard sticker on the discard container prior to transport.
- Place the discard container and other materials in a biohazard bag and autoclave.
- Remove shoes or shoe covers, outer clothing, respirator, and gloves and disinfect or autoclave (preferable).
- Wash hands, arms and face; shower if necessary.

Blood Spills

- Don 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 and autoclave.

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 Biosafety Office.

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.
- Report incident to supervisor and Biosafety Office (352) 392-1591.
- Obtain medical attention as required.

Immediate Life threatening or Serious Injuries

These injuries include burns, eye injuries, chemical exposures, head injuries, and similar traumatic injuries. Protocols are described in more detail on the Human Resources website. Once medical attention is received, supervisor (or employee) should contact Workers Comp Office at 352-392-4940.

- Remain calm.
- Call for EMERGENCY RESPONSE - MEDICAL EMERGENCY 9-1-1.
- Calling 911 automatically alerts the UF Police Department.
- Initiate lifesaving measures, as required.
- If the person is alert and responsive, but cannot move, leave the person there; medical responders will don 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 doff their PPE. Meet emergency responders outside the lab in the corridor.
- If the person is unconscious, drag the person into the clean area and doff their PPE. Doff your own PPE and exit into the corridor where you will wait for emergency responders.
- Have another lab member meet responders at the entrance to escort them to the lab.

Non-life threatening injuries (M-F 7:30 – 5:00 pm)

Inform supervisor and contact Workers Comp Office at 352-392-4940. Workers Comp Office will direct you to the appropriate medical facility.

Non-life threatening injuries (after normal business hours)

If you are injured while performing your assigned job duties after hours, the two facilities listed below are available. Simply identify yourself as a UF employee who was injured on the job and show them your Gator 1 Card and personal insurance card. After you have been treated, please immediately contact the Workers' Compensation Office at (352) 392-4940 and follow the directions.

- Emergency Physicians Medical Center
  352-872-5111
  2445 SW 76th St, Suite 110 (At the intersection of Tower Road and SW 24th Avenue).
  OPEN FOR AFTER HOURS CARE:
  Monday - Friday 5 p.m. - 8 p.m.
  Saturday 12 p.m. - 4 p.m.
  Sunday 12 p.m. - 4 p.m.

- Emergency Medical Center
  352-331-4357
  6121 NW 1st Place (Across from Sears at the Oaks Mall).
  OPEN FOR AFTER HOURS CARE:
  Saturday 8 a.m. - 6 p.m.

Outside of the Gainesville area: seek treatment at the nearest medical provider.

An emergency room should only be used for life-threatening injuries or for after-hour care of injuries that require immediate attention, such as burns, eye injuries, chemical exposures, head injuries, and similar traumatic injuries.

Occupational Medicine Programs

Environmental Health & Safety at UF is responsible for policy development and administration of the Occupational Medicine (OCCMED) program. The program goal is to ensure employee health and manage job-related injuries. Details of the program are located on the EH&S Department of Occupational Medicine website

- The OCCMED Program provides for pre-placement and other health assessments depending upon the type of work in which an employee will be engaged.
- The program offers general and work-specific health assessment of employees at the time of hire as well as periodic medical monitoring thereafter.
- It includes the Bloodborne Pathogen Program, Animal Contact Program, Respiratory Protection Program, BioPath Program, and Vaccine Program. Researchers who work with specific agents will require a health assessment based on the requirements of one of these programs. The OCCMED program also covers workplace injuries with no charge to the employee. The cost is covered by the Worker's Compensation Program.

Bloodborne Pathogen (BBP) Program

Bloodborne pathogens are pathogenic microorganisms that may be present in human blood and other potentially infectious materials (OPIM) that can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).

To curb the spread of BBPs in occupational settings, the Occupational Safety and Health Administration implemented the Bloodborne Pathogens Standard (29CFR 1910.1030) in 1991. The standard was written into the Florida administrative code in 1993 and UF instituted its BBP program based on the OSHA standard in that same year. UF requires BBP Program participation by all employees and non-employees (students, volunteers, affiliates, etc.) who have occupational exposure to human blood, other potentially infectious materials or non-human primates. All who need BBP training also need Biomedical Waste (BMW) Training, and that training is provided in conjunction with BBP training.

Basic OSHA BBP program requirements are:

- Development and annual review of an Exposure Control Plan
- Annual site-specific training of all participants geared to their educational and professional level
- Hepatitis B vaccinations provided by employer
- Post-exposure health care services
- Record-keeping of training and immunization status

Additionally, OSHA outlines specific content requirements within each category above.

BBP training at UF is accomplished through departmental BBP training coordinators to comply with the OSHA site-specific training component. These trainers identify those in their department who need the training, add site-specific information to the training provided by the Biosafety Office to make the training more relevant and effective for their departmental personnel, track the training and provide documentation of that training via a list of those trained to the Biosafety Office.

If you need BBP training, contact your BBP departmental trainer. If you don't know who that trainer is, contact bso@ehs.ufl.edu with your name, UFID and departmental affiliation, and request the name and contact information for your BBP Training Coordinator.
Department chairman or division directors are designated as the responsible party to ensure department members remain in compliance with the BBP program requirements. To that end, the department head

must familiarize him/herself with the material, assign a BBP trainer for the department and return the Receipt Acknowledgement and Training Coordinator Designation form to the Biosafety Office each year by February 1st.

Responsibilities of the BBP departmental trainer:

- Attend the BBP-Train-the-Trainer session in person or on-line. All BBP trainers are required to take the BBP-Train-the-Trainer session to update their training for the year and to provide them with the training tools, techniques and documentation instructions they will need to complete their task.
- Identify and contact those who need the training. All employees, students, and affiliates at risk of exposure to bloodborne pathogens must participate in annual bloodborne pathogen training. This includes those who handle human blood, tissues, primary human cell lines, and certain human body fluids. The program may include principal investigators, nurses, physicians, laboratory workers, residents, students, and others. Please note that at UF there are secretaries, custodial workers, and teachers included in the at risk group due to the nature of the work they perform.
- Conduct the training. You can give a live session using the BBP/BMW PowerPoint presentation developed by the Biosafety Office or using your own material (following BSO review and approval). You can also use the on-line Sakai BBP sessions provided by the Biosafety Office. You must enroll, track, and document the training of those trained by this method. You should always supplement the material presented with information about site-specific hazards that aren't addressed in the training.
- Offer the Hepatitis B immunization series to all at-risk employees. New employees or new participants in the BBP program who decline or accept the series are required to complete and sign the Training and Vaccination - Acceptance/Declination statement prior to performing job duties that involve exposure. Trainers are provided with a list of those trained in the department the previous year. That list has a column indicating whether the Hep B immunization information or declination is on file. If "No" is indicated in that column, the person must submit the Training and Vaccination form to the Biosafety Office. If they want to get the vaccination, they should take the completed form to the SHCC.
- Document the training with the Biosafety Office by returning the List of People Trained form, even if people take the training online via Sakai. The several on-line BBP sessions developed by the Biosafety Office on Sakai are convenient and have made the task semi-automatic. However, the departmental BBP training coordinators must track the training of those enrolled in the course and provide the Biosafety Office with a list of people trained for proper Biosafety Office and myUFL BBP training documentation.

BBP Recordkeeping:

The Biosafety Office maintains the complete BBP/BMW training database for employees, volunteers and other non-employees. Although BBP training is not completed through myUFL, employees' BBP training dates are uploaded into the myUFL system on a regular basis from Biosafety Office reports. The BBP/BMW training dates for volunteers or other non-employees are not available through myUFL.

Animal Contact Medical Monitoring Program

According to the US Public Health Service, an occupational health program is required for institutions that employ personnel who have animal contact to protect employees, students, and volunteers from exposure to animal related illnesses. The UF Florida Animal Contact Medical Monitoring Program grew out of the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The requirements of the program are based upon those outlined in the Public Health Services document, Guide for the Care and Use of Laboratory Animals and Occupational Health and Safety in the Care and Use of Research Animals published by the National Research Council.

Program Information

Individuals who will be working with animals or who will be working in proximity to animals are required to participate in the medical monitoring program; they are provided with animal contact medical monitoring information and immunizations as part of their pre-placement health assessment. In addition, the Institutional Animal Care and Use Committee (IACUC) verifies that all personnel listed on new and continuing projects are registered with the UF Animal Contact Medical Monitoring Program. The IACUC

staff notifies the PI of any personnel who are not cleared for animal contact. Principal Investigators are responsible for ensuring that all personnel (including employees, students, colleagues, collaborators, and volunteers) involved with their IACUC-approved project are provided program information. Investigators who do not respond to requests for registration may have their approval rescinded by the IACUC.

All individuals listed on an IACUC protocol, even those without animal contact, must complete risk assessment form that includes contact information and a health questionnaire. A health assessment (physical examination, medical history, blood serum banking and immunizations) may be required based upon information provided on the risk assessment form. This risk assessment is provided at no cost to the employee. Visitors and volunteers must provide documentation of participation in an animal contact / occupational medicine program or they must register in the UF Animal Contact Program. Contact the EH&S Biosafety Office to discuss program requirements for individuals involved in isolated, non-recurrent exposures. The primary responsible party at UF (principal investigator, research director, student research coordinator, etc.) shall be responsible for assuring compliance with the notification requirements for all visitors.

Elements of the Program

- A Risk Assessment form is required to be filled out by everyone working with animals at UF or entering UF animal facilities. This form includes contact information and a health questionnaire that will be evaluated by UF's Occupational Medicine Physician or Licensed Health Care Professionals to assess the risk of exposure and determine whether additional information and/or interaction is necessary.
- A Renewal Risk Assessment form is required every three years or when any new species is contacted. The new risk assessment submission supersedes previous approvals; all species (old and new) must be included on renewal. This update will allow UF's Occupational Medicine Physician or Licensed Health Care Professionals to evaluate and, if necessary, address potential health risks resulting from a change in health status or changes to the type and frequency of exposure to animals.
- Specific requirements
    1. Tetanus Immunization within 10 years – All participants
    2. Rabies Immunization Series/Booster or Positive Titer within 2 years – All individuals handling unvaccinated carnivores or their tissue.
    3. Respirator Clearance and Fit Test – All individuals required by the Q-Fever Policy or as medically necessary to prevent allergic reactions.
    4. Serum Banking – All individuals who work with non-human-primates, all who handle blood from alligators or birds housed outdoors, all who are required by the Q-Fever Policy and all pre-menopausal females who handle cats or cat waste.
    5. TB Screening within 12 months – All individuals who enter any room with non-human primates.
    6. Medical consultation may be required as determined by the Occupational Medicine Physician. Examples are individuals with chronic disease, work-related injuries or illness, environmental or animal allergies.

Respiratory Protection Program

Laboratory personnel who work with agents that are transmitted by aerosols or with certain chemicals or acute toxins may require a respirator at some stage of the research project.

- The EH&S Occupational Medicine Respiratory Protection Program provides for health assessments that will determine an employee's fitness for respirator use.
- Use of N95/99 or Powered Air-purifying respirators (PAPRs) will be recommended by the Biosafety Office based on the type of agents handled by the researchers.
- The Respiratory Protection Program requires initial medical clearance along with initial and annual training and fit tests for N95 respirators. Employees entering this program must be evaluated by a physician or other licensed health care professional. The purpose of the evaluation is to screen employees for pre-existing conditions not conducive to respirator use. The medical clearance is established by the Student Health Care Center.

- Annual training for N95 respirators must be completed before the N95 fit test is done. This N95 training is completed online.
- The N95 respirator fit test will be completed by an Environmental Health & Safety representative; contact respirators.ehs@connect.ufl.edu or (352) 273-2163 or (352) 273-2118
- PAPR training must be performed annually by the PI or lab supervisor. The Biosafety Office trains the PI or lab supervisor who is then authorized to train laboratory staff.

BioPath Medical Monitoring Program

BioPath is the term utilized to describe the Biohazards Medical Monitoring Program for the University of Florida Occupational Medicine team. BioPath provides occupational health oversight for employees, students, visitors, and volunteers in instances where it has been determined that such oversight is necessary due to the potential for exposure to biohazards. The purpose of the program is to prevent illness and injury from biohazards. A medical monitoring program for biohazards, based on underlying assessments of possible risk, is mandated by several regulatory agencies (e.g. OSHA, NIH, AAALAC, CDC, etc.) and is therefore part of UF's compliance obligations.

The BioPath program is comprised of a number of different component and/or services; participation in these is determined by individual risk assessment(s). The components/services include:

- Health Assessment
- Serum Banking
- Respiratory Protection Clearance
- Vaccinations
- Medical Testing

The goals of the BioPath program are to:

- Identify and provide assistance, as appropriate, in management of medical conditions that may place an individual at an increased risk of adverse health effects from their work with biohazardous agents.
- Conduct baseline, periodic, exit, or problem-specific health assessments or testing.
- Educate individuals about their risks, how to minimize the risk(s) and protect their health.
- Provide preventative vaccines as appropriate.
- Establish medical clearances for personal protective equipment when required.

The UF Biosafety Office, in consultation with the UF IBC and the SHCC Occupational Medicine team, determines if participation in the BioPath program is required. Enrollment in the BioPath program is required for all those working in a BSL3 laboratory and for those individuals with access to Tier 1 Select Agents. Other participants may include individuals working with certain risk group 2 agents. Initial medical screening should be completed before starting activities with potential for biohazard exposure. This offers the best health protection and provides the Occupational Medicine Service providers with a baseline health assessment. Volunteers or visitors with potential exposure to biohazards are also expected to meet the same requirements as UF employees or students, either through participation in UF's program or by providing documentation to the UF SHCC of coverage under their institution's occupational medicine program.

Individuals requiring these services should identify to the Student Health Care Center (SHCC) medical provider, a) the type of work they do, b) what biohazards are involved, c) their work environment, d) their health status, (including pre-existing conditions such as immune system deficits, pregnancy etc.), e) their participation in any other occupational medicine program and f) any questions or concerns they have regarding their health and work-related exposures. The medical assessment questionnaire assists in capturing this information.

It is the responsibility of the supervisor or Principal Investigator to ensure that persons requiring participation in the biohazard medical monitoring program complete and submit the required forms for enrollment in the program. The supervisor or Principal Investigator acknowledges and signs the Authorization Form. The following are the BioPath responsibilities:

Departments:

- Assist EH&S in identifying employees who are required to participate in the Program by assigning and monitoring job duties.
- Inform personnel of the requirement for participation in the Program and assist personnel with completing the authorization request forms.
- Provide fiscal information for SHCC to complete required services. There are fees assessed by the Student Health Care Center for these services. This cost is borne by the individual's department, not the applicant.
- Ensure that all guests (vendors, visitors, contractors etc.) submit documentation and enroll in the program as necessary.
- Ensure compliance for those within their department

Environmental Health and Safety:

- Reviews and verifies all information submitted for authorization.
- Determines and/or assigns the appropriate respiratory protection.
- Authorizes the request for participation in the Program.
- Documents, monitors and ensures compliance for annual follow-up

Student Health Care Center:

- Reviews submitted documentation (authorization and participant forms)
- Performs health assessment and determines whether on-site evaluation and/or physical examination are required.
- Performs medical services as needed.
- Maintains all medical records associated with this program.

Participants:

- Comply with all requirements of the program.
- Complete Medical Assessment Questionnaire
- Complete required initial and annual health assessments.
- Report all potential exposures.

References:

- Department of Health and Human Services, National Institutes of Health, Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).
- Department of Health and Human Services, Centers for Disease Control and Prevention, 42 CFR Part 73, Possession, Use, and Transfer of Select Agents and Toxins (Select Agent Program).
- AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care

Vaccination Policy for Research Personnel

All University of Florida employees who may be at risk for occupational exposure to certain biological agents and toxins must follow this vaccination policy. The complete policy is located on the Biosafety Website: http://www.ehs.ufl.edu/programs/bio/vaccination-policy/

Required Vaccinations:

If there is a licensed va:ccine available and the Advisory Committee for Immunization Practices (ACIP) provides specific vaccination recommendations for the type of work performed, OR the vaccine is required by federal regulations, the employee is required to be vaccinated prior to working with the agent. UF requires vaccination for work with the following agents based on ACIP recommendations: *Bacillus anthracis*, Hepatitis A, Japanese encephalitis virus, *Neisseria meningitidis*, poliovirus, rabies virus, vaccinia virus, *Salmonella typhi*, tetanus (for animal contact) and influenza virus (for work with Risk Group 3 influenza viruses). See ACIP Recommended/UF Required FDA-Licensed Vaccines.

Strongly Recommended Vaccinations:

If there is a licensed vaccine available for the agent(s) that are being worked with that does not fall under Required Vaccinations above, and a risk assessment performed by the Biosafety Office recommends vaccination for the work performed, the employee is strongly encouraged, although not required, to receive the vaccination prior to working with the agent. Vaccination may be recommended based on risk

assessment for work with the following agents: tetanus, diphtheria and pertussis toxins or toxin-producing strains of *Corynebacterium diphtheriae*, *Clostridium tetani*, and *Bordatella pertussis*; human papillomavirus; influenza virus (for Risk Group 2 influenza viruses); *Streptococcus pneumoniae*, varicella zoster virus and Hepatitis B virus. See FDA-Licensed Vaccines that may be Recommended).

Investigational New Drug (IND) Vaccinations:
When an employee may be at risk for occupational exposure to a highly pathogenic agent for which no licensed vaccine is available, but for which investigational products are available via an Investigational New Drug (IND) protocol through the Special Immunizations Program (SIP) administered by the US Army Medical Research and Material Command, the IND vaccines will be made available on a voluntary basis with informed consent. The SIP program currently has vaccinations against the following agents: *Francisella tularensis*, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus and Rift Valley Fever virus.

Required Vaccination Procedure:

- You must document your decision to accept or decline the vaccine by submitting an Acceptance/Declination/Request to Waive Required Licensed Vaccines Form to the Biosafety Office.
- Review the agent fact sheets and vaccine information sheets
- Schedule a consultation appointment with the Occupational Health Physician through the Student Health Care Center (SHCC) to review immunization options.

If you have previously been vaccinated AND are current on all recommended vaccine boosters you must provide either the date (month and year) of your vaccination(s)/booster(s) AND the signature of the physician/medical provider (or authorized representative) that administered the vaccine; OR laboratory evidence of immunity which must be provided to the UF Occupational Medicine Provider for interpretation and verification. All vaccinations will be provided in consultation with the UF Occupational Health Physician according to ACIP recommended dosing schedules at no cost to the individual. If immunogenicity data is available, the data will be used to determine at what point the individual is considered "protected" and approved for work with the agent (See Dosing Schedules and Immunogenicity Data – Required Vaccines).
If there is no immunogenicity data available, an individual will be considered "protected" and approved for work with the agent two weeks after the final vaccine dose.

If there is a disagreement about the applicability of the ACIP recommendation, the UF Biological Safety Office shall make the final decision with input from the UF Institutional Biosafety Committee (IBC). If you have questions regarding the applicability of the ACIP recommendations to your specific work duties, contact the Biosafety Office to discuss your concerns.

While **waivers** are not typically given, you may request a waiver using the Acceptance/Declination/Request to Waive Required Licensed Vaccines Form. You must provide a justification for the waiver request. The request will then be evaluated by a group consisting of the UF Occupational Medicine provider, UF attorney's office, the IBC and the Biological Safety Office. If there are fewer than three of the four groups in agreement, the ACIP recommendation will prevail. If your waiver request is not approved and you decline vaccination, you will not be able to perform the duties that triggered the vaccine requirement.

Strongly Recommended Vaccine Procedure:

- You must document your decision to accept or decline the vaccine by submitting an Acceptance/Declination of Recommended Licensed Vaccines Form to the Biosafety Office. Note: For acceptance/declination of the Hepatitis B vaccine – please use the UF Bloodborne Pathogen Training/Vaccination Form Acceptance/Declination Statement
- Review the agent fact sheets and vaccine information sheets

Investigational New Drug (IND) Vaccine Procedure:

- If work will be performed with an agent for which an IND vaccine exists, you must document your decision to Accept or Decline the IND vaccine by submitting a completed, signed Acceptance/Declination of IND Vaccines form to the Biosafety Office.
- Review the agent fact sheets and vaccine information sheets.
- Schedule a consultation appointment with the Occupational Health Physician through the Student Health Care Center (SHCC) to review immunization options.
- If you are requesting to receive an IND vaccine, contact the Biosafety Office to initiate the process.

Packaging, Shipping and Transport of Biological Materials

The shipping and transport of dangerous goods is a highly regulated activity. A large number of people will handle or be in proximity to your package as it travels to its destination. All that protects these people from any hazard within the package is the information you provide on or with your package and the packaging itself. All who ship or transport biological materials at UF must be certified to do so and renew the certification every two years or when regulations change. Contact the Biosafety Office at 352-392-1591 for training and certification.

Regulations that apply to the packaging and shipment of biological materials:

- U.S. Department of Transportation, 49 CFR Parts 171-180 and amendments
- U.S. Public Health Service, 42 CFR Part 72, Interstate Shipment of Etiologic Agents
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne Pathogens
- International Air Transport Association (IATA), Dangerous Goods Regulations
- U.S. Postal Service, 39 CFR Part 111, Mailability of Etiologic Agents, Mailability of Sharps and Other Medical Devices, and Publication 52, Acceptance of Hazardous, Restricted or Perishable Matter
- International Civil Aviation Organization, Technical Instructions for the Safe Transport of Dangerous Goods by Air
- United Nations, Recommendations of the Committee of Experts on the Transportation of Dangerous Goods

All North American airlines and FedEx, the largest shipper of infectious materials, use the IATA regulation (also referred to as the Dangerous Goods Regulation or DGR) as their standard. Meeting the conditions of this standard will ensure meeting the provisions of the other US regulations.

Many biological materials fall into the category of dangerous goods for shipping purposes. All individuals involved in the transport of dangerous goods or the preparation of dangerous goods for transport must be trained to do so properly and safely. In addition, the Biosafety Office requires safe transport of items within facilities and around campus. These topics are covered in the Shipping and Transport of Biological Materials training provided by the Biosafety Office as well as on the EH&S website. Additional details regarding Transport of Biological materials on campus is located on the EH&S website.

General guidelines for transport of Biological Materials on campus:

- Double contain the items in plastic leak-proof containers within sturdy outer packaging.
- Include absorbent material within the containers as well as padding to minimize movement of the container(s) within the outer packaging.
- Wipe the outer container with an appropriate disinfectant before removing it from the laboratory and apply a biohazard sticker if applicable.
- Place your name and contact information on the package.

Individuals transporting biohazardous agents should be knowledgeable about handling spills. Further, UF policy states that dangerous goods are not to be transported in your personal vehicle. This is both a safety and liability issue. A state vehicle must be used.

Training

A training certificate from UF EH&S must be obtained to ship biological materials. Training ensures successful shipments to the recipient since carriers or Federal regulators may open, delay or reject the shipment if not packaged/labeled correctly. In addition, violations of the shipping regulations may result in civil penalties of $250 - $27,500 per violation per day, and/or criminal penalties for willful violations, up to $500,000 and 5 years in jail. To register for the on-line Shipping and Transport of Biological Materials course send an email bso@ehs.ufl.edu and provide your first and last name and UFID. Participants will receive a certificate of training completion.

- Training is valid for 2 years and is a federal requirement & is designed to protect yourself, your co-workers, and the public – drivers, airline staff, crew, pilots, passengers, and package recipients.
- All individuals involved in the transport of dangerous goods or the preparation of dangerous goods for transport must abide by the International Air Transport / International Civil Aviation and Dept. of Transportation regulations.

Training Learning Objectives

- Classifying the material – Is it regulated? Is it forbidden for transport?
- Identifying the material – select the proper shipping name
- Choosing the right packaging
- Packaging correctly
- Marking & Labeling the shipment correctly
- Supplying additional required documentation – dangerous goods declaration forms
- Making shipping arrangements – i.e. permits, customs documents for overseas shipments
- Transporting materials safely around UF – hand carrying & vehicle transport

Biological Material Subject to Shipping & Transport Regulations

In the context of shipping regulations, Dangerous Goods are “Articles or substances which are capable of posing a risk to health, safety, property or the environment & which are shown in the list of dangerous goods in the Regulations or which are classified according to these Regulations.” (49 CFR Parts 100-185 & IATA 1.0).

Biological Materials Under This Definition:

- Biological toxins
- Infectious substances
- Diagnostic specimens
- Biomedical waste
- Cultures
- Genetically Modified Organisms

Other Regulated Biological Material:

- Plants
- Plant pests
- Insects
- Cell cultures
- Live animals

Other Regulated Items Accompanying Biological Material Shipment:

- Dry ice
- Environmental pollutants (formalin)
- Alcohol
- Fixative solutions

If shipping radioactives, call 392-7359. If shipping chemicals, call 392-8400.

Transport of Biological Materials on Campus
In addition, the Biosafety Office requires safe transport of items within facilities and around campus. These topics are covered in the Shipping and Transport of Biological Materials training provided by the Biosafety Office as well as on the EH&S website. Additional details regarding Transport of Biological materials on campus is located on the EH&S website.

General guidelines

- Double contain the items in plastic leak-proof containers within sturdy outer packaging.
- Include absorbent material within the containers as well as padding to minimize movement of the container(s) within the outer packaging.
- Wipe the outer container with an appropriate disinfectant before removing it from the laboratory and apply a biohazard sticker if applicable.
- Place your name and contact information on the package.

Individuals transporting biohazardous agents should be knowledgeable about handling spills. Further, UF policy states that dangerous goods are not to be transported in your personal vehicle. This is both a safety and liability issue. A state vehicle must be used.

Permits

All exotic or potentially harmful plant-associated arthropods, controlled biological agents, plant pests, noxious weeds, invasive plants, genetically-engineered field released crops, regulated livestock pathogens, imported etiologic agents, or imported animal-source material are regulated by federal or state agencies (USDA APHIS, CDC, EPA, FDA, FDACS, etc.). These agents also require registration with the Biosafety Office by submission of a biological agent registration form and a copy of the current permit/notification and permit conditions that have been granted by that agency. The Principal Investigator is responsible for obtaining and maintaining valid permits.

CDC

A CDC Import Permit is required for import of etiological agents causing disease in humans, non-sterilized human or animal tissues/fluids known or suspected to contain disease agents, hosts/vectors known or suspected to contain disease agents. Additional information and permit application materials are located on the CDC website under Etiologic Agent Import Permit Program (EAIPP).

USDA

A USDA/APHIS Veterinary Permit is required for import of materials derived from (livestock/poultry) animals or exposed to (livestock/poultry) animal-source materials, including: animal tissues, blood, cells or cell lines of livestock or poultry origin, RNA/DNA extracts, hormones, enzymes, monoclonal antibodies for IN VIVO use in non-human species, certain polyclonal antibodies and antisera, bulk shipments of test kit reagents, arthropod vectors of livestock diseases, and microorganisms infectious to livestock including bacteria, viruses, protozoa, and fungi. In addition, a permit is also required for the interstate movement of microorganisms infectious to livestock/poultry including bacteria, viruses, protozoa, fungi, arthropod vectors of livestock/poultry diseases, and tissues, blood, serum, or cells from known infected livestock/poultry. Note: A courtesy letter to the Florida Department of Agriculture and Consumer Services Division of Animal Industry is required for possession or use of any of the State of Florida reportable animal diseases.

- A USDA/APHIS Plant, Organism and Soil Permit is required for import or interstate movement of plant pests, plant pathogens, biological control agents, bees, plant pest diagnostic laboratories, soil microbe isolation laboratories, federal noxious weeds and parasitic plants.
- A USDA Biotechnology Regulatory Services Notification or Permit is required for the import, interstate movement, or field release of genetically-engineered plants, arthropods, and plant-associated microorganisms.

U.S. Fish and Wildlife Service

The U.S. Fish & Wildlife Service issues permits under various wildlife laws and treaties at different offices at the national, regional, and/or wildlife port levels. Permits are required for certain live animals, including bats. For permit overview, application forms and determination as to whether a permit is required, the US Fish and Wildlife Service Permit webpage is a useful resource.

Florida Department of Agriculture and Consumer Services

A Division of Plant Industry Permit is required for the import into Florida of: arthropods, plant pathogens, nematodes, noxious weeds, genetically altered (insects, nematodes, plants, plant pests) organisms and biological control agents.

Bioagent Export Control

Export of Etiologic Agents of Humans, Animals, Plants and Related Materials is regulated by the U.S. Department of Commerce, Dept. of State, and Dept. of the Treasury. Export to certain countries is prohibited. A wide variety of etiologic agents of human, plant and animal diseases, including genetic material, and products which might be used for culture or production of biological agents, will require an export license. Furthermore disclosing (including oral or visual disclosure) of controlled information to a non-U.S. person, in the U.S. (also known as a deemed export) or abroad; performing technical assistance, training, or other defense services for, or on behalf of a non-U.S. person, whether in the United States (also known as a deemed export) or abroad is also controlled. For a complete listing of agents, general information, checklists and training, please see the UF Office of Research Website for Export Control.

Registration Information for Researchers

All UF research projects involving the following must be registered with the Biosafety Office and approved by the Biosafety Office or Institutional Biosafety Committee (IBC):

- Known human, animal, or plant pathogens or pathogenic material; BSL-2 or BSL3
- Suspected human or animal pathogens or pathogenic material
- Primary human tumor cells
- Cell lines transformed with a virus
- All use or generation of recombinant or synthetic nucleic acid, including cloning of PCR products.
- Dual Use Research of Concern experiments
- Biological toxins having an LD_{50} of ≤100 μg/kg body weight
- Biological materials regulated by state or federal permits
- Select Agents

State and federal requirements, as well as many granting agencies, require that the University monitor the use of biohazards. The registration is a means to initiate a risk assessment of the project, and to ensure that these materials are handled properly and disposed of appropriately. Project registration categories include Biological Agents, Recombinant and Synthetic Nucleic Acid Molecules, and Acute Toxins. Approved projects must be renewed annually to retain their approved status.

Biological Agent Registration

Use of the following materials requires that the principal investigator complete and submit the Biological Agent registration form for approval:

- All human, animal, or plant pathogens that require BSL-2 or BSL-3 containment and handling.
- Unknown human and animal pathogens; these are considered BSL-2 until identified.
- Cell lines or cultures that have been immortalized with a virus (e.g. EBV), or are primary human tumor cells.
- Research use of human blood, cells, or other tissues that are known to be positive for any human disease agent

Recombinant and Synthetic Nucleic Acid Registration

Use of the materials listed below requires that the principal investigator complete and submit the Recombinant or Synthetic Nucleic Acid registration form for approval. In the context of the NIH Guidelines, Recombinant and Synthetic Nucleic Acid molecules are defined as either: (i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids; (ii) 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 (iii) molecules that result from the replication of those described in (i) or (ii) above. In addition to the above UF requires the following to be registered:

- All projects that involve recombinant or synthetic nucleic acid technology experiments that are designed to integrate into DNA, are replication competent or able to replicate in a living cell or code for a vertebrate toxin with an LD_{50} of <100 nanograms/kilogram body weight.
- Projects that involve the creation of transgenic animals and plants. An exception to the registration requirement is the use of purchased or transferred (i.e. from another investigator, colleague, or collaborator) transgenic rodents.
- Human Gene Therapy Projects e.g. the transfer of recombinant or synthetic nucleic acid, or DNA or RNA derived from recombinant or synthetic nucleic acid, into human research subjects.

Acute Toxin Registration

Use of the following materials requires that the principal investigator complete and submit the Acute Toxin registration form for approval.

- All biological toxins with a mammalian LD_{50} of ≤ 100µg/kg body weight*, as well as the organisms (both natural and recombinant) which produce these toxins. The Biosafety Office has compiled a Toxin Table with the LD_{50} values for numerous toxins.
- All Select Agent Toxins (including quantities exempt from federal regulations). The Select Agent and Toxins Table (also see Table 4) lists these toxins. Exempt levels of select toxins are located in Table 5.

*Please note that LD_{50} values may be obtained from a number of sources. For specifics on route of application (i.v., i.p., s.c.), animals used, and variations on the listed toxins please see references below. All laboratories using acute toxins must develop lab-specific SOPs that include written safety protocols, security measures, inventory control, posted emergency procedures, and specialized inactivation or disposal procedures must be developed. A Standard Operating Procedure Template for documenting this information has been developed. The SOPs should be readily available as printed copies in the laboratory where the toxin is used.

For additional information, please refer to the following references:

- Gill, D.Michael, 1982.Bacterial toxins: a table of lethal amounts; Microbiological Reviews 46:86-94
- Stirpe, F., Luigi Barbieri, Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; Biotechnology; 10: 405-412
- Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the RTECS. 1997. Doris V. Sweet, ed., U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; Cincinnati, Ohio

Administrative and Technical Change Amendments

Any and all changes to registered projects must be documented and provided to the Biosafety Office. These changes include, but are not limited to, the following:

- Addition or deletion of project personnel or project location; termination of project
- Addition of a host, target recipient, gene insert, or methodology to existing Recombinant or Synthetic Nucleic Acid registration
- Addition of a non-viral vector to existing Recombinant or Synthetic Nucleic Acid registration
- Addition of a viral vector to existing Recombinant or Synthetic Nucleic Acid registration
- Addition of a biological agent, host, or methodology to existing BA registration
- Addition of a toxin, host, or methodology to existing AT registration

Human Gene Therapy Projects

These projects require additional documentation as part of the registration process:

- Study Protocol
- Informed Consent Document
- Investigator Brochure
- NIH Appendix M Points to Consider in the NIH guidelines.
- Copies of relevant RAC correspondence
- Institutional Review Board (IRB) review. The IBC requests that all human gene therapy trials go through a UF IRB. IRB approval is not required prior to IBC submission. If UF has an INSTITUTIONAL conflict of interest, such as owning the technology, the Western Institutional Review Board (WIRB) can be the IRB of record, but the UF IRB must review and comment as well.
- Project site listing: manufacture (if applicable) area, storage area, administration area, as well as laboratory.

Laboratory Biosafety Levels and Checklists

The National Institutes of Health (NIH) and Centers for Disease Control and Prevention (CDC) describe four biosafety levels for work involving infectious microbes and biological agents (see tables 2, 3). Biosafety Levels are designated in ascending order commensurate with risk and degree of protection provided to personnel, the environment and the community. The levels outline combinations of work practices, safety equipment, administrative controls, and laboratory facility design and features. Standard microbiological practices are common to all laboratories. Special microbiological practices outlined for each Biosafety Level enhance worker safety, environmental protection and risks associated with handling the agents.

- Essential elements of the four biosafety levels, designated Biosafety Levels (BSL) 1-4*, are described in the CDC/NIH BMBL 5th edition.
- Four biosafety levels are described for activities involving infectious disease work with commonly used experimental animals and are designated Animal Biosafety Levels (ABSL) 1-4*.
- Physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving plants and plant-associated microorganisms are outlined in Appendix P of the NIH Guidelines and are designated Biosafety Level (BL) 1—Plants, BL-2P, BL-3P and BL-4P*. Additional guidance is available from "A Practical Guide to Containment Greenhouse Research with Transgenic Plants and Microbes."

***Note:** No BSL-4, ABSL-4 or BL-4P experiments are permitted at the University of Florida.

The checklists for CDC/NIH guidelines for each Biosafety Level are on the Biosafety Office website: http://www.ehs.ufl.edu/programs/bio/forms/biosafety-levels-checklists/. These checklists are used by the Biosafety Office to ensure compliance of each laboratory during laboratory inspections. Individual laboratories should use these checklists to periodically evaluate their work practices.

Special Issues

HIV Research Laboratories

This policy is designed to protect employees who conduct research with HIV. Researchers who handle, manipulate, or assay live HIV cultures are covered under this policy. Prior to beginning work in an HIV research lab, an employee shall be provided, at no cost to the employee, the following:

- A baseline serum sample shall be stored with the UF Student Health Care Center. This is a requirement to work in the HIV lab.
- A confidential HIV test shall be offered to the employee. The HIV test is available at the UF Student Health Care Center (SHCC). The test must be offered by the employer, but may be declined by the employee. This test shall include HIV counseling. Results of the test shall be provided to the employee in person in a face-to-face meeting with a health care professional. No exceptions.
- All results of HIV testing shall remain completely confidential and shall be stored in a separate section in the employee's medical record. At no time will the employer or any administrator or official of UF have access to the employee's confidential record concerning HIV testing.
- If the HIV researcher also works with human blood or other potentially infectious material, all other aspects of the UF Bloodborne Pathogen Program under the OSHA Bloodborne Pathogen standard shall be implemented for that employee. The employer shall offer free HBV immunization, annual training, and have a written exposure control plan in the workplace.
- Annual serum banking shall be offered to the employee, but may be declined.
- Annual HIV tests shall be offered to the employee, but may be declined.
- Annual training is required regarding HIV and post-exposure prophylaxis, including the risks of chemoprophylaxis.

Post-exposure prophylaxis

An exposure to an HIV culture in a research laboratory is considered a high-risk exposure (HIV Status Code 2) according to Public Health Service CDC guidelines. The following shall be implemented:

- If the exposure is to intact or broken skin, or in the event of a puncture wound, immediately wash the affected area with water for 15 minutes. Soap may be used, if immediately available. If the exposure is to the eyes, they shall be rinsed for 15 minutes in an eyewash station. Other exposed mucous membranes (nose and mouth) shall be rinsed for 15 minutes with water.
- The employee shall proceed directly to the ER. It is important for the employee to seek treatment within the first two hours of exposure.
- An employee with an exposure to HIV will be medically evaluated immediately, free of charge.
- The current Public Health Service (CDC) chemo-prophylactic treatment/post-exposure prophylaxis (PEP) shall be recommended and offered to an employee with a high-risk exposure, within the first two hours of exposure. The medical use of chemoprophylaxis is a decision that shall be made by a health care professional in conjunction with the employee, based upon the most current CDC recommendations, the nature of the exposure event, and other medical factors. Shands pharmacy is well stocked with the appropriate medication.
- Baseline tests and informed consent statements shall be required for PEP.
- Counseling concerning the risks and benefits of the chemo-prophylactic treatment shall be provided to the employee at the time of the exposure event.
- The employee may contact an infectious disease specialist, if desired.

All HIV exposures that occur in the workplace are covered under Worker's Compensation. SHCC and UF Medical Plaza personnel are familiar with the documentation to process this type of Worker's Compensation claim for UF employees. Post-exposure follow-up, including offering HIV testing and counseling, shall be provided in accordance with the CDC recommendations and the OSHA Bloodborne Pathogen rule. The SHCC and UF Medical Plaza protocols on HIV exposures will be followed.

References

- UF Bloodborne Pathogen Program Compliance Materials
- OSHA. 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens, Final Rule, Dec. 6, 1991
- CDC. Public Health Service Guidelines for the Management of Health-Care Worker Exposures to HIV and Recommendations for Postexposure Prophylaxis; MMWR 1998; 47(no. RR-7)

Non-Human Primates (monkeys) B Virus Information

These guidelines are to protect workers including veterinarians, laboratory workers, and others who come into contact with, or handle tissues derived from, old world non-human primates (NHP), particularly macaque monkeys. For additional details, refer to the UF Animal Contact Program, and Centers for Disease Control and Prevention Website.

B virus, formerly called Cercopithecine herpes virus 1 is now known as Macacine herpesvirus 1. It is also known as herpes B virus, monkey B virus or *Herpesvirus simiae*. The virus is endemic among monkeys of the genus Macaca which includes "Old World" monkeys such as rhesus and pig-tailed macaques, cynomolgus monkeys, and others.

Infected monkeys may be asymptomatic or may have mild lesions on the mouth, face, lips, and or genitals. The lesions heal spontaneously but may appear again sporadically in the same way that cold sores do in humans. In humans, B virus causes acute, usually fatal encephalitis. Any monkey handler who has an exposure event and then notices skin lesions or symptoms such as itching, pain, or numbness near the wound or exposure site should notify the supervisor and seek medical attention immediately. Transmission of B virus occurs through monkey bites, scratches, or contact with infected monkey tissues, cells, or fluids, including blood, saliva, urine, and feces. All old-world primates, regardless of their origin, should be treated as positive for B virus.

Those who work in direct contact with non-human primates or equipment used to contain, transport, or handle these animals or enter the rooms housing these animals must wear the personal protective equipment (PPE) described in the UF Animal Contact Program and this Biological Safety Manual. Use of PPE is not optional. UF has adopted the CDC's "Guidelines for Prevention of Herpesvirus Simiae (B Virus) Infection in Monkey Handlers" published in 1987. These handling practices are required by Animal Care Services and Environmental Health & Safety. Any known or suspected exposure to old-world primate fluids requires immediate medical care. The CDC and NIH have published a post-exposure protocol, "Recommendations for Prevention of and Therapy for Exposure to B Virus (Cercopithecine Herpesvirus 1)" for reference.

- In the event of a mucous membrane or eye exposure, immediately (within 5 minutes) irrigate the area with free-flowing water for 15 minutes.
- In the event of a bite or scratch, immediately (within 5 minutes) wash the wound with soap and water for 15-20 minutes. Gently massaging the wound is helpful. Length of washing time is more important than the use of soap.
- Seek medical attention immediately. Post exposure prophylaxis and follow-up will be carried out according to CDC guidelines under the care of a university physician who is an infectious disease specialist.
- A bite or scratch from non-human primates: contact the following physician/specialist regarding B virus exposure: Dr. Kenneth Rand Work Phone Dr. Kenneth Rand, Work: (352) 265-0111 x44875, Pager: (888) 543-1806, Cell 352-222-4613.
- The physician will evaluate the injury and may decide to culture the wound for B-virus or collect blood for a baseline titer against B-virus, or use prescription drugs for preventative therapy. The physician directing the care of the patient will contact the Director of Animal Care Services for instructions regarding the need for cultures or serology from the monkey inflicting the injury upon the patient.

Symptoms suggestive of B virus infection should be reported immediately to the medical consultant. When the possibility of B virus illness is seriously considered, appropriate diagnostic studies should be performed and specific antiviral therapy should be instituted. The physician may wish to consult Dr. Scott Schmid at (404) 639-0066; cell: 404-725-5652 at Measles, Mumps, Rubella, and Herpes (MMRH) Branch, Division of Viral Diseases, CDC, and Dr. Julia Hilliard at (404) 413-6550; cell: 404-358-8168, at National B Virus Resource Center at Georgia State University, Atlanta, GA for laboratory assistance.

Q fever Control

The objective of Q fever control is to protect University faculty, staff, students, volunteers and visitors from exposure to the Q fever agent *Coxiella burnetii*. University policy stipulates that sheep and goats coming to UF for biomedical research purposes must test negative for Q fever within one month prior to the shipment. Sheep and goats coming to UF for agricultural purposes will be held in an outdoor isolated quarantine area until Q fever negative test results are obtained (contact EH&S for guidance on Q fever testing). All newly arriving animals will be held in a quarantine area and segregated from other animals. Access to quarantined animals will be restricted to essential personnel. Sheep and goats will be required to have a second Q fever negative test prior to being housed indoors or used for biomedical research or invasive surgical procedures. Animals confirmed positive will be euthanized and disposed of as biohazardous material; no tissues may be collected from positive animals. All indoor housing, research, and/or procedure areas for sheep and goats will be confined to areas having no recirculation of air to other rooms. These rooms will be posted with a biohazard sign.

All rooms in the Animal Care Services vivarium housing sheep or goats shall be negative pressure relative to vivarium corridor(s). Participation in the Animal Contact Medical Monitoring Program is required for all individuals working with, or in close proximity to, sheep and goats or for those entering indoor housing, research, and/or procedure areas. Follow-up assessments are conducted on a periodic basis, as well as in the event of an exposure to a Q fever positive animal.

Employees, students, or UF affiliates who develop a febrile illness while working with sheep and goats (or their tissues or fluids) will be directed to seek immediate medical care at the SHCC Occupational Medicine Service. Initial training is required for all individuals that will work with, and around, sheep and goats. The awareness training will cover information about Q fever found in the Animal Contact Program Handbook, and methods to reduce Q fever exposure must be completed before an individual is allowed to work with sheep and goats, as described below:

- Indoor housing, procedure, and research areas require disposable or on site-laundered jumpsuits, coveralls, or scrubs, booties or dedicated footwear, eye protection, surgical mask or HEPA-filtered/N95 respirator (recommended), and gloves.
- Obstetrical procedures or surgery/necropsy of pregnant animals conducted indoors will require the use of a HEPA-filtered/N95 and hair cover. Note that the use of a HEPA-filtered/N95 respirator requires enrollment in the EH&S Respiratory Protection Program.
- IFAS or Veterinary Medical Center personnel contacting placental tissue or amniotic fluid (i.e. at parturition or abortion) should wear coveralls, boots, face mask or HEPA-filtered/N95 respirator (recommended), and gloves. Personnel must wash and change prior to leaving the facility.
- Sheep and goat surgical/necropsy procedures require, on site-laundered scrubs, leak proof/moisture repellant surgical gown, booties or dedicated footwear, goggles, surgical mask or HEPA/N95 (required for surgery on pregnant animals or obstetrical procedures) and hair cover.
- All disposable PPE shall be left on site in biohazard bags. All reusable PPE shall be appropriately disinfected. Surgical scrubs and gowns shall be autoclaved prior to laundering. Biosafety Level 2 practices will be followed for sheep and goat research, including research with sheep and goat tissues.
- Special care shall be taken to properly contain and inactivate materials or tissue having contact with amniotic fluid.

Aborted fetuses should be removed immediately for disposal as biohazardous material and the ewe or doe retested for Q fever. EH&S Biosafety personnel shall perform annual inspections of all sheep and goat facilities and practices. EH&S will audit work practices, PPE, and engineering controls.

Failure to comply with the above policies and procedures will result in the rescinding of an investigator's animal use approval and ability to procure animals.

All those who work with, or in close proximity to, sheep and goats or those entering sheep and goat indoor housing, research, and/or procedure areas shall be required to undergo a pre-placement or initial Animal Contact Medical Monitoring Risk Assessment. The risk assessment includes:

- A questionnaire about the location, frequency, and type of work with sheep and goats
- A medical history questionnaire

This work-related information will be evaluated by UF Occupational Medicine Physicians or Licensed Health Care Professionals at the Student Health Care Center (SHCC) to determine potential health risks to you and whether further clinical interaction or preventive steps may be necessary to protect your health. All individuals working with, or in close proximity to sheep and goats will be required to provide a sample of blood for serum storage (serum banking). This should be performed at the SHCC.

Depending on the location, frequency, and type of work with sheep and goats, you may be required to complete the filtering face piece respirator medical questionnaire and obtain clearance from the SHCC for a filtering face piece respirator. To wear this type of respirator, a fit test is required by EH&S Occupational Medicine. Fit tests are required on an annual basis. If a Q fever positive animal is identified during the course of research, a teaching project or clinical workup, all potentially exposed individuals shall undergo further medical evaluation that may include Q fever titer testing.

Persons with a high risk for developing Q fever

Individuals identified by way of the risk assessment as being at an increased risk for developing Q fever shall be scheduled for a medical consultation/assessment at the SHCC. They will be advised that it is not recommended that they work under conditions that may expose them to Q fever. The reasons for this will be thoroughly explained during the health consultation. The following conditions indicate an increased risk for developing Q fever or complications from Q fever:

- Valvular heart disease
- Pregnancy
- Prosthetic heart valves
- Liver disease
- Altered immune system

Disinfectants Appropriate for Sheep and Goat Work

- Surfaces in surgical and laboratory areas: fresh-made 10% solution of household bleach, 10% solution of H2O2 or 5-10% solution of phenolic-based Lysol concentrate.
- Large Contaminated Items that cannot be Autoclaved: fresh-made 10% solution of bleach plus detergent.
- Housing Facilities: fresh-made 5% solution of household bleach, 5% solution of H_2O_2, 5% solution of phenolic-based Lysol concentrate.
- Large scale decontamination of facilities with paraformaldehyde or vaporized hydrogen peroxide may be performed by a trained professional, after approval from the EH&S Biosafety Office.

Disinfectants not appropriate for use for sheep and goat work: Ethanol, 1% phenol, 1% formalin, Quartenary ammonium compounds, Wexcide®, Broadcide®.

Tuberculosis Research Laboratories

Laboratory personnel working with *M. tuberculosis* are three times more likely to get infected with the agent as compared to those not working with it; it is the fourth most commonly reported laboratory infection. *M. tuberculosis* has a low infective dose: 10 bacilli by inhalation. Multidrug resistant (MDR) TB strains are one of the emerging pathogens and CDC has recently issued interim guidelines for handling these in clinical and research laboratories. Exposure to *M. tuberculosis* bacilli is likely when handling infected sputum, gastric lavage fluid, CSF, urine, and various tissues. Aerosols pose the most important hazard. Accidental needle-stick inoculation is also possible. Non-human primates (NHP) infected with *M.*

tuberculosis are a potential source of human infections. A litter of infected guinea-pigs and mice are likely to produce aerosols. *M. bovis* may be acquired from infected cattle. Direct contact, ingestion, parenteral inoculation and aerosols when handling clinical material and cultures are a potential source of infection with NTM (non-tuberculous mycobacteria). The Public Health Agency of Canada site is an excellent resource for PSDS for these agents.

Biosafety recommendations:

- Based on risk assessment, handling *M. tuberculosis* may be recommended at BSL- 2 or 3 and ABSL-2 or 3 containment and practices.
- Researchers working at BSL-3 are required to enroll in the BioPath Program. Medical Alert Cards are provided for working with Respiratory/Contact agent (R/C).
- NIOSH respiratory protection program is mandated for all individuals required to wear respiratory protection (N95/97 or PAPRs). Fit testing for respirators is absolutely necessary before commencing work requiring respirators, and annually thereafter. (See Occupational Medicine Program of this manual or the EH&S OCCMED website).
- Tuberculin skin testing (TST) using protein purified derivative (PPD) is performed annually/semi-annually, based on risk assessment by the Biosafety Office, in previously skin-test negative personnel. If previously positive, annual follow-up medical evaluation is required.
- For respiratory fit test and PPD testing, the researcher should enroll in the BioPath program. .
- Working with NTM requires BSL-2 containment and practices. ABSL-2 is recommended for animal work.

Vaccinia and Other Pox Virus Research Laboratories

All open manipulations of Pox virus must be confined to a BSC. When open manipulations are in progress, unvaccinated personnel many not enter the vaccinia BSL2 laboratories, however when open manipulations are not ongoing (i.e. cultures are secured in incubators, freezers, etc.) unvaccinated personnel may enter the laboratory but must be escorted at all times.

Tables

Table 1: Classification of Infectious Microorganisms by Risk Group

Risk Group (RG)	WHO Laboratory Safety Manual 3rd Ed. 2004	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules May, 2013
RG1	(No or low individual and community risk) A microorganism unlikely to cause human/animal disease.	Agents that are not associated with disease in healthy adult humans.
RG2	(Moderate individual risk; low community risk) A pathogen that can cause human/animal disease but is unlikely to be serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
RG3	(High individual risk, low community risk) A pathogen that usually causes serious human/animal disease that does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual but low community risk).
RG4	(High individual and community risk) A pathogen that usually causes serious human/animal disease and that can be readily transmitted from one individual to another. Effective treatment and preventive measures are not usually available.	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

References:

Section II of the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, 2009,

WHO Biosafety Manual, 3rd Edition, 2004

NIH Guidelines, 2013

Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

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

Reference: Section IV of the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, 2009

Table 3: Summary of Recommended Animal Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals Are Used

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

Reference: Section V of the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, 2009

Table 4: Select Agents and Toxins

HHS AND USDA SELECT AGENTS AND TOXINS
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS

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

Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
- Far Eastern subtype
- Siberian subtype

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

OVERLAP SELECT AGENTS AND TOXINS

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

USDA SELECT AGENTS AND TOXINS

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

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

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

*Denotes Tier 1 Agent

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

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

12/4/12

Table 5: Permissible Toxin Amounts

Although they must still be registered by the Biosafety Office, in small quantities these select agent toxins are exempt from select agent regulations, provided the amount under control of a principal investigator, treating physician or veterinarian does not exceed, at any time or in any form, the amounts indicated in the table below.

HHS Toxins	Amount
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Staphylococcal enterotoxins (Subtypes A, B, C, D, and E)	5 mg
T-2 toxin	1000 mg
Tetrodotoxin	100 mg

Table 6: Summary of NIH Guidelines; Sections III-D, III-E, III-F

Section	Subsection	Research Application Description	Comments
III-D-1		Experiments using Risk Group (RG) 2, 3, or 4, or Restricted Agents as host-vector systems	USDA/APHIS permit may be required for work with certain plant or animal pathogens. See http://www.aphis.usda.gov/.
	III-D-1-a	Recombinant or synthetic nucleic acid molecules into RG2 agents	Usually conducted at BSL-2/ABSL-2
	III-D-1-b	Recombinant or synthetic nucleic acid into RG3 agents	Usually conducted at BSL-3/ABSL-3
	III-D-1-c	Recombinant or synthetic nucleic acid into RG4 agents	Usually conducted at BSL-4/ABSL-4[1]
	III-D-1-d	Recombinant or synthetic nucleic acid into restricted agents	Containment determined on a case-by-case basis following NIH/OBA review
III-D-2		Experiments in which DNA from Risk Group 2, 3, or 4, or Restricted Agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems	
	III-D-2-a	DNA from RG2, RG3, or RG4 agents	BSL-2 containment for cloning from RG2 or RG3 pathogens; specific lowering to BSL-1 may be approved by the IBC BSL-2 for cloning from RG4 agents only after demonstration that a totally & irreversibly defective fragment of the agent's genome is present in a given recombinant; otherwise BSL-4 required.
	III-D-2-b	DNA from restricted agents	Containment determined on a case-by-case basis following NIH/OBA review
III-D-3		Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems	
	III-D-3-a	Infectious/defective RG2 viruses with helper virus	Usually conducted at BSL-2
	III-D-3-b	Infectious/defective RG3 viruses with helper virus	Usually conducted at BSL-3
	III-D-3-c	Infectious/defective RG4 viruses with helper virus	Usually conducted at BSL-4[1]
	III-D-3-d	Infectious/defective restricted poxviruses with helper virus	Containment determined on a case-by-case basis following NIH/OBA review
	III-D-3-e	Viruses not covered in III-D-3-a through III-D-3-d	Usually conducted at BSL-1
III-D-4		Experiments involving whole animals in which the genome has been altered by stable introduction into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecules -modified microorganisms tested on whole animals	
	III-D-4-a	Recombinant or synthetic nucleic acid molecules from any source except for > 2/3 of eukaryotic viral genome	Usually conducted at BSL-1; viral vectors must not lead to transmissible infection
	III-D-4-b	Recombinant or synthetic nucleic acid molecules involving whole animals and not covered by III-D-1 or III-D-4-a	Appropriate containment decided by the IBC

	III-D-4-c-(1)	Generating transgenic rodents that require BSL-1 containment	Covered under Section III-E-3
	III-D-4-c-(2)	Purchase or transfer of transgenic rodents	Exempt under Section III-F-6, Appendix C-VI
III-D-5		Experiments involving whole plants – genetically engineering plants by recombinant or synthetic nucleic acid molecules methods, using or propagating such plants, using plants with microorganisms or insects containing recombinant or synthetic nucleic acid molecules	
	III-D-5-a	Exotic plant pathogens with recognized potential for serious detrimental impact on ecosystems	Usually conducted at BSL-2+P/BSL-3P
	III-D-5-b	Readily transmissible exotic agents in which the complete and functional genome may be reconstituted *in planta*	Usually conducted at BSL-2+P/BSL-3P
	III-D-5-c	Readily transmissible exotic agents in the presence of their arthropod vector	Usually conducted at BSL-4P[1]
	III-D-5-d	Sequences encoding potent vertebrate toxins introduced into plants or associated organisms	Usually conducted at BSL-3P
	III-D-5-e	Microbial pathogens of insects or small animals associated with plants	Usually conducted at BSL-2+P/BSL-3P
III-D-6		Experiments involving > 10 liters of culture	Containment to be decided by IBC; see Appendix K for containment conditions
III-D-7		Experiments involving influenza viruses	Conducted at the containment level corresponding to the RG of the virus that is the source of the majority of segments
	III-D-7-a	Human H2N2 (1957-1968)	BSL-3+ for viruses containing H2 hemagglutinin (HA) segment BSL-2 for H2 HA gene in cold-adapted, live attenuated vaccine strains and for H2N2 genes other than HA
	III-D-7-b	Highly pathogenic avian influenza H5N1	Usually conducted at BSL-3+
	III-D-7-c	1918 H1N1	Usually conducted at BSL-3+
	III-D-7-d	Antiviral susceptibility for genes from viruses in III-D-7-a through III-D-7-c	Higher containment may be required if any of the genes are resistant to both classes of current antiviral agents (adamantanes and neuraminidase inhibitors)

Section	Subsection	Research Application Description	Comments
III-E		All components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes	BSL-1
III-E-1		Formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus	BSL-1, provided that cells lack helper virus for the specific families of defective virus being used
III-E-2		Nucleic acid molecule-modified plants or recombinant or synthetic nucleic acid-modified microorganisms associated with plants not covered in sections III-A, III-B, III-D or III-F	
	III-E-2-a	Recombinant or synthetic nucleic acid modified plants that are not noxious weeds and nucleic acid-modified non-exotic microorganisms (e.g. Rhizobium and Agrobacterium spp.)	BSL-1P
	III-E-2-b-(1)	Plants modified by recombinant or synthetic nucleic acid molecules that are Noxious weeds or can interbreed with noxious weeds in the immediate area	BSL-1+P/BSL-2P
	III-E-2-b-(2)	Plants containing complete genome of non-exotic infectious agent	BSL-1+P/BSL-2P
	III-E-2-b-(3)	Plants associated with recombinant or synthetic nucleic acid -modified non-exotic microorganisms that have potential for serious detrimental impact on ecosystems	BSL-1+P/BSL-2P
	III-E-2-b-(4)	Plants associated with recombinant or synthetic nucleic acid -modified exotic microorganisms that have no potential for serious detrimental impact on ecosystems	BSL-1+P/BSL-2P
	III-E-2-b-(5)	Recombinant or synthetic nucleic acid -modified arthropods or small animals associated with plants or arthropods or small animals with recombinant or synthetic nucleic acid modified microorganisms associated with them if the microorganism has no potential for serious detrimental impact on ecosystems	BSL-1+P/BSL-2P
III-E-3		Creation of transgenic rodents	BSL-1; experiments requiring BSL-2 or higher covered under section III-D-4
	III-E-3-a	Breeding of BSL-1 transgenic rodents	Exempt under Section III-F-6, Appendix C-VII

Section	Subsection	Research Application Description	Comments
III-F		Exempt experiments involve recombinant or synthetic nucleic acid molecules that:	
III-F-1		Synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and (2) are not designed to integrate into DNA, and (3) do not produce a toxin that is lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight. If a synthetic nucleic acid is deliberately transferred into one or more human research participants and meets the criteria of Section III-C, it is not exempt	
III-F-2		Are not in organisms or viruses and have not been modified or manipulated	
III-F-3		Consist entirely of exact recombinant or synthetic nucleic acid from single nonchromosomal or viral DNA source (one or more segments may be synthetic equivalent)	
III-F-4		Consist entirely of nucleic acids from prokaryotic host when propagated only in that host (or closely related strain of the same species) or when transferred to another host by well-established physiological means	
III-F-5		Consist entirely of nucleic acids from a eukaryotic host (excluding DNA from viruses) when propagated only in that host (or closely related strain of the same species)	
III-F-6		Consist entirely of DNA from different species that that exchange DNA by known physiological processes (one or more segments may be synthetic equivalent)	See Appendix A for list of natural exchangers
III-F-7		Genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.	
III-F-8		Do not present significant risk to health or environment	Appendix C
	App C-1	Recombinant or synthetic nucleic acid containing < ½ of any eukaryotic viral genome in tissue culture	Exceptions: Experiments described in Sections III-A or III-B, those involving RG3, 4, or restricted agents, large-scale experiments, or cloning of toxin molecule genes coding for biosynthesis of molecules toxic for vertebrates
	App C-II	*E. coli* K-12 host-vector systems	Same exceptions as C-1
	App C-III	*Saccharomyces* host-vector systems	Same exceptions as C-1

	App C-IV	*Kluyveromyces Host-Vector Systems*	
	App C-V	*Bacillus subtilis* or *Bacillus licheniformis* host-vector systems	Same exceptions as C-1
	App C-VI	Extrachromosomal elements of listed gram positive organisms propagated and maintained in gram positive organisms	See App C-V for listSame exceptions as C-1
	App C-VII	Purchase or transfer of transgenic rodents	Only applies to rodents requiring BSL-1 containment
	App C-VIII	Generation of BL1 Transgenic Rodents via Breeding	

Reference: NIH Guidelines

Table 7: Animal Experiments Covered Under the NIH Guidelines

ACTIVITY	MINIMUM BSL	SECTION
CREATION OF TRANSGENIC ANIMALS		
Creation of transgenic rodents	BL1	III-E-3
Creation of transgenic rodents	BL2 or higher	III-D-4-b
Creation of transgenic animals other than rodents	BL1/BL1-N	III-D-4-a
Creation of transgenic animals other than rodents	BL2/BL2-N or higher	III-D-4-b
Creation of recombinant DNA modified arthropods	BL1	III-D-4-a
Creation of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Creation of knock-out rodents	BL1	III-E-3
Creation of knock-out rodents	BL2 or higher	III-D-4-b
BREEDING OF TRANSGENIC ANIMALS		
Breeding rodents from one strain (propagation/colony maintenance)	BL1	Exempt (III-F-4)
Breeding rodents from one strain (propagation/colony maintenance)	BL2 or higher	III-D-4-b
Breeding rodents from two strains (generating new strain)	BL1	III-E-3
Breeding rodents from two strains (generating new strain)	BL2 or higher	III-D-4-b
Breeding of transgenic animals other than rodents	BL1	III-D-4
Breeding of transgenic animals other than rodents	BL2 or higher	III-D-4
Breeding of recombinant DNA modified arthropods	BL1	Exempt (III-F-4)
Breeding of recombinant DNA modified arthropods	BL2 or higher	III-D-4-b
Breeding of knockouts (propagation)	BL1	Exempt (III-F-4)
Breeding of knockouts (propagation)	BL2 or higher	III-D-4-b
Breeding of knockouts from two strains (generating new strain)	BL1	III-E-3
Breeding of knockouts from two strains (generating new strain)	BL2 or higher	III-D-4-b
EXPERIMENTS WITH TRANSGENIC ANIMALS		
Experiments with transgenic rodents	BL1	III-D-4-a* (see note)
Experiments with transgenic rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with transgenic animals other than rodents	BL1	III-D-4-a
Experiments with transgenic animals other than rodents	≥ BL2 set by IBC	III-D-4-b
Experiments with recombinant DNA modified arthropods associated with plants	BL1	III-E-2-b-(5).
Experiments with recombinant DNA modified arthropods associated with plants	BL2 or higher	III-E-2
Experiments with recombinant DNA modified arthropods not associated with plants	BL1	III-D-4-a
Experiments with recombinant DNA modified arthropods not associated with plants	BL2 or higher	III-D-4-b
*The purchase or transfer of transgenic rodents requiring BL1 containment is exempt under Appendix C-6. Subsequent use of these animals is also exempt providing the experimental protocol does not involve the use of recombinant DNA. If the protocol does involve the use of recombinant DNA then the research is covered under III-D-4-a. All experiments involving the use of other transgenic animals at any Biosafety Level and the use of transgenic rodents requiring BL2 or higher containment are subject to the *NIH Guidelines*. See above for applicable sections.		

EXPERIMENTS WITH R-DNA IN AN ANIMAL (TRANSGENIC OR OTHERWISE)		
Experiments with r-DNA modified microbes in any animal (transgenic or otherwise)	BL1/BL1-N	Not permitted at BL1*
Experiments with RG2 r-DNA modified microbes in any animal (transgenic or otherwise)	BL2/ BL2-N	III-D-1-a
Experiments with RG3 r-DNA modified microbes in any animal (transgenic or otherwise)	BL3/ BL3-N	III-D-1-b
Experiments with RG4 r-DNA modified microbes in any animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-c
Experiments with r-DNA modified restricted agent in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Experiments with r-DNA modified animal pathogens in an animal (transgenic or otherwise)	BL4/BL4-N	III-D-1-d
Introduction of less than 2/3 of eukaryotic viral genome into a non-human vertebrate or invertebrate	BL1/BL1-N	III-D-4-a
Propagation of animals containing viral vector sequences not leading to transmissible infection	BL1/BL1-N	III-D-4-a
Experiments with R-DNA involving whole animals not covered by Sections III-D-1or III-D-4-a	Set by IBC	III-D-4-b
* Other than viruses which are only transmitted vertically, the experiments may not be conducted at BL1. A minimum of BL2 or BL2-N is required.		
CLONING ANIMALS		
Cloning animals	BL1 or higher	Not covered
PURCHASE OR TRANSFER OF TRANSGENIC ANIMALS		
Purchase or transfer of transgenic rodents	BL1	Exempt (Appendix C-6)
Purchase or transfer of transgenic rodents	BL2 or higher	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL1	III-D-4
Purchase or transfer of transgenic animals other than rodents	BL2 or higher	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL1	III-D-4
Purchase or transfer of recombinant DNA modified arthropods	BL2 or higher	III-D-4
PLANT EXPERIMENTS WITH ANIMALS OR ARTHROPODS		
Experiments with microorganisms or insects containing recombinant DNA with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	III-D-5-a or III-D-5-b
Experiments with exotic infectious agents in the presence of arthropod vectors	BL4-P	III-D-5-c
Experiments with microbial pathogens of insects or small animals associated with plants with the potential for detrimental impact to ecosystems.	BL3-P or BL2-P plus biological containment	III-D-5-e
Small animals associated with recombinant DNA-modified plants.	BL1	III-E-2
Experiments with rDNA-modified arthropods or small animals associated with plants	BL1	III-E-2-b-(5).
OTHER		
Transfer of a drug resistance to microorganisms compromising the use in veterinary medicine	Set by NIH (case by case)	III-A-1-a

Reference: Animal experiments covered under the NIH Guidelines for Research Involving Recombinant DNA Molecules

Table 8: Summary of Biosafety of Viral Vectors

Viral Vectors	Adeno-Associated Virus	Adenovirus	Lentivirus	Retrovirus (MuMLV)	Poxvirus (Vaccinia)	Herpesvirus (HSV-1)
Nucleic Acid	ssDNA	dsDNA	ssRNA	ssRNA	dsDNA	dsDNA
Envelope	N	N	Y	Y	Y	Y
Genome Size (kb)	4.5	36	9.7	8.3	192	152
Integration	<10%	No (episomal)	Yes (oncogenic potential)	Yes(oncogenic potential)	NO	No (episomal)
Cloning Capacity (kb)	<5	~8-10	~8	~8	~30	~30
Transmission	Aerosol	Aerosol	Bloodborne	Bloodborne	Aerosol; Direct Contact	Direct Contact
Generation of RCL	Negligible	Yes (low risk)	Yes	Yes	No	No
Risk Group (Parent Virus)	1	2	2/2+	Ecotropic:RG1 Ampho/Xeno- tropic: RG2	2	2
Biosafety / Animal Biosafety Level*	BSL1/ABSL1 (non-inflammatory; non-pathogenic)	BSL2/ABSL1 (High inflammatory potential)	BSL2+: handling viral stocks ;BSL2 following neg RCL; ABSL2, ABSL1 following viral shedding data or if using LVV-tranduced cells	Ecotropic:BSL-1 Ampho/Xenotropic:BSL-2	BSL2/ABSL2	BSL2/ABSL2 (High inflammatory potential; strong tropism for neurons)
Requirements	Standard Microbiological practices	BSL1/ABSL1 practices; negative pressure lab; Procedures with aerosol/splash potential in certified BSC; appropriate PPE:gloves, gowns, face protection; sharps precautions; appropriate disinfection; autoclave available	BSL2/ABSL2 practices; BBP training (including ACS personnel); 'safe sharps'; plastic labware; RCL/shedding data to be submitted to biosafety office	BSL/ABSL-1/2 practices	BSL2 practices; entry to pox lab resricted to individuals with smallpox vaccination withing last 10yrs; all waste in the lab treated as biohazardous; dress in-dress out policy including N95 for unvaccinated personnel	BSL2 practices

*Biosafety level higher if transgene is an oncogene, toxin, can alter host range, or increase virulence of the organism etc.

Table 9: Inactivation of Select Agent Toxins

Toxin	Chemical Inactivation	**Autoclaving** 121°C for 1 hour, liquid cycle, slow exhaust
Abrin <100mg*	Mix liquid samples with equal volume of 10% (v/v) NaOCL or 0.5 mol/L NaOH and expose for 30 minutes.	Yes
Botulinum neurotoxins <0.5mg*	Mix liquid samples with equal volume of 10% (v/v) NaOCL or 0.5 mol/L NaOH and expose for 30 minutes.	Yes
Conotoxin <100mg*	Mix liquid samples with equal volume of full-strength NaOCL and expose for 1 hour.	No
Diacetoxyscirpenol <1000mg*	Mix 1 volume of sample with 9 volumes of NaOCL/NaOH solution (1:1 v/v mixture of full strength bleach with 0.5 mol/L NaOH) and expose for 48-72 hours.	No
Ricin <100mg*	Mix liquid samples with equal volume of 10% (v/v) NaOCLor 0.5 mol/L NaOH and expose for 30 minutes.	Yes
Saxitoxin <100mg*	Mix liquid samples with 20% (v/v) bleach and expose for 30 minutes or mix with 0.25% (w/v) NaOCL/1% (w/v) NaOH solution and expose for 30 minutes.	No
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E) <5mg*	Mix liquid samples with equal volume of at least 20% (v/v) NaOCL or 0.5 mol/L NaOH and expose for 30 minutes.	Yes
T-2 toxin <1000mg*	Mix 1 volume of sample with 9 volumes of NaOCL/NaOH solution (1:1 v/v mixture of full strength bleach with 0.5 mol/L NaOH) and expose for 48-72 hours.	No
Tetrodotoxin <100mg*	Mix liquid samples with 20% (v/v) bleach and expose for 30 minutes or mix with 0.25% (w/v) NaOCL/1% (w/v) NaOH solution and expose for 30 minutes.	No

NaOCL – sodium hypochlorite; NaOH – sodium hydroxide

*Select agent toxins are exempt from the select agent registration requirements provided the amounts under control of the principle investigator do not exceed, at any time, the amounts indicated above.

Resources

Biosafety Resources**:** http://www.ehs.ufl.edu/programs/bio/

Respirator fit testing: http://www.ehs.ufl.edu/programs/ih/respirator/

Animal Contact Program: http://www.ehs.ufl.edu/programs/bio/animal/

Other Occ. Med. concerns: http://www.ehs.ufl.edu/programs/occmed/

Minors in Research: http://www.ehs.ufl.edu/Bio/minors.htm

Institutional Biosafety Committee: http://www.ehs.ufl.edu/programs/bio/ibc/

Select Agents: http://www.ehs.ufl.edu/programs/bio/select_agents/

Infectious Agents:
http://www.absa.org/riskgroups/index.html
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

NIH Guidelines for Research Involving Recombinant DNA Molecules (March 2013 revisions):
http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

NIH Office of Biotechnology Activities: http://oba.od.nih.gov/oba/index.html

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th ed. (2009):
http://www.cdc.gov/biosafety/publications/bmbl5/

Appendices

Appendix A. Acronyms, Abbreviations, Terms and Definitions

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ABSL	Animal Biosafety Level
ACS	Animal Care Services
APHIS	Animal and Plant Health Inspection Service
BBP	Bloodborne pathogen
BEA	Biological etiological agent
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BMW	Biomedical waste
BRS	Biotechnology Regulatory Service
BSC	Biological safety cabinet
BSL	Biological Safety Level
BSO	Biosafety Officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CJIS	Criminal Justice Information Service
DGR	Dangerous Goods Regulation
DNA	Deoxyribonucleic acid
DURC	Dual-use research of concern
EBV	Epstein - Barr virus
EH&S	Environmental Health & Safety
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FDACS	Florida Department of Agriculture and Consumer Services
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEPA	High-efficiency particulate air
HHS	Health and Human Services
HIV	Human immunodeficiency virus
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
IND	Investigational New Drug
IRB	Institutional Review Board
LAI	Laboratory Acquired Infection
MSDS	Material Safety Data Sheet
NHP	Non-human primate
NIH	National Institutes of Health
NIH Guidelines	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
NIOSH	National Institute for Occupational Safety and Health
NSABB	National Science Advisory Board for Biosecurity
OBA	Office of Biotechnology Activities

OCCMED	Occupational Medicine Program
OPIM	Other potentially infectious material
OSHA	Occupational Safety and Health Administration
PAPR	Powered Air Purifying Respirator
PI	Principal Investigator
PPE	Personal protective equipment
PPQ	Plant Protection and Quarantine
PSDS	Pathogen Safety Data Sheet
RAC	Recombinant DNA Advisory Committee
rDNA	Recombinant DNA
RG	Risk Group
SHCC	Student Health Care Center
SOP	Standard operating procedure
SRA	Security Risk Assessment
UF	University of Florida
USDA	United States Department of Agriculture
WHO	World Health Organization
WIRB	Western Institutional Review Board

Administrative Controls	Institutional policies that have been established and are enforced by UF to ensure the safety of laboratory workers, the public, the environment and the institution.
Amphotrophic	A pathogen that has a wide host range and can infect more than one species or cell line.
Animal and Plant Health Inspection Service (APHIS)	A component of the U.S. Department of Agriculture (USDA) that administers the Animal Welfare Act and oversees compliance with the USDA Select Agent Program and the importation of agents and materials that may pose a threat to U.S. agriculture. Within APHIS, the Animal Care Unit is responsible for ensuring compliance with the animal welfare regulations.
Antisepsis	The process of disinfecting living tissue or skin to reduce or remove transient microbial flora.
Biohazard	Any biological material (e.g., pathogens, biologically-derived toxins, etc.) or its components that present a real or potential hazard of illness or injury to humans, plants and/or animals.
Biohazardous agent	A living organism that has the capacity to produce deleterious effects because of its infectious nature. Biohazardous agents include, but are not limited to, various viruses, Chlamydia, bacteria, fungi, yeast, and algae, as well as plants and animals and their products that contain any of these agents.
Biological safety cabinet (BSC)	A ventilated cabinet used as an engineered control that serves as the primary containment for operations involving biohazardous agents or other biologically-derived materials, particularly when there is a potential for aerosol generation.
BioPath	The Biohazards Medical Monitoring Program for the University of Florida. BioPATH provides occupational health oversight for employees, students, visitors and volunteers in instances where it has been determined that such oversight is necessary due to the potential for exposure to biohazards.

Biosafety Level (BSL)	The level of containment required to perform biohazardous operations safely. Work practices and techniques, safety equipment, and laboratory facilities appropriate for the operations are based on the potential hazards imposed by the agents used and for the laboratory function and activities.
Bloodborne pathogen (BBP)	A pathogenic microorganism present in human blood, tissues or body fluids, which can cause disease in humans. Such pathogens include hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).
Centers for Disease Control and Prevention (CDC)	An agency of the Department of Health and Human Services. The CDC oversees compliance with the CDC Select Agents Program and the importation of agents and materials that may pose a threat to U.S. public health.
Contaminated	The presence, or reasonably anticipated presences, of blood or other potentially infectious materials on an item or surface.
Decontamination	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	The physical or chemical means of eliminating most, if not all, pathogenic microorganisms, excluding spores.
Ectotrophic	Describes an organism that gets its nutrients from the outside surface of its host.
Engineering Control	A device (e.g., biosafety cabinet, sharps disposal container, self-sheathing needle, centrifuge safety cups) that isolates or removes a biohazard from the workplace, monitors critical physical parameters or provides a specific surface.
Exposure	Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious or biological toxin materials that may result from the performance of a worker's duties.
Fomite	An inanimate object (e.g., doorknob) that may be contaminated with infectious organisms and serve in their transmission.
Infectious	Used to describe an agent, microorganism, or material that is capable of invasion and multiplication in body tissue resulting in cellular injury and/or disease.
Infective dose	The amount of a pathogen (measured in number of microorganisms) required to cause an infection in the host.
LD_{50}	LD stands for "lethal dose." LD_{50} is the amount of material, given all at once, which causes the death of 50% of a group of test animals. LD_{50} is the measure of acute toxicity of a material.
Oncogene	A gene that has the potential to cause cancer.
Oncogenic virus	A virus that causes cancer.
Other potentially infectious material (as defined in 29 CFR1910.1030)	1) Any of the following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood and all body fluids in situations where it is difficult or impossible to differentiate between body fluids. 2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead). 3) An HIV-containing cell or tissue culture, organ culture or HIV-, HCV- or HBV-containing culture medium or other solution; blood, organs or other tissues from

	experimental animals infected with HIV, HCV, HBV or other human pathogens.
Parenteral	The introduction of nutrition, a medication, or other substance into the body via a route other than the mouth or rectum, especially via infusion, injection or implantation.
Pathogen	Any agent (usually living) capable of producing disease.
Pathogenicity	Refers qualitatively to the ability of an organism to cause disease.
Personal protective equipment (PPE)	Specialized clothing or equipment worn by a worker for protection against a hazard. General work clothes (*i.e.,* uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered PPE.
Principal Investigator (PI)	The one individual who is designated by the university to direct a project or program and who is responsible to the university for the scientific and technical direction of that project or program.
Select agent (SA)	A microorganism or toxin listed in one of the following regulations: 1) 42 CFR 73 – Possession, Use, and Transfer of Select Agents (for humans); 2) 9 CFR 121 – Possession, Use, and Transfer of Select Agents (for animals); or 3) 7 CFR 331 – Possession, Use and Transfer of Select Agents (for plants) and not subject to the current rules of exemption.
Sterilization	The physical or chemical means of rendering items free of all microorganisms, including spores.
Synthetic Nucleic Acid	In accordance with the NIH guidelines: 1) 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 2) molecules that result from the replication of those describes in (1) or this definition.
Tier 1 Select Agents	A subset of select agents or toxins designated in the select agent regulations as “Tier 1” because these agents and toxins present the greatest risk of deliberate misuse with the most significant potential for mass casualties or deleterious effects on the economy, critical infrastructure, or public confidence.
Universal Precautions	An approach to infection control in which all human blood and certain human bodily fluids are treated as if known to be infectious for HIV, HCV, HBV and other bloodborne pathogens.
Vector	In epidemiology, any agent (person, animal or microorganism) that carries and transmits an infectious pathogen into another living organism.
Virulence	The quantitative measurement of an organism’s ability to cause disease.
Zoonotic	A disease of animals (e.g., rabies) that can be transmitted to humans.

Appendix B. Regulatory Authorities

	Policy Level	Policy	Description/Purpose
Occupational Health	University	BioPath: Biohazards Medical Monitoring Policy	Establishes the procedures, requirements, organizational responsibilities, guidance, safety and health precautions governing tasks involving occupational exposure to certain biological agents for all individuals at the University of Florida who may be potentially exposed to these biological agents including bacteria, viruses, toxins, prions, and cells, tissues, animals or vectors that could harbor these agents.
		HIV Research Laboratory Occupational Medicine Policy	Designed to protect employees who conduct research with HIV and covers all researchers who handle, manipulate or assay live HIV cultures.
		Q Fever Control Policy	Designed to protect University of Florida faculty, staff, students, volunteers and visitors from exposure to the Q fever agent (*Coxiella burnetii*).
		Vaccinia Immunization Policy	Clarifies the requirements for all personnel who work in an area where vaccinia, orthopoxviruses, or animals vaccinated with those agents are used, handled or housed.
		Respiratory Protection Policy	Prevent adverse health effects from the inhalation of hazardous airborne contaminants through the administration of a comprehensive Respiratory Protection Program.
		Personal Protective Equipment Policy	Establishes the minimum requirements for the selection and proper use of personal protective equipment.
	Federal	U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standards; 29 CFR 1910.1030	Prescribes safeguards to protect workers against health hazards related to bloodborne pathogens; it contains provisions dealing with exposure control plans, engineering and work practice controls, Hepatitis B vaccination, hazards communication and training, and recordkeeping. The standard imposes requirements on employers of workers who may be exposed to blood or other potentially infectious materials such as certain tissues and body fluids.
		U.S. Department of Labor, OSHA Occupational Exposure to Hazardous Chemicals in Laboratories; 29 CFR 1910.1450	Also referred to as the Laboratory Standards. Requires that the employer have a written chemical hygiene plan (CHP) that includes provisions for worker training, chemical exposure monitoring where appropriate, medical consultation when exposure occurs, criteria for the use of PPE and engineering controls, special precautions for particularly hazardous substances and a requirement for a Chemical Hygiene Officer (CHO) responsible for implementing the CHP.

		Environmental Protection Agency (EPA) Registered Sterilizers, Tuberculocides, and Antimicrobial Products Against Certain Human Public Health Bacteria and Viruses	Includes listings of EPA's registered antimicrobial products effective against certain bloodborne/body fluids pathogens (*i.e., Mycobacteria tuberculosis*, HIV-1, Hepatitis B and C viruses) as well as products classified as sterilizers. The use of EPA registered products effective against human bloodborne pathogens listed are in compliance with OSHA's Bloodborne Pathogens Standards.

Comprehensive Biosafety Guidelines	Federal	Center for Disease Control and Prevention (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition	The BMBL describes the combinations of standard and special microbiological practices, safety equipment, and facilities constituting Biosafety Levels (BLS) 1-4, which are recommended for work with a variety of infectious agents in various laboratory settings. The advisory recommendations are intended to provide a voluntary guide or code of practices as well as goals for upgrading operations.
Recombinant and synthetic	Federal	NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)	Specifies the practices for constructing and handling recombinant and synthetic nucleic acid molecules and provides guidance on physical and biological containment practices for work involving recombinant and synthetic nucleic acid molecule research *in vitro*, in plants, and in animals as well as guidance for protocols involving the transfer of recombinant or synthetic nucleic acid molecules into human research participants.
Plant, Plant Pests, Noxious Weeds, and GE Plants Regulations	State	State of Florida Division of Plant Industry	As a regulatory agency of the Florida Department of Agriculture and Consumer Services (FDACS), the Division of Plant Industry works to detect, intercept and control plan and honey bee pests that threaten Florida's native and commercially grown plants and animal resources.
		Florida Statute, Title XXXV – Agriculture, Horticulture and Animal Industry, Chapter 581 – Plant Industry	Regulates the introduction into or release within Florida of any plant pest, noxious weed, genetically engineered plant or plant pest, or any other organism which may directly or indirectly affect the plant life of the state of Florida as an injurious pest, parasite or predator (581.083)
		Chapter 586 – Florida Honey Certification and Honeybee Law	Regulates the production, sale and interstate shipment of honey as well as the introduction of honeybee pests or unwanted honeybees into the state of Florida.
		Chapter 5B-57, Florida Administrative Code – Introduction or Release of Plant Pests, Noxious Weeds, Arthropods, and Biological Control Agents	The purpose of this policy is to control the introduction into, or movement or spread within the state of Florida of any plant pest, noxious weed, or arthropod, and to establish procedures under which the field release of plant pests, noxious weeds, arthropods or biological control agents or non-native species plantings are permitted.

		Chapter 5B-64, Florida Administrative Code – Aquatic Plant Importation, Transportation, Non-nursery Cultivation, Possession and Collection	Regulates the eradication, control, or prevention of introduction and dissemination of noxious or prohibited aquatic plants through the importation, transportation, non-nursery cultivation, collection, sale, or possession of aquatic plants.
	Federal	United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS)	Regulates the importation, interstate movement, and field testing of genetically engineered plants and organisms that are or might be plant pests under the Plant Protection Act (PPA) and animal biologics (e.g., viruses, serums, toxins for animal vaccines) under the Virus, Serum and Toxins Act.
Plant, Plant Pests, Noxious Weeds, and GE Plants Regulations	Federal	USDA APHIS Plant Protection and Quarantine (PPQ)	Safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the U.S. and supports the trade and exports of US agricultural products. Administers permits required for the importation, transit, domestic movement and environmental release of organisms that impact plants, and the importation of plants and plant products under authority of the Plant Protection and Honeybee Acts.
		7 CFR 330 – Federal Plant Pest Regulations; General; Plant Pests; Soil, Stone and Quarry Products	Under authority of the Plant Protection Act, restricts and/or regulates the importation, entry, exportation, or movement in interstate commerce of any plant, plant product, biological control organism, noxious weed, article (including baggage, mail, garbage, earth, stone and quarry products) to prevent the introduction into or the dissemination within the U.S. or a plant pest or noxious weed.
		USDA Biotechnology Regulatory Service (BRS)	Implements the APHIS regulations for certain genetically engineered organisms that may pose a risk to plants health. Through APHIS, BRS regulates the introduction (importation, interstate movement, or environmental release) or certain genetically engineered organisms through its permitting and notification process under the authority of 7 CFR 340.4 – Permits for the Introduction of a Regulated Article.
		Food and Drug Administration (FDA) Statement of Policy: Foods Derived from New Plant Varieties ("The 1992 Policy.")	Clarifies the FDA's interpretation of the application of the Federal Food, Drug and Cosmetic Act with respect to human foods and animal feeds derived from new plant varieties including varieties that are developed using recombinant DNA technology (bioengineered foods). These regulations are intended to ensure that relevant scientific, safety and regulatory issues are resolved prior to the introduction of such products into the marketplace.
		40 CFR Part 174 – Procedures and Requirements for Plant-Incorporated Protectants	Sets forth regulatory requirements, criteria, and procedures applicable to plant-incorporated protectants (PIPs) under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA). When applied to PIPs, the definitions and regulations in this part supersede the regulations found in parts 150-180 of this chapter to the extent that the regulations conflict. Unless otherwise superseded by this part, the regulations in parts 150-180 of this chapter apply to PIPs.

Animal, Animal Products, GE Animals and Animal Pathogen Regulations	State	State of Florida Division of Animal Industry	The Division of Animal Industry is responsible for enforcing animal health regulations in Florida and protecting the state from animal pests and diseases, which could have major economic and public health consequences.
		Chapter 5C-3, Florida Administrative Code – Importation of Animals	The purpose of this rule is to specify, detail and clarify the importation requirements, by species, for animals and certain animal products, into Florida from other states.
		Chapter 5C-23, Florida Administrative Code – Transporting Animal Carcasses/Refuse	This rule provides regulations, licensing/permitting and record requirements, standards for equipment and penalties for non-compliance with equipment standards, regarding the transporting of animal carcasses and/or refuse within Florida.
		Chapter 5C-25, Florida Administrative Code – Humane Euthanasia of Livestock	This rule provides acceptable options for the deployment of approved euthanasia methods.
Animal, Animal Products, GE Animals and Animal Pathogen Regulations	State	Florida Statute Chapter 585, Part II – Disease Inspection, Control and Eradication	Authorizes the Division of Animal Industry to (i) restrict, regulate, or prohibit the movement or transportation of animals found, determined, or suspected by it to be carriers of any contagious, infectious, or communicable disease, or of the vectors of such disease; (ii) govern the introduction of animals into or within the state; (iii) govern the disposal or destruction of carcasses of animals which are condemned or die from or while afflicted with any contagious, infectious, or communicable disease.
	Federal	USDA APHIS Veterinary Services (VS)	Administers animal health permits to import controlled material or transport organisms (including animal pathogens) or vectors or animal products and by-products (VS 16-3 permit); import cell cultures and their products (VS 16-7 permit); import or transport live animals, semen and embryos (VS 17-129 permit).
		FDA Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs	Explains the process by which the FDA is regulating genetically engineered animals and provides a set of recommendations to producers of GE animals to help them meet their obligations and responsibilities under the law.
Select Agent Regulations	Federal	Federal Select Agent Program	Jointly comprised of the Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins and the USDA APHIS Agricultural Select Agent Program, the Federal Select Agent Program oversees the possession, use and transfer of biological select agents and toxins, which have the potential to pose a severe threat to public, animal and plant health or to animal and plant products.
		42 CFR 73 – Select Agents and Toxins	Implements the provisions of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 setting forth the requirements for possession, use and transfer of select agents and toxins that pose a severe threat to public health and safety, to animal health or to animal products. Overlap select agents and toxins are subject to regulation by both the CDC and APHIS.
		7 CFR 331 – Possession, Use and Transfer of Select Agents and Toxins	Implements the provisions of the Agricultural Bioterrorism Protection Act of 2002 setting forth the requirements for possession, use and transfer of select agents and

			toxins that pose a sever threat to plant health and plant products.
		9 CFR 121 – Possession, Use and Transfer of Select Agents and Toxins	Implements the provisions of the Agricultural Bioterrorism Protection Act of 2002 setting forth the requirements for possession, use and transfer of select agents and toxins that pose a severe threat to public health and safety, to animal health or to animal products.
		National Science Advisory Board for Biosecurity (NSABB)	A federal advisory committee chartered to provide advice, guidance, and leadership regarding biosecurity oversight of dual use research, defined as biological research with legitimate scientific purpose that may be misused to pose a biological threat to public health and or national security.
		United States Government (USG) Policy for Oversight of Life Sciences Dual Use Research of Concern (DURC)	The purpose of this policy is to establish regular review of USG-funded research with certain high-consequence pathogens and toxins for its potential to be DURC to preserve the benefits of life sciences research while minimizing the risk of misuse of knowledge, information, products or technologies provided by such research.

Select Agent Regulations	Federal	Department of Defense (DOD), Department of the Army (DA), AR-50-1	Applies to DOD-funded etiological agent research. This regulation establishes DA policies, assigns responsibilities, and prescribes procedures for the Army Biological Surety Program. It is Army policy that biological select agents and toxins (BSAT) in the possession or custody of the Army shall be properly safeguarded against the theft, loss, diversion, or unauthorized access or use, and that operations with such agents are conducted in a safe, secure and reliable manner.
Export Control Regulations	Federal	15 CFR 730-774 – Export Administration Regulations (EAR)	The EAR are issued by the United States Department of Commerce, Bureau of Industry and Security (BIS) under laws relating to the control of certain exports, reexports, and activities. BIS maintains the Commerce Control List and Category 1 of the CCL pertains to "Materials, Chemicals, Microorganisms and Toxins" which includes Human and Zoonotic Pathogens and Toxins (Category 1C351), Animal Pathogens (Category 1C352), Genetic Elements and Genetically Modified Organisms (Category 1C353) and Plant Pathogens (Category 1C354).
		U.S. Department of State International Traffic in Arms Regulations (ITAR)	The ITARs controls the import/export of defense-related articles and services that are listed on the United States Munitions List (USML). Category XIV of the USML includes "Toxicological Agents, Including Chemical Agents, Biological Agents, and Associated Equipment."

Biomedical Waste Regulations	University	Biological Waste Disposal Policy	Intended to provide guidance and insure compliance with NIH/CDC guidelines, the State of Florida Administrative Code 64E-16, and restrictions of the local County landfill.
	County	Alachua County Health Department Biomedical Waste Program	The Florida Department of Health in Alachua County manages the Biomedical Waste Program and provides permitting, inspection, regulatory and environmental assessment and surveillance services.
	State	Section 381.0098 of the Florida Statute – Biomedical Waste	Establishes the Florida Department of Health as the regulating authority regarding the packaging, transportation, storage and treatment of biomedical waste; establishes permits and fees for persons who generate, store or treat biomedical waste
		Chapter 64E-16, Florida Administrative Code – Biomedical Waste	Provides minimum sanitary practices relating to the management of biomedical waste, including segregation, handling, labeling, storage, transport and treatment. Applies to all facilities that generate, transport, store or treat biomedical waste to ensure that the waste is properly handled to protect public health.
Transportation Regulations	Federal	U.S Department of Transportation 49 CFR 171-178 – Hazardous Materials Regulations	Regulates the shipment of hazardous material including biohazardous material and select agents and toxins; provides guidance on hazards communication, emergency response information, packaging requirements, training requirements, and security plans related to the shipping of hazardous materials in commerce.
Transportation Regulations	Federal	U.S. Public Health Service, Department of Health and Human Services 42 CFR 71 – Foreign Quarantine	Subpart F (Importations) of 42 CFR 71 is intended to prevent the introduction, transmission, and spread of communicable human disease resulting from importations of various animal hosts or vectors or other etiological agents from foreign countries into the U.S.; provides import regulations and requirements for non-human primates (NHPs), infectious biological agents, infectious substances and vectors, and African rodents and other animals that may carry the monkeypox virus.
	International	United Nations (UN) Recommendations on the Transportation of Dangerous Goods	The UN subcommittee of Experts on the Transport of Dangerous Goods develops recommended procedures for the transport of all dangerous goods (excluding radioactive material), by all modes of transportation. These recommendations are used by the International Civil Aviation Organization (ICAO) to develop regulations for the safe transport of dangerous goods by air which are published as ICAO Technical Instructions.

		ICAO Technical Instructions for the Safe Transport of Dangerous Goods by Air	The Technical Instructions contain a comprehensive set of requirements; among other things, they provide for the classification of dangerous goods and list these goods. The instructions require that all dangerous goods be packaged and, in general, restrict the quantity per package according to the degree of hazard and type of aircraft (*i.e.*, passenger or cargo) to be used; they also give the packing methods to be used and the packagings permitted, together with the specifications for those packagings and the stringent testing regime that must be followed in addition to requirements for markings and labels for packages and documentation for consignments.
		International Air Transport Association (IATA) Dangerous Goods Regulations	The IATA has developed regulations that contain all of the requirements of the ICAO Technical Instructions, but include additional restrictions.
Additional Regulations	University	Minors in Laboratories, Clinics and Animal Facilities Policy	Identify when minors will be allowed to work or conduct research in a University of Florida laboratory, greenhouse, clinic area or animal facility.
		University of Florida Procedure for Disposal of Clean Lab Ware	Defines and establishes how to package and dispose of clean labware.
		Laboratory Closeout Policy	Addresses laboratory closures and associated disposition of hazardous materials.
	National	National Sanitation Foundation (NSF)/ANSI Standard 49 for Biosafety Cabinetry	Includes basic requirements for the design, construction and performance of Class II (laminar flow) biosafety cabinets to provide personnel, product and environmental protection, reliable operation, durability, cleanability, noise level and illumination control, vibration control and electrical safety. The standard includes detailed test procedures including recommendations for installation, field certification and decontamination procedures.