Palm Beach State College

Florida's First Public Community College

BIOLOGICAL SAFETY MANUAL

2010

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INTRODUCTION

Palm Beach State College is committed to providing a safe and healthy learning, teaching and working environment. The goals of Palm Beach State College's Biological Safety Program are to protect the staff and students from exposure to infectious agents, to prevent environmental contamination, to enhance the academic atmosphere, and to comply with federal, state and local regulations.

The Biological Safety Manual provides College Safety guidelines for those working with biohazards. This manual outlines general policies and procedures for using and disposing of infectious or potentially infectious materials according to applicable regulatory guidelines and Palm Beach State's policy and procedures. Laboratories must comply with the biological Safety practices and procedures outlined in this manual. Laboratory Specialists must contact the Safety Manager if they are uncertain how to categorize, handle, store, treat or discard any biologically derived material. Laboratory Specialists should use the information in this manual to develop site specific safety procedures for their program or laboratory.

ADMINISTRATION AND RESPONSIBILITIES

The Biological Safety Program is designed to assist Laboratory Personnel in the selection of safe laboratory controls and practices that will ensure a safe working and learning environment for Students, Faculty and Staff of Palm Beach State College. The Safety Manager in conjunction with the Program Chairpersons and Lab Specialists develops and conducts appropriate training programs to promote techniques for the safe handling and disposal of potentially infectious and biohazardous materials. The Safety Manager conducts laboratory Safety audits to ensure compliance with policy and procedures and will investigate all reported accidents which may result in personnel or environmental exposure to biohazardous materials.

Deans/Department Chairs

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their schools or departments. They should be aware of and approve all academic work conducted under their auspices. Deans and Department Chairs must ensure departmental compliance with applicable laws, regulations and guidelines covering the use of biological agents in the programs they oversee.

Laboratory Specialists/ Instructors

Laboratory Specialists and instructors are ultimately responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratory areas to ensure a safe work environment for staff and students. This responsibility may not be shifted to inexperienced or untrained personnel. Instructors must develop specific standard operating procedures for their projects and outline proper emergency procedures in case of accidental exposure of personnel or the environment to biological hazards. Laboratory Specialists must provide training to all personnel working in the lab so they have a complete understanding of the lab procedures and techniques and document that they have had the appropriate Safety training. All protocols involving work with potentially infectious agents must be submitted to the program Deans for review and approval.

Employees/Students

Employees/Students are responsible to comply with Palm Beach State College guidelines and procedures required for the tasks performed and to report unsafe conditions to the Instructor, Laboratory Specialist, or Program Chairperson. They should seek guidance when they are uncertain how to handle, store or dispose of any hazardous or biohazardous material. They should not begin working in the laboratory until all Technical and Safety training has been completed.

CLASSIFICATION OF BIOHAZARDS

Bacteria, viruses, fungi or other infectious agents are used and studied in the laboratories. Since several of these agents are pathogenic to humans, animals, or other forms of life, their use poses risks, which vary with each agent and the way it is used. The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled *Biosafety in Microbiological and Biological Laboratories (BMBL),* recommends that work be done using one of four levels of containment (see Biosafety Levels). The *NIH Guidelines for Working with rDNA* also classifies pathogenic agents into one of four risk groups according to specific criteria (see Risk Groups). It is Palm Beach State College policy that all laboratories adhere to these CDC and NIH guidelines.

Microorganisms

Common microorganisms that cause disease have been classified by the CDC and NIH according to their risk associated with severity of disease. A list of those organisms used at Palm Beach State College can be found in Appendix A. The New or unknown pathogens that have not been assessed by CDC/NIH must go through a risk assessment to determine their Biosafety containment level. Laboratory Specialists and Instructors must seek approval of any project involving a pathogenic agent with the Program Dean and receive their approval before work is initiated. Following receipt of the completed written approval.

Genetically Engineered Organisms.

The in-vitro incorporation of segments of genetic material from one cell into another is known as recombinant DNA (rDNA) technology has resulted in altered organisms which can manufacture products such as vaccines, hormones, interferons and enzymes. Genetically engineered organisms are used for treatment of diseases, to clean up hazardous waste and spills, and plants can be made resistant to cold, disease, pests and drought. However, rDNA technology carries with it the potential for harm. A genetically altered organism may be directly pathogenic or toxic and if released into the environment and the risks associated with recombinant DNA technology need to be assessed by the Deans and Instructors prior to inclusion into the curriculum.

Human Blood, Blood Products, Body Fluids and Tissues

Programs or projects that utilize human blood, blood products or potentially infectious body fluids are governed by the OSHA Occupational Exposure to Bloodborne Pathogens Standard. This federal regulation mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help reduce occupational exposure to human blood and other potentially infectious materials which may cause Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. Programs that use human blood, blood products, body fluids or tissues must comply with the Palm Beach State College Bloodborne Pathogen Exposure Control Plan. The completed plan must be readily available in the laboratory for all workers.

Prions

Some progressive neurological diseases are caused by unconventional proteinaceous infectious particles called prions. Prions have been associated with transmissible degenerative diseases of the central nervous system in humans (Creutzfeldt-Jacob, kuru) and animals (mad cow disease, transmissible encephalopathy of mink and scraLaboratory Specialist in sheep and goats). These unconventional agents are resistant to destruction by chemical (10% formalin, glutaraldeyhye, 70% ethanol, iodine) and physical (UV light, ionizing radiation, boiling) procedures. While there have been no documented cases of laboratory-acquired infections, extreme precautions should be taken when handling material from infected or potentially infected humans and animals. The use of this agent needs to be approved and use should be established using a risk assessment that accounts for the nature and host range of the agent, as well as the nature of the procedures and concentration and quantity of the agent.

Tissue Cultures
Cell cultures derived from humans or animals known to be infected with a pathogen, as well as cultures known or suspected to contain infectious microorganisms (e.g., herpes virus or EBV-transformed cultures) should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices. Repositories such as the American Type Culture Collection (ATCC) can provide information on some of the cell lines used in the lab. In addition, mammalian cell cultures may carry unsuspected oncogenic, allergenic or infectious particles. It is impractical, if not impossible, to screen such cultures for all potentially harmful microorganisms; even well-characterized lines with a history of safe use can become contaminated by adventitious, possibly infectious, microorganisms. For this reason, it is prudent to treat all mammalian cultures as potentially infectious and to use Biosafety Level 2 facilities and work practices whenever working with them.

Viral Vectors
These vectors provide a broad spectrum of uses in both basic and clinical sciences in allowing both the transient and long term expression of almost any gene of interest in specific tissues either in culture or in vivo. Recent research using viral vectors has proven that viral reagents have broad potential applications for the study of disease and normal cellular processes. Special care should be given to the design, risk assessment (Section 2 of NIH Guidelines), and handling of virus vectors containing genes that make growth-regulating products, products released into the circulation, products that may have a general effect on the host-immune system.

LABORATORY INFECTIONS

A laboratory-acquired infection is defined as one that resulted from laboratory work, whether it occurred in a laboratory worker or in another person who happened to be exposed as a result of research or clinical work with infectious agents. If you are immune compromised you are at a much higher risk of acquiring infections and you should meet with an occupational health physician or your personal physician for a medical consultation to determine your risk of infection. Also if you are pregnant, you should discuss the kind of work and materials that you are exposed to with your physician to determine the risk to you and/or your fetus.

Modes of Transmission
Microorganisms can enter the body through the mouth, the respiratory tract, broken or intact skin and the eyes. It should be noted that in laboratory-acquired infections, the route may not be the same as when the disease is acquired naturally. Infectious materials and cultures of microorganisms accumulate in large amounts in clinical and microbiological laboratories and since it is necessary to transfer them from one container to another and to manipulate them in various ways, the potential hazards are increased when working with materials. **That is why it is so important to have documented and deliberate standard operating procedures that are enforced by Deans, Program Chairs, Instructors and Laboratory Specialists, to make certain that nobody is exposed to biohazards.**

Infections preceded by overt personal accidents, include:

1. Inoculation (resulting from pricking, jabbing or cutting the skin with contaminated instruments such as hypodermic needles, scalpels and glassware; and from animal bites or scratches).
2. Ingestion (resulting from mouth-Laboratory pipettes, eating, drinking and smoking, which are why these practices are not permitted in the lab).
3. Splashing into the face and eyes.
4. Laboratory spillage and direct contact.

Infections not preceded by personal accidents:

1. Aerosols, droplets and fomites. These are speculated to be responsible for up to 80 percent of all laboratory-acquired infections. Aerosols are defined as a cloud of very small liquid droplets produced whenever energy is applied to a liquid, and such liquid is allowed to escape into the environment. It has been shown that if the liquid contains infectious agents, these would be distributed in the aerosol and would remain viable for some time. The larger droplets (greater than 0.1 mm in diameter) will settle quickly and contaminate the surfaces upon which they come to rest. The smaller droplets can remain in the air for sometime before being evaporated or settling on surfaces. The infectious agents in the droplets remain in a dried state as "droplet nuclei" or fomites. The smaller the number of organisms and amount of dried material, the longer they will remain airborne, and they are moved around buildings by air currents generated by ventilation and people traffic. It has been shown that many laboratory techniques using both simple and complex mechanical equipment, as well as laboratory accidents, produce aerosols. These include: use of microbiology loops, laboratory pipettes, syringes and needles, opening tubes and bottles, use of centrifuges and blenders, harvesting of eggs and other virological procedures, and breakage of culture tubes and vials.

Using Infectious Materials

1. Infectious materials must be clearly identified and stored in such a manner as to preclude accidental exposure. This normally includes double containment and labeling all samples stored in the lab and freezer/refrigerator where these samples are kept. A number of infectious agents have been documented as causes of laboratory-acquired infections.

RISK ASSESSMENT

It is the responsibility of the Program Deans and Chairpersons to ensure that risk assessments are conducted to determine the proper work practices and containment requirements for work with biohazardous material prior to materials being added to laboratories and curriculums. The risk assessment process should identify features of microorganisms as well as host and environmental factors that influence the potential for biohazard exposure. The Program Deans and Chairpersons should ensure that the laboratory is in compliance with established guidelines and regulations. The following resources are used to assist in the risk assessment, NIH Recombinant DNA Guidelines, WHO Biosafety Manual and the Biosafety in Microbiological & Biological Laboratories, 4th ed. (CDC/NIH). When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Factors to consider when evaluating risk include the following:

- **Pathogenicity**: The more severe the potentially acquired disease, the higher the risk. Salmonella, a Risk Group 2 agent, can cause diarrhea, septicemia if ingested. Treatment is available. Viruses such as Ebola, Marburg, and Lassa fever cause diseases with high mortality rates. There are no vaccines or treatment available. These agents belong to Risk Group 4.
- **Route of transmission**: Agents that can be transmitted by the aerosol route have been known to cause the most laboratory-acquired infections. The greater the aerosol potential, the higher the risk of infection. Work with Mycobacterium tuberculosis is performed at Biosafety Level 3 because disease is acquired via the aerosol route.
- **Agent stability:** The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.
- **Infectious dose**: Consider the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units. An individual's immune status can also influence the infectious dose.

- **Concentration:** Consider whether the organisms are in solid tissue, viscous blood, sputum, etc., the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.
- **Origin:** This may refer to the geographic location (domestic or foreign), host (infected or uninfected human or animal), or nature of the source (potential zoonotic or associated with a disease outbreak).
- **Availability of an effective prophylaxis or therapeutic intervention:** Effective vaccines, if available, should be offered to laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.
- **Medical surveillance:** Medical surveillance programs may include monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, and annual physicals.
- **Experience and skill level of at-risk personnel:** Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to working with biohazardous materials. Laboratory workers must go through additional Safety Training before they are allowed to work with high risk materials or in a designated facility.

Risk Groups

Infectious agents are classified into risk groups based on their relative hazard. The table below presents the basis for the classification of biohazardous agents by risk group.

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	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)

The risk groups designation along with the risk assessment are the steps used to determine the appropriate procedures and containment to use while working with a particular agent in the lab. Laboratories and animal facilities are classified according to their design features, construction and containment facilities to safely work with biological agents and infectious materials. These designations, called **Biosafety levels (BSL) and animal Biosafety levels** (ABSL) provide appropriate containment for the various risk group agents.

CONTAINMENT BARRIERS

The term "containment" is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. **The purpose of containment is to reduce or eliminate exposure of laboratory workers and the outside environment to potentially hazardous agents.** The three elements of containment include facility design, safety equipment and laboratory practice and techniques.

Primary containment is the protection of personnel and the immediate laboratory environment from exposure to infectious agents. It is accomplished by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials. It is accomplished by providing by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of work practices, safety equipment and facility design to provide adequate containment.

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. Program Deans and Chairpersons must designate a person who will be responsible for providing or arranging for appropriate training of personnel. Each laboratory should develop an operational manual which identifies specific hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, Safety Equipment and management practices.

Safety Equipment (Primary Barriers). Safety Equipment includes biological Safety cabinets, enclosed containers (centrifuge cups) and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The Biological Safety Cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. The BSC provides personnel, product and environment protection. Safety Equipment also may include items for personal protection such as personal protective clothing, respirators, face shields, Safety glasses or goggles. Personal protective equipment is often used in combination with other Safety Equipment when working with biohazardous materials.

Facility Design (Secondary Barriers). The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. Facilities must be designed to meet the requirements of the laboratory's function and the recommended Biosafety Level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary

barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

BIOSAFETY LEVELS

The CDC describes four Biosafety levels (BSLs) which consist of combinations of laboratory practices and techniques, Safety Equipment, and laboratory facilities. The recommended Biosafety Level for an organism represents the conditions under which the agent can be ordinarily handled safely. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Biosafety Level 1 is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended except for a hand washing sink. Agents can be used safely on the open bench. The standard microbiological practices are as followed:

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.

2. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

4. Mouth Laboratory pipetting is prohibited; mechanical Laboratory pipetting devices are used.

5. All procedures are performed carefully to minimize the creation of splashes or aerosols.

6. Work surfaces are decontaminated at least once a day and after any spill of viable material.

7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and federal regulations before removal from the facility.

8. An insect and rodent control program is in effect.

Biosafety Level 2 (Currently, Palm Beach State College operates at a Level 2) is applicable to work done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as Biosafety cabinets. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as hand washing and waste decontamination facilities must be available. In addition to BSL 1 procedures, level 2 also requires the following special practices:

1. Access to the laboratory is limited or restricted by the Laboratory Specialist when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The Laboratory Specialist has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The LABORATORY SPECIALIST establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the Biosafety Level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

5. Biosafety procedures are incorporated into standard operating procedures or in a Biosafety manual adopted or prepared specifically for the laboratory. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

6. The Laboratory Specialist ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

7. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, Laboratory Pipettes, tubes, and scalpels.

    a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently

located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes that re-sheathe the needle, needleless systems, and other Safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

8. Cultures, tissues, specimens of body fluids, or other potentially infectious materials are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.

9. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

10. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Laboratory Specialist and the Safety Manager Office. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

11. Animals not involved in the work being performed are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

12. Properly maintained biological Safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

13. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intravenously, and harvesting infected tissues from animals or embryonic eggs.

14. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge Safety buckets are used, and if these rotors or Safety buckets are opened only in a biological Safety cabinet.

15. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

16. Protective laboratory coats, gowns, aprons, or uniforms designated for laboratory use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-

laboratory areas (e.g., cafeteria, library, and administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; personnel should never take it home.

17. Gloves must be worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the laboratory. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

Laboratory Facilities (Secondary Barriers)

18. Provide lockable doors for facilities that house select agents (as defined in 42 CFR 72.6).

19. Consider locating new laboratories away from public areas.

20. Each laboratory contains a sink for hand washing.

21. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

22. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.

23. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

24. Install biological Safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological Safety cabinets to operate outside their parameters for containment. Locate biological Safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological Safety cabinets' air flow parameters for containment.

25. An eyewash station is readily available.

26. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

27. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without re-circulation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel working with these agents (i.e., Mycobacterium tuberculosis, St. Louis encephalitis virus and Coxiella burnetii) include auto-inoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment. Secondary barriers include controlled access to the laboratory, specialized ventilation system that minimizes the release of infectious aerosols, and a double door autoclave for decontaminated waste from the facility. **PALM BEACH STATE COLLEGE DOES NOT CURRENTLY APPROVE THE USAGE OF ANY LEVEL 3 AGENTS OR CONDUCT ANY OPERATIONS OR EXPERIMENTS AT LEVEL 3.**

Biosafety Level 4 is applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Primary hazards to workers include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and auto-inoculation. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III biological Safety cabinet or a full-body, air-supplied positive pressure personnel suit. The facility is generally a separate building or a completely isolated zone within a complex with specialized ventilation and waste management systems to prevent release of viable agents to the environment. **At this time, Palm Beach State College does not have this kind of facility and there are only a few BSL 4 containment facilities in the US such as CDC and Plum Island. PALM BEACH STATE COLLEGE DOES NOT CURRENTLY APPROVE THE USAGE OF ANY LEVEL 4 AGENTS OR CONDUCT ANY OPERATIONS OR EXPERIMENTS AT LEVEL 4.**

Animal Biosafety Levels

There are four animal Biosafety Levels (ABSLs), designated Animal Biosafety Level 1 through 4, for work with infectious agents in animals. The levels are combinations of practices, Safety Equipment and facilities for experiments on animals infected with agents that produce or may produce human infection. These levels are comparable to the BSL levels with additional measures taken for handing animals and zoonotic disease transmission. Further information can be found in the CDC book or contact the BSO.

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

Animal Biosafety Level 2 is suitable for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

Animal Biosafety Level 3 is suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease.

Animal Biosafety Level 4 is suitable for addressing dangerous and exotic agents that pose high risk of like threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) will be used to reduce employee exposure to hazards when engineering and administrative controls are not feasible or effective. Program Deans, Chairpersons, Instructors and Laboratory Specialists are required to have knowledge of all exposures to hazards in their workplace and need to determine if PPE should be used to protect their employees and students. The PPE must be appropriate for the task and users must be trained to understand the use and limitations of the protective gear. The PPE used in laboratories includes laboratory coats and gowns, eye protection, face shields and the appropriate gloves.

Coat/Gowns

The lab coat is used to protect street clothing against biological or chemical spills, as well as to provide some additional body protection. The specific hazard and the degree of protection required must be known before selecting coats for lab personnel. The CDC/NIH guidelines for bio-containment practices recommend the use of a lab coat, wrap-around gown, smock, or scrub suits while working in research labs. The lab coat can be disposable or should be washed regularly either on site or through a commercial laundry service. Contaminated lab coats should not be taken home or worn outside of the laboratory.

Eye/Face Protection Equipment

Goggles and face shields should be worn whenever procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of tissues, and manipulations of infectious materials. The Laboratory Specialist has the responsibility to assess the potential for eye/face injuries, to train employees on the uses and limitations of PPE, to provide the type of protection required, and to ensure that the appropriate eye/face PPE is available and used by laboratory personnel. All eye/face protection devices must meet the requirements set forth in the ANSI Z87 standard.

Foot Protection

Safety shoes should be worn in any area where there is a significant risk of dropping heavy objects on the foot. For most biological lab use, comfortable shoes such as tennis shoes or nurses shoes should be worn. Although we live in Florida, **sandals and other types of open-toed shoes are not permitted in labs using biohazards or chemicals, due to the potential exposure to infectious agents or toxic materials as well as physical injuries associated with the work.**

Gloves

Skin contact is a potential source of exposure to infectious materials; it is important that the proper steps be taken to prevent such contact. Gloves should be replaced periodically, depending on frequency of use and permeability to the substance handled. Gloves should be taken off carefully to avoid contaminating hands and never reuse disposal gloves. Latex gloves are commonly used in the labs but may cause allergic reactions in certain sensitized individuals. Alternative gloves such as latex free or nitrile gloves should be available for use in the lab. Glove use should never replace the need for hand washing. Gloves should also be worn whenever it is necessary to handle rough or sharp-edged objects, and very hot or very cold materials. The type of glove materials to be used in these situations includes, leather, aluminum-backed gloves, and other types of insulated glove materials.

Respirators

Respirators can only be used when it is not possible to minimize or eliminate exposure to a contaminant through other means. Respirators are used in biological laboratories when there is a potential for exposure through inhalation or in some cases to animal allergies. The selection and use of respirators must be done in accordance with 29 CFR§1910.134 and Palm Beach State's Respiratory Protection Policy. All individuals

issued respirator must go through training, medical screening and fit testing to be approved to wear a respirator.

Cleaning and Maintenance

It is important that all PPE be kept clean and properly maintained. Cleaning is particularly important for eye and face protection where dirty or fogged lenses could impair vision. PPE should be inspected, cleaned, and maintained at regular intervals so that the PPE provides the required protection. Personal protective equipment shall not be shared between employees until it has been properly cleaned and sanitized. PPE will be distributed for individual use whenever possible.

SAFETY TRAINING

The key to the prevention of laboratory acquired infections and accidents, is to provide Safety Training to keep all staff well informed about the recognition and control of laboratory hazards. An effective Safety program begins with the Deans, Chairpersons and Laboratory Specialists, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Laboratory Specialists play the key role in training staff in good laboratory techniques. Certain Safety training is required by government regulations. For instance, OSHA requires annual training for those working on blood or other potentially infectious materials and the Florida Administrative Code requires annual training for those generating biological waste. All training programs need to be sited specifically and records need to be maintained within the department for review at any time.

Annual Training

As per Florida Statutes, All instructors, lab workers, and any other staff exposed to Biological Hazards associated with working within the lab, conducting experiments, or exposure through lab oversight, will receive annual Safety Training. Lab Specialists or other knowledgeable persons dedicated by the Dean will deliver the Safety Training for instructors and lab workers. This plan will be used as the basis of the content of the Training. Every person trained needs to receive a copy of this plan and sign off that they have received proper training.

New Employee Training

As per Florida Statutes, All NEW instructors, lab workers, and any other staff exposed to Biological Hazards associated with working within the lab, conducting experiments, or exposure through lab oversight, will receive initial Safety Training. Lab Specialists or other knowledgeable persons dedicated by the Dean will deliver the Safety Training for NEW instructors and lab workers. This plan will be used as the basis of the content of the Training. Every NEW person trained needs to receive a copy of this plan and sign off that they have received proper training.

Training Materials

All instructors, lab workers, and any other staff exposed to Biological Hazards associated with working within the lab, conducting experiments, or exposure through lab oversight, will receive annual Safety Training which, at a minimum, covers the following topics: Classification of Biohazards, Laboratory Infections, Risk Assessment and approval for new experiments or procurement of new microorganisms, Containment Barriers, Biosafety Levels, Personal Protective Equipment, Decontamination Procedures, Spill and Emergency Procedures, Overview of Lab Equipment, Biological Waste Disposal. Other topics can be added at the discretion of the Dean and Chairpersons as needed.

Training Records

As per Florida Statutes, all Training records will be available for review upon request. Training records will be maintained by the Lab Specialists and stored within the Department for annual audit review.

DECONTAMINATION PROCEDURES

Sterilization, disinfection, and antisepsis are all forms of decontamination. Sterilization implies the killing of all living organisms. Disinfection refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. Antisepsis is the application of a liquid antimicrobial chemical to living tissue.

Chemical Disinfectants

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

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

Disinfectants Commonly Used

1. Iodophors

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

2. Hypochlorites (bleach)

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

3. Alcohols (ethanol, isopropanol)

    • The effective dilution is 70-85%.
    • Effective against a broad spectrum of bacteria and many viruses.
    • Fast acting.
    • Leaves no residue.
    • Non-corrosive.

• Not effective against bacterial spores.

Dilution of Disinfectants

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

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

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

LABORATORY SPILL PROCEDURES

A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment includes: disinfectant (such as 10% chlorine bleach), a package of paper towels, gloves and goggles, biohazard bags, and forceps to pick up broken glass and possibly solidifier if necessary. Kits can be made up or bought commercially. Please contact the Lab Specialist you have questions about the use or location of a spill kit.

Lab Cleanup Procedures

With due care, small spills can be easily cleaned up and contained with the supplies in the spill kit. Laboratory personnel should follow the procedures below for large spills and the explicit instructions outlined by the Laboratory Specialists.

1. Depending on the nature of the organisms or size of spill, hold your breath, leave the room immediately, and close door. Turn on ultra-violet light if these are present.

2. Warn others not to enter the contaminated area. If possible place warning signs.

3. Immediately remove contaminated clothing, place it in the biohazard bags provide for transporting solid wastes to the autoclave, and seal the bag.

4. Wash exposed areas of the body with soap and warm water or take a shower if necessary. Autoclave contaminated clothing immediately upon leaving and securing the contaminated area. Avoid contact with other individuals as much as possible to prevent additional exposure.

5. Inform the Laboratory Specialists responsible for the area and the Safety Manager soon as possible.

6. Wait at least thirty minutes to one hour for aerosols to settle and for the UV to act before re-entry into the contaminated area.

7. Put on a long sleeved gown, mask, eye protection preferably goggles, rubber gloves, and shoe cover before re-entering the contaminated room. For a high risk agent, a jumpsuit with tight-fitting wrists and a self-containing breathing apparatus (SCBA) respirator might be necessary.

8. Pour an appropriate decontaminate solution around the spill. Paper towels soaked with disinfectant may be used to cover the area. Avoid pouring the disinfectant directly onto the spill to minimize the generation of aerosols.

9. Let stand approximately thirty minutes to allow an adequate contact time.

10. Discard all materials from the spill in a biohazard bag and decontaminate any equipment that will be reused (i.e. dust pan).

11. All accidents, exposures, and potential hazards should be reported to the Safety Manager. In severe emergencies, telephone communication should be used to secure immediate medical care, decontaminating procedures or facility repairs.

Spills in a Biological Safety Cabinet

A spill that is confined to the interior of the Biological Manager Cabinet (not a Laminar Clean Bench) should present a little or no hazard to personnel in the area.

1. Chemical disinfectant procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminates from the cabinets.

2. The operator should wear Safety goggles, gloves and other appropriate personnel protective equipment during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant.

3. Immediately after a spill, the cabinet should be allowed to run for at least ten minutes to allow the cabinet to purge any airborne contaminants. Chemical decontamination procedures should be conducted while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

4. Spray or wipe walls, work surfaces and equipment with appropriate disinfectant. Germicide should at least have a minimum contact time of ten minutes.

5. Flood the top work surface, tray, and if a BSL 1 cabinet, wipe the drain pans and catches basins below the work surface with a sponge or cloth soaked in a disinfectant. For BSL 2 cabinets, drain the tray into the cabinet base, take out the tray and remove exhaust grill work and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a disinfectant. Then replace in position and drain disinfectant from cabinet base into appropriate container and autoclave waste liquid. Gloves, cloth or sponge should be discarded in a biohazard container and autoclaved.

6. For BSL 3 or greater materials spill, decontaminate cabinet with formaldehyde and other appropriate means.

7. After contaminated gloves and clothing have been removed, wash arms, hands and face with soap and water.

LABORATORY EQUIPMENT

Autoclaves
Steam sterilization is a proven and economical process of killing microorganisms through the application of moist heat, time and pressure. Heat damages the cell's essential structures including the cytoplasmic membrane rendering the cell no longer viable. Autoclaves are classified as pressure vessels and because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Please read the autoclave procedure section to prevent injuries or accidents when using the autoclaves on campus and to ensure that they are used properly.

Biological Safety Cabinet
The common element to all classes of Biological Safety Cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particulates of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases, but will remove particles smaller or bigger than .3 microns. The Biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually.

1. Class I cabinets protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or to the outside via the building exhaust.

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

3. Class III cabinets (sometimes called Class III glove boxes) were designed for work with infectious agents that require Biosafety Level 4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

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

Operation of Class II Biological Safety Cabinet

1. Turn on cabinet fan 15 minutes before beginning work.

2. Disinfect the cabinet work surface with 70% ethanol or other disinfectant.

3. Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)

4. Work as far to the back (beyond the air grill) of the BSC workspace as possible. Do not work in a BSC while a warning light or alarm is signaling.

5. Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a light and place it to the rear of the workspace. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs.

6. Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling.

7. Wear gloves when there is potential for skin contact with infectious material.

8. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.

9. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.

10. Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If a UV light is used, due to its limited penetrating ability, surfaces should be dust-free and the UV light tube should be wiped frequently with alcohol to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.

11. The UV lamp should never be on while an operator is working in the cabinet.

12. Minimize traffic around the Biosafety cabinet and avoid drafts from doors and air conditioning.

Centrifuge Containment

- Examine centrifuge tubes and bottles for cracks or stress marks before using them.

- Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.

- Centrifuge Safety buckets and sealed rotors protect against release of aerosols.

- If high concentrations or large volume of infectious agents are used, open centrifuge only in a BSC.

Vacuum Systems

All vacuum lines used on supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BL2 and above, a vacuum line filter should be used.

Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the Biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BL2 and BL3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

Vacuum Line Filter
A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment.

AUTOCLAVE PROCEDURES

Autoclaving Procedures
Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. A combination of pressure, heat and time is needed to efficiently decontaminate waste loads so perimeters must be made for each type of waste. Using an autoclave improperly can cause serious injury or contamination and can ruin the mechanical components. Before using the autoclave ensure that it is operating properly and determine the appropriate exposure time for the load. Appendix C has a SOP that can be placed near the autoclave.

Autoclave Log
Everyone using the autoclave needs to complete the Autoclave Use Log and when biological indicators are used the Biological Indicator Log needs to be completed. It is very important to complete these logs so there is a record of people using the autoclave in case it malfunctions or items are left in the unit.

Container Selection

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

Preparation and Loading of Materials

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

Cycle Selection

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

Time Selection

- Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- Bags of biological waste should be autoclaved for 30-50 minutes to assure decontamination.

Removing the Load

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

Monitoring

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. Operators should ensure that each autoclave is routinely monitored as follows:

1. *Temperature Monitoring* - check indicating thermometers during each complete cycle to ensure the attainment of a minimum temperature of two hundred fifty degrees Fahrenheit (250ºF) or (121ºC) for at least one-half (1/2) hour or longer, depending on quantity and compaction of the load and in order to achieve sterilization of the entire load. Remember that greater time and/or temperatures may be necessary to effectively sterilize a load.

2. *Heat Sensitive Tape Monitoring* - use heat-sensitive tape or other device for each load that is processed to indicate the load has undergone the steam sterilization process. Remember this tape only indicates that the proper temperature has been reached, but does not indicate it was heated for the proper time.

3. *Biological Indicator Monitoring* - use a biological indicator such as *Bacillus stearothermophilus* placed at the center of a load processed under standard operating conditions to confirm the attainment of adequate sterilization conditions. If the autoclaves are run weekly, then the indicators should be performed monthly. If the frequency is less, than indicators are run quarterly or with each load as appropriate.

Annual Safety Inspection of Autoclaves
All autoclaves mechanical and monitoring systems should be serviced and checked annually by a service provider to ensure that the unit is running correctly. Check with the autoclave manufacture for service providers in the area.

EMERGENCY PROCEDURES

All accidents, exposures and needlesticks must be reported to the Department Dean or Chairperson, Human Resources and the Safety Manager for correct follow-up and risk assessment. Depending on the injury and the materials used in the lab, different approaches can be taken on the type of first aid that can be utilized. When working with human material or know pathogens, seek medical attention immediately. Do not use strong disinfectants or scrub brushes that can abrade the skin as this can cause addition damage and increase the chances of pathogens penetrating the body and cause illness.

Severe Injuries

1. Call 911 for assistance and transportation to the nearest emergency room.

2. Lab assistants should accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.

3. Report accident to the Department Dean or Chairperson, Human Resources and the Safety Manager.

Sharp Injury

1. Allow the wound to bleed under steady stream of water and wash area with soap and water.

2. Seek medical attention immediately by going to the Worker Compensation clinic or nearest local emergency room.

3. Report the accident to the Department Dean or Chairperson, Human Resources and the Safety Manager, and seek additional medical assistance if necessary.

Splashes to the Eye/Body

1. For eyes, immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. For body, immediately remove contaminated clothing and drench skin in shower for 15 minutes.

2. Seek medical attention by going to the Student Health Services, Worker Compensation clinic or the local emergency room.

3. Report the accident to the Department Dean or Chairperson, Human Resources and the Safety Manager, and seek additional medical assistance if necessary.

Fires Involving Biological Materials

1. Without placing yourself in danger, put biological materials in secure location, such as incubator or freezer.

2. Activate the building fire alarm and leave the building at once

3. Call the fire department from a safe location, and call Palm Beach State College Security.

4. Meet the fire department outside and direct them to the fire location.

MEDICAL SURVEILLANCE

Medical surveillance may be necessary for laboratory personnel that use agents known to cause disease in humans or are working with animals that are susceptible to human diseases. The Occupational Health program will provide health assessments, medical test and immunizations for certain at-risk employees. Baseline serum samples may be appropriate for those working with BSL 2 or 3 agents if the agents can be monitored for serological changes to determine if an exposure has occurred in the laboratory.

TRANSPORTING BIOLOGICAL MATERIALS

Infectious agents and other dangerous goods must be transported according to the applicable regulations. The shipment of biological specimens, infectious agents and other biological materials are regulated by the International Air Transport Association (IATA), US Department of Transportation (DOT), US Postal Services (UPS) and other agencies. Carrying dangerous goods on person, in luggage or private automobile is strictly prohibited and those who violate the rules are subject to significant fines and criminal prosecution.

Shipment Classification

Biological materials are shipped according to the following classification; category A or B, biological product, genetically modified organisms, medical waste, and human specimens. Category A&B are infectious substances with category A causing fatal disease in humans or animals and category B has less severe consequences. Biological products are items manufactured and distributed for healthcare purposes and are considered a low probability of containing pathogens. Biological products are defined as materials used in the prevention, treatment of cure of disease in humans or animals, examples include; vaccines, blood products, therapeutic serums, and antitoxins. Genetically modified organisms (GMO) that are not infectious substances but are capable of altering animals or plants in a way that is not normally the result of natural reproduction can be transported as a Miscellaneous Hazard. GMO that are infectious or carried by an animal host are regulated for transportation. Patient specimens are materials for which there is minimum likelihood that pathogens are present.

Packaging Materials

Potentially hazardous materials must be package to withstand leakage of contents, shocks, temperature and pressure changes and other conditions that may occur during transportation procedures. Biological materials must be packed with the triple packaging principle to include; primary receptacle, leak proof secondary container with absorbent material and durable outer container. The packages must comply with the package instructions from IATA such as 602 for infectious substance. Buying certified packages from suppliers that are specific for the materials you want to ship will ensure compliance with the packing requirements.
The proper shipping name, labels and UN markings must also be on the package before sending out for shipment. If using dry ice or liquid nitrogen with your shipment, these materials must be declared and packages properly labeled. Dry ice should never be placed in a sealed container or the package may be at risk of exploding.

BIOLOGICAL WASTE DISPOSAL

Proper biological waste disposal will ensure safety for laboratory personnel, custodial staff, waste transporters, and the general public. The Florida Administrative Code 64E-16 on Biological Waste sets forth waste disposal regulations for biomedical and biohazardous waste generation, treatment and disposal. These rules must be followed by all who generate biowaste at Palm Beach State College.

Biological waste includes infectious and non-infectious waste that may be generated in the laboratory, clinical and campus setting. Examples of infectious or potentially infectious waste include human and animal pathogens, recombinant DNA, human blood and tissue. Examples of non-infectious waste include needles/syringes used for teaching purposes or in chemical labs, and materials that has not been contaminated with disease causing agent, such as petri dishes, media and animal carcasses.

Waste Guidelines

1. All biological waste except sharps must be placed in biohazardous bags. Biohazardous materials **must not be placed in the regular trash stream.**

2. Biohazardous waste must be identified and segregated from other solid waste at the point of origin. Sharps must be segregated from non-sharps biological waste.

3. All biohazardous waste known to contain infectious agents should be rendered inactive through autoclaving or other decontaminating method before leaving the generating facility.

4. All surfaces and materials contaminated with spilled or leaked biological waste must be cleaned and disinfected with one of the following methods:

    a. Autoclave at 121^{0}C, 15 psi for at least 15 minutes.
    b. Household beach solution diluted 10%, one part bleach to nine parts water.
    c. Chemical germicides that are registered by the Environmental Protection Agency as approved disinfectants and are tuberculocidal when used at recommended dilutions.

5. All biohazardous material from a spill must be placed in an appropriate container and disposed of as biological waste.

6. Each department generating biological waste must obtain supplies needed for managing the waste such as biohazardous bags, boxes, liners, clear autoclave bags, and sharp containers.

7. Each biohazardous box should have a liner in place before putting red biohazardous bags into the box. The liner should not be used as a bag. All biohazardous bags placed in the box should be closed after use; bags should not be left opened.

8. All non-biohazardous biological waste should be placed in a clear autoclave bag and autoclaved.

9. All infectious waste should be decontaminated by using an autoclaved or chemical inactivation before leaving the facility and must be properly labeled with Laboratory Specialists name and location and sealed with tape before being stocked for final treatment and disposal.

10. All bags have to be labeled unless placed in a secondary container then only the liner and outside container has to be labeled.

11. If biological waste is mixed with chemicals or radiation, it must be managed as hazardous or radioactive waste prior to disposal.

12. Infectious mixed waste should be rendered non-infectious before being handled as chemical or radioactive waste.

13. All employees who generate or handle biological waste must be trained annually on the proper procedures and policy for biological waste.

Animal Waste

1. Animal waste and carcasses that are generated in the laboratory should be picked up for disposal by the Waste Management Company. Animal carcasses should not be placed in the regular trash stream.

2. Biohazardous plastic bins are available to store **only** animal carcasses and sharps containers for pickup. Biohazardous bags should not be put into the bin; the bags must be placed into a lined box.

3. Biological waste should be stored in designated locations to be sent out for disposal.

Labeling

1. Biohazardous waste bags, boxes, and sharps containers must be labeled with the generator's name and location where the waste was generated. The information on the box must be legible and written with an indelible marker.

2. Biohazardous waste bags, boxes and outer containers must be labeled with the date when the waste was **first generated**. The sharps container should be labeled with the date when it is **sealed**.

3. If a biohazard bag or sharps container is placed into a larger box or container prior to transport, the label for the exterior container must comply with the above information. Inner bags and inner sharps containers are exempt from the labeling requirements above.

4. Outer containers must be labeled with the transporter's name, address, registration number, and 24-hour telephone number prior to transport.

5. All packages containing biological waste must be visibly identifiable with the international biological hazard symbol and one of the following phrases: "BIOLOGICAL WASTE," "BIOHAZARDOUS WASTE," "BIOHAZARD," "INFECTIOUS WASTE," OR "INFECTIOUS SUBSTANCE." The symbol must be red, orange, or black and the background color must contrast with that of the symbol or comply with requirements cited in **Subpart Z** of **29 CFR§1910.1030(g)(1)(C)**.

Storage and Containment

1. Storage of biological waste at the generating facility **must not exceed 30 days**. The 30-day period must commence when the first item of biological waste is placed into a red bag, or when a sharps container housing only sharps is sealed.

2. Indoor storage areas must have restricted access. They must be located away from pedestrian traffic, be vermin and insect free, and must be maintained in a sanitary condition. They must be constructed of smooth, easily cleanable materials that are impervious to liquids. Waste containers in the lab should remain closed when not in use with a lid.

3. Outdoor storage areas, including containers and trailers, must, in addition to the above criteria, be conspicuously marked with the international biological hazard symbol, and be secured against vandalism and unauthorized entry. The international biological hazard symbol on an outdoor storage area must be a minimum of six inches in diameter.

4. Biological waste shall be packaged and sealed at the point of origin in impermeable red biohazard bags or sharps containers. Packages of biological waste shall remain sealed until picked up by the waste management company for treatment and disposal. Ruptured or leaking packages of biological waste shall be placed into larger container without disturbing the original seal.

5. All outer containers must be rigid, leak resistant, and puncture resistant. Reusable outer containers must be constructed of smooth, easily cleanable materials and must be decontaminated after each use by an approved method. The international biological hazard symbol must be at least six inches in diameter on outer containers 19" X 14".

Transfer Requirements

1. Persons handling packages or spills of biological waste must wear appropriate personal protective equipment as specified in **Subpart Z** of **29 CFR§1910.1030(d)(3)**, which includes, but is not limited to gloves, gowns, laboratory coats, and face shields or masks and eye protection.

2. Ruptures or leaking packages of biological waste must be repackaged prior to transport.

3. Contract personnel, appropriately trained by the contractor, will remove biological waste containers for disposal.

4. Only transporters registered with the Florida Department of Health must be used to transport biological waste.

Permits and Exemptions

1. All biological waste generating facilities must obtain a permit from the County Health Department on an annual basis if the waste is more than 25 lbs in a 30-day period.

2. Facilities generating less than 25 lbs of waste in a 30-day period are exempted from the permit for three years.

3. The generating department or program will maintain permit and exempt information.

4. Copies of the permit will be sent to each generating facility to post in a conspicuous place.

Recordkeeping

Records of waste disposal and management must be maintained for three years and must be available for review by the County Health Department. All records pertaining to Biological Waste must be kept within the generating department and available to review at any time.

Responsibility of Generating Department

The **Lab Specialists** will be responsible for Waste Storage and Disposal Compliance and will ensure that all requirements are met. Lab Specialists will monitor Storage areas and will coordinate all waste disposal activities. Lab Specialists will contact Biohazardous Waste Contractor for pick up, will sign all manifests and ensure that records are stored and maintained within generating department as per regulatory requirements, and available at any time for internal or external audits.

The **Program Deans and Chairpersons** are responsible for oversight to ensure that the proper management, storage, and disposal of all biological or biological waste generated by their Departments is done properly. Biological Waste Programs that are improperly managed must be corrected immediately.

The **Program Instructors and other Staff** are responsible for compliance with Waste Storage and Disposal requirements and must comply with the Palm Beach State College Program. Non Compliance with the program is a violation of Regulatory and organizational requirements.

Enforcement and Penalties
Any one in violation of F.A.C. 64E-16, or who interferes with, hinders, or opposes any County Health Department employee in the discharge of his duties, is chargeable with a misdemeanor of the second degree. If any violation occurs, the County Health Department may deny, suspend, or revoke any biological waste permit or impose an administrative fine of up to $2500 per day for each violation of the FAC 64E-16.

Appendix A

Palm Beach State College

Biological Agents List

Location	Program	Microorganisms
Palm Beach State College PBG Campus	BioTechnology	Listeria monocytogenes – ATCC 19115 Listeria innocua – ATCC 33090 Staphylococcus aureus – ATCC 25923 Rhodococcus equi – ATCC 6933 Salmonella enteritidis subsp. Enteritidis – ATCC 6539 Salmonella enteritidis subsp enteritidis Enteritidis – ATCC 13076 Salmonella enteritiolis subsp enteritidis Typhimurium – ATCC 14028 E. Coli 0157:H7 - ATCC 35158 CHO Cells – ATCC CCL-61 Melanoma Cells – ATCC CRL-1675
Palm Beach State College PBG Campus	Micro Biology	Bacillus subtilis E coli Micrococcus luteus Staph epi Staph aureus Strep pyogenes Strep pneumonia Proteus mirabilis Neisseria subflava Enterobacter cloacae Shigella enteric ssp enteric Serratia marcescens
Palm Beach State College Boca Campus	Micro Biology	Alcaligenes faecalis Bacillus subtilis Bacteroides fragiles Citrobacter diversus Citrobacter freundii Enterobacter cloacae Enterococcus faecalis Escherichia coli Klebsiella pneumonia Micrococcus luteus Morganella morganii Neisseria lactamica Proteus mirabilis Proteus vulgaris

		Pseudomonas aeruginosa Serratia marcescens Staphylococcus aureu epidermidis Streptococcus pyogen Mycobacteria phlei
Palm Beach State College Lake Worth Campus	Microbiology	Bacillus subtilis Escherichia coli Serratia sp. Enterobacter aerogenes Micrococcus Staphylococcus epidermidis Clostridium butyricum Staphylococcus aureus Streptococcus pyogenes Candida sp. Aspegillus niger Penicillum sp. Proteus vulgaris Salmonella spp. Shigella spp.