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Approved and issued by order of:

Wendy Ash Graves
Director, Environmental Health and Safety

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POLICY MAINTENANCE SECTION

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Biological Safety Manual

Florida Atlantic University
Office of Environmental Health and Safety
May 2023
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1. INTRODUCTION

Florida Atlantic University (FAU) is committed to providing a safe and healthy environment for our faculty, students, staff, and visitors. The goals of FAU’s Biological Safety Program are to: 1) Protect the researchers, staff, students and visitors from exposure to biological agents or recombinant/synthetic nucleic acids; 2) Prevent environmental release of any biological agents; 3) To enhance the research atmosphere; and 4) To comply with federal, state and local regulations. In pursuit of this goal, the following Biological Safety Manual is provided to assist FAU researchers, staff, students, and visitors on maintaining safety while working with potentially dangerous biological materials. Biological materials include but are not limited to: Recombinant/ Synthetic Nucleic Acids; Microorganisms (bacteria, viruses, fungi, parasites, prions, and rickettsia), Biological Toxins or Venoms; Human- or Nonhuman Primate-Derived Materials; and Field Work with Animals.

The Biological Safety Program is administered by the Biological Safety Officer within the Office of Environmental Health and Safety, along with the Institutional Biosafety Committee, which is housed within the Office of Research Integrity in the Division of Research. This Biological Safety Manual has been developed as part of the overall FAU Biosafety Program. The manual provides University safety guidelines and outlines general policies and procedures for working with and disposing of biohazardous materials, according to applicable regulatory guidelines.

All laboratories housed in facilities owned/leased by FAU must comply with the biological safety practices and procedures outlined in this manual. Principal Investigators (PIs) or Laboratory Managers/Supervisors must contact the Biological Safety Officer if they are uncertain of how to categorize, handle, store, treat or discard any biologically derived material. PIs should use this manual to develop site specific safety procedures for their laboratory.
2. ADMINISTRATION AND RESPONSIBILITIES

Biosafety is a cooperative effort between Florida Atlantic University, its employees, students, volunteers, and affiliate organizations. The Biosafety Officer, the Institutional Biosafety Committee (IBC), PIs, technicians, students, postdocs, and administrative personnel all must work in concert to minimize the risk of exposure, injury or illness associated with activities involving potentially biohazardous materials.

2.1. Environmental Health and Safety (EH&S)

EH&S provides services, advice, and compliance assistance to ensure employees, students and visitors follow safe work practices when working in research laboratories. The Biological Safety Program within EH&S monitors compliance with University safety policies and procedures regarding potentially infectious and biohazardous materials. The Biological Safety Program is designed to assist PIs and laboratory personnel in the selection of appropriate laboratory controls and practices that will ensure a safe working and learning environment for the University.

2.2. Biosafety Officer

The Biosafety Officer (BSO) is responsible for:
1) Reviewing activities and facilities for proper biohazard control; apply relevant laws, standards, and guidelines.
2) Taking measures necessary to ensure that all biohazardous activities comply with the policies and practices established by the IBC.
3) Providing supplemental training to PIs, supervisors, postdocs, students, and other laboratory workers in proper laboratory safety and other training that may be required per CDC, NIH, OSHA, or other regulatory agencies.
4) Reporting any significant problems, trends, and non-compliance violations of regulations, policies, or practices to the IBC.
5) Assisting the PI and laboratory staff in identifying potential biohazards and relevant risks (including protocols, techniques, and practices), and in selecting potential alternatives, appropriate personal protective equipment (PPE) and use of other exposure controls.
6) Interacting with PIs to determine weaknesses and deficiencies within the Biosafety Program and work to correct.
7) Responding to any exposures/accidents involving biohazardous material. Support follow-ups to these incidents and assist the PI with investigations.
8) Assisting and advising facilities administrators on engineering controls for laboratory modifications and for new laboratory construction.
9) Advising the IBC, PIs, and laboratory workers on biohazard security, biosafety, and technical compliance issues.
10) Together with EH&S, organizing and conducting periodic laboratory inspections and post-approval monitoring.

2.3. Division of Research/Research Integrity

The Division of Research oversees funding for research projects that are awarded to investigators at FAU. Within the Division of Research is the Office of Research Integrity, which coordinates and oversees the Institutional Animal Care and Use Committee (IACUC), the Institutional Review Board (IRB) and the IBC.

2.4. Institutional Biosafety Committee (IBC)
The IBC is seated within the Office of Research Integrity in the Division of Research. The IBC is charged by the Vice President for Research to formulate policy and procedures related to the use of biohazardous agents, including: bacteria, viruses, parasites, fungi, prions, rickettsia (infectious to humans, animals or plants); biologically active agents (toxins or venoms); human cells, tissues and fluids (including cell lines); nonhuman primate cells, tissues and fluids (including cell lines); field research with animals; and recombinant/synthetic nucleic acid molecules. As mandated by the National Institutes of Health (NIH; NIH Guidelines), experiments involving work with recombinant/synthetic nucleic acid molecules must be reviewed by the IBC. There are certain experiments that are exempted from the NIH Guidelines, but these low risk projects must still be registered with the IBC so the committee can keep track of recombinant/synthetic nucleic acid work on campus (please visit the FAU IBC website for more information about what work requires registration with the IBC).

2.5. Deans/Department Chairs

Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their respective schools/departments. They should be aware of and approve all research conducted under their purview. The must ensure departmental compliance with applicable laws, regulations and guidelines covering the use of biological agents in their facility.

2.6. Principal Investigators

Principal Investigators (PIs) are ultimately responsible for identifying potentially infectious/biohazardous materials in their laboratories and performing appropriate risk assessments on these materials to implement specific control measures thus ensuring a safe work environment. They will:

1) Ensure that all work is conducted in accordance with established policies and guidelines described in this document.
2) Ensure that all employees under his/her supervision are adequately trained in good microbiological practices and have received safety training appropriate to the work conducted in the laboratory.
3) Develop, review, and approve laboratory-specific and/or protocol-specific procedures, consulting with the BSO when necessary.
4) Provide training and relevant information to all employees regarding biohazards that are present in the lab.
5) Develop risk assessments for specific biohazards in the laboratory (in consultation with the BSO when necessary) and ensure that all employees have access to the assessments.
6) Ensure that employees are aware of any special requirements, such as vaccinations, required to work with and around specific biohazards.
7) Ensure prompt reporting of any job-related injuries, exposures, or illnesses.
8) Inform the Department Chair and the BSO of any serious, or potentially serious, accidents, or incidents involving exposure to biohazardous materials, including accidental releases and illnesses.
9) Act, in a timely manner, upon requests and/or directives from the IBC or BSO concerning laboratory safety and work with biohazards.
10) Ensure that appropriate containment devices and other engineering controls are in place, operating correctly and certified (if necessary) for use; and ensure that employees have been trained to use such equipment in a proper manner.
11) Ensure that appropriate PPE is available, that the employees are adequately trained in donning and doffing, and that the PPE is being utilized.
12) Ensure that proper decontamination of the laboratory and/or equipment is conducted prior to any needed inspections, calibrations, or repairs.
13) Ensure proper disposal of all biohazardous material, including any sharps.
14) Keep an up-to-date inventory of biohazards and respective amounts.
15) Maintain proper labeling of the labs.

2.7. Employees/Lab Workers

All employees/lab workers performing work with infectious/biohazardous materials must accept a shared responsibility for conducting their work in a safe manner. Ultimately, each employee is responsible for his/her own safety. Employees/Lab Workers must:
1) Ensure that all work is conducted in accordance with established policies and guidelines described in this document and/or via specific laboratory SOPs.
2) Report all hazardous conditions to the PI and/or the BSO.
3) Report any job-related injuries, exposures, or illnesses to the PI and/or BSO and seek medical treatment immediately.
4) Refrain from operating any equipment or instrument without proper instruction and/or training.
5) Wear and maintain personal protective equipment necessary for each task.
6) Properly utilize engineering controls.
7) Participate in required training programs.

2.8. Laboratory Owners of 3rd Party Labs

Lab owners of 3rd party labs that occupy space on the FAU campus must comply with FAU safety policies/manuals. These include, but are not limited to: Laboratory Safety Manual, Biological Safety Manual, Chemical Hygiene Plan, Bloodborne Pathogens Exposure Control Plan; Biomedical Waste Program Manual; and Hazard Communication Program Manual. All personnel must complete the appropriate training modules through the FAU EH&S training program, as well as training through other FAU programs as necessary (e.g. IACUC).

3. BIOSAFETY REQUIREMENTS

3.1. The Occurrence of Laboratory Acquired Infections

One of the primary goals of Biosafety is to prevent Laboratory Acquired Infections (LAIs). A LAI is an infection, either symptomatic or asymptomatic, resulting directly from work in a laboratory (research or clinical) using infectious agents. That infection can be either to the person doing the work, or another person in the laboratory that is exposed.

Published reports of LAIs first appeared around the start of the 20th century. It is impossible to know exactly how many LAIs have occurred over the years because of the lack of a universal reporting system, either at the federal, state, or local level. By 1978, four studies by Pike and Sulkin collectively identified over 4,000 LAIs over 48 years (1930-1978) (1-4). These studies found that the ten most common causative agents of overt infections among workers were Brucella sp., Coxiella burnetti, hepatitis B virus (HBV), Salmonella typhi, Francisella tularensis, Mycobacterium tuberculosis, Blastomyces dermatitidis, Venezuelan equine encephalitis virus, Chlamydia psittaci, and Coccidioides immitis. The
authors acknowledged that the 4,079 cases did not represent all LAIs that occurred during this period since many laboratories chose not to report overt cases or conduct surveillance programs to identify sub-clinical or asymptomatic infections. In addition, reports of LAIs seldom provided data sufficient to determine incidence rates, complicating quantitative assessments of risk. Similarly, there were no distinguishable accidents or exposure events identified in more than 80% of the LAIs reported before 1978. Studies did show that in many cases the infected person worked with a microbiological agent or was in the vicinity of another person was handling an agent.  

A more recent study was conducted by Harding and Byers (5), who categorized over 1,200 LAIs over 22 years (1979-2001). These studies were conducted by survey of biomedical, clinical, and industrial laboratories and review of the research literature on LAI occurrences. One of the most important findings in these studies is that only 20% of reported LAIs have the specific route of exposure/infection known. Still, many exposures have had a root cause of poor basic biosafety practices in the laboratory. It is important to understand the primary routes of infection for the specific agent that is being worked with in the laboratory. However, it is also important to understand that working in a laboratory with large volumes and high concentrations of specific agents can circumvent normal routes of infection and result in exposures.

Exposures can occur through overt personal accidents that include:

- **Self-inoculation** (resulting from pricking, jabbing, or cutting the skin with contaminated instruments such as needles, scalpels, and glassware; and from animal bites and scratches)
- **Ingestion** (resulting from mouth-pipetting, eating, drinking, and smoking)
- **Splashing into the face and eyes**
- **Spills that can come into direct contact with skin** (especially when there are cuts or scrapes present).

Exposures can also occur through passive mechanisms including:

- **Contact with aerosols, droplets, or fomites.** Aerosols are defined as a suspension of fine solid particles or liquid droplets in air or another gas. If the liquid contains infectious agents, these agents would be distributed in the aerosol and would remain viable for some time. These aerosols can remain airborne for quite some time, can be moved around by air currents/ventilation, and can be inhaled or ingested and potentially cause infection. Larger droplets can settle quickly and contaminate surfaces in the area upon which they come to rest. The surfaces are then fomites—inanimate objects that, when contaminated with infectious agents, can transfer disease to a host.
- **Many laboratory techniques, using both simple and complicated mechanical equipment, as well as laboratory accidents, can produce aerosols.** These include use of microbiology loops, pipettes, syringes, and needles; opening tubes and bottles; use of centrifuges, blenders and sonicators; harvesting of eggs and other virological procedures; and breakage of tubes and vials.

Infectious materials must be clearly identified and stored in such a manner as to preclude accidental exposure. This normally includes secondary containment and labeling of all samples stored in the laboratory. Several infectious agents have been documented to cause laboratory-acquired infections. The American Biological Safety Association has assembled a comprehensive list of all published LAIs, which can be accessed here: [https://my.absa.org/LAI](https://my.absa.org/LAI).

Several governmental regulations exist to help mitigate the potential problems of LAIs. However, it falls upon the PI to comply with regulations as well as currently established guidelines, to create a safe work area for the laboratory. It is important for all laboratory workers to understand that several organisms,
which would normally be innocuous, could cause severe and even life-threatening infections in people who are immunocompromised in some way.

3.2. Biosafety Requirements

Appendix A shows a chart for determining requirements of PIs and lab personnel based upon the use of various agents in the laboratory. Details of requirements are further described in the subsequent sections.

3.2.1. Registration for the use of Biohazardous Materials

All PIs working with biological hazards (viruses, bacteria, fungi, rickettsia, parasites, prions, biological toxins and venoms, recombinant/synthetic nucleic acids, human or nonhuman primate cells, tissues or fluids, and mammalian cells treated with biological vectors) are required to complete and submit an IBC Registration through SciShield (formerly BioRaft) (https://fau.bioraft.com/). The IBC and Biosafety Officer must maintain accurate information regarding the use of biohazardous materials by FAU personnel. IBC policy requires a new IBC Registration be submitted every three (3) years. Additionally, if any changes occur in agents, funding, personnel or procedures, the PI is required to file an amendment to the Registration in SciShield.

Most work with biological agents at FAU will not require review by the full IBC. However, the IBC is required to review work with recombinant/synthetic nucleic acids as directed by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines). There is some work that is exempted from the NIH Guidelines, but FAU still requires registration of the biological material work with the IBC and review by the BSO. The IBC does reserve the right to review any research with agents listed in Risk Group 2 or higher (https://my.absa.org/Riskgroups). Please note that any work with any potentially hazardous biological materials requires registration in SciShield and review and approval by the BSO.

The review process for protocol registration is described in detail within the IBC policies and procedures (available on the IBC Website).

3.2.2. Recombinant/Synthetic Nucleic Acid Experiments

As indicated above, registration with the IBC is required for all work involving recombinant/synthetic nucleic acids. Refer to the IBC Protocol Registration Form for specific information on types of experiments and compliance with NIH Guidelines.

3.2.3. Human Gene Transfer Experiments

Proposed clinical trials involving human gene transfer have specific requirement for approval. In addition to review by the IBC, these studies must be reviewed by the FAU IRB and registered with NIH OSP.

3.2.4. Human Blood, Body Fluids, Tissues, Cells and Other Potentially Infectious Materials (OPIM)

The Occupational Safety and Health Administration (OSHA) has created the Bloodborne Pathogens Standard (29 CFR § 1910.1030) to minimize or eliminate exposure to infectious agents that may be present in human blood, tissues, or body fluids (bloodborne pathogens). The Standard applies to all
employers that have employees that are occupationally exposed to human blood or OPIM. OPIM include:

- Human (or nonhuman primate) cells or tissue cultures (including cell lines)
- Any unfixed tissue or organ, other than intact skin, from a human (or nonhuman primate)
- Human (or nonhuman primate) body fluids, except urine, feces, saliva, or tears, unless visibly contaminated with blood
- Organ cultures
- HIV- or HBV-containing fluids, as well as other pathogens including, but not limited to, Hepatitis C Virus, Malaria parasites, Brucella, Arboviruses, HTLV-1 and hemorrhagic fever viruses.
- Blood, organs, fluids, or tissues from experimental animals infected with bloodborne pathogens

The employer is required to develop a bloodborne pathogen exposure control plan that outlines who may be occupationally exposed and how to minimize or eliminate exposures. All employees that fit within the guidelines of potential occupational exposure are required to take a bloodborne pathogen training session prior to beginning work, and then annually thereafter. Florida Atlantic University’s exposure control plan is available online.

3.2.5. Research Animals

All experiments involving animals must be conducted in accordance with established federal laws and guidelines and must be approved by the FAU Institutional Animal Care and Use Committee (IACUC), prior to initiation. Animal research that involves a biological hazard (see 3.2.1 above for a list) must also be approved by the IBC.

3.2.6. Biological Safety Cabinets

BSCs are required for working with agents classified in Risk Group 2 and above when work will involve the potential generation of aerosols and splashes. BSCs protect the worker, the material, and the environment from potential exposures. Personnel that utilize BSCs must be properly trained by the PI or the laboratory supervisor. Alternatively, training can be provided through the Biosafety Office if requested. PIs should notify the BSO if planning on purchasing, moving, transferring, or discarding a BSC. BSCs require annual certification. Please see section 6.2.2 for more detailed information regarding BSCs.

3.2.7. Training

Training is an essential part of a comprehensive biosafety program. All laboratory workers should have general laboratory safety training and, if necessary, any specialized training related to the work they are going to perform. All training is accessed through the Percipio platform, which can be found on the EH&S training website (https://www.fau.edu/ehs/training/index.php). All laboratory workers need to demonstrate good microbiological practices. For specific work with specific agents, the PI is responsible for providing appropriate training. If so desired, the BSO can work with the PI on developing specific training regimens appropriate for the agent. Table 1 below lists the training requirements.

Table 1. Required Safety Training
### Work Type | Required Training
---|---
All Laboratory Workers | Laboratory Safety, Fire Safety and Prevention, Portable Fire Extinguisher Training, Hazard Communication, Hazardous Material Handling and Storage, Hazardous Waste Generator
Laboratories working with infectious or potentially infectious material (any human/nonhuman primate materials; mammalian whole animals and cells/cell lines used in recombinant/synthetic nucleic acid studies) | Bloodborne Pathogens Training, Biosafety Hazardous Waste Handling and Disposal, CITI Initial Biosafety Training (Through Research Integrity)
Laboratory workers Packaging, Shipping, Transporting or Receiving Biohazards (Infectious agents, hazardous biological toxins, and human clinical specimens) and Dry Ice | Dependent upon the shipping method: DOT 1-4, Shipping Hazardous Materials: Ground And/Or IATA 1-5: Shipping Hazardous Materials: Air

## 4. BIOSAFETY

### 4.1. General Principles

Biological Safety, or Biosafety, is defined as: The application of knowledge, techniques, and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. This can be accomplished through two main means:

- **Primary Containment** – the protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

- **Secondary Containment** – the protection of the environment external to the laboratory from exposure to biohazardous material or other biohazards through a combination of facility design and operational practices.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four Biosafety Levels (1-4) define the level of containment necessary to protect personnel and the environment. Biosafety Level 1 (BSL-1) is the least restrictive, while Biosafety Level 4 (BSL-4) requires a special containment laboratory or facility.

The recommended biosafety level(s) for an organism or toxin represents the conditions under which the agent can ordinarily be safely handled. **The laboratory PI is specifically and primarily responsible for**
assessing risks and for appropriately applying the recommended biosafety level(s) (see Assessments: Section 3.3).

The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. Most laboratory-acquired infections (90%) have occurred because of non-adherence to proper procedures, including good microbiological practices. Everyone working with infectious agents or potentially infectious materials must be aware of the pertinent risks. In addition, they must be trained and proficient in the practices and techniques required for handling such material. It is the responsibility of the Principal Investigator or laboratory supervisor to provide and/or arrange for appropriate training of personnel in their laboratory.

4.2. Routes of Infection

When working in a biological research environment, it is not unreasonable to expect that a laboratory person working with infectious materials is more likely to become infected than are members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body’s defense system. The following routes of infection have been reported for laboratory-acquired infections:
Mucous membranes: Exposures to mucous membranes of the eyes, nose, mouth and cuticles through splashes or splatters

Ingestion: Mouth pipetting, eating, drinking, smoking in the lab

Inhalation: Breathing in respirable sized aerosols (<5 mm), centrifuge leaks, spills, pipetting, etc.

Percutaneous: Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, abrasions, or eczema

Contact (indirect transmission): Via hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment, etc.) or by failure to wash hands after working.

Most of the laboratory-acquired infections reported in the literature point to spills, splashes and accidents involving needles or other sharp objects as the source of exposure. The general laboratory procedures outlined in this manual address those issues and provide guidance in handling infectious or potentially infectious materials. Table 2 lists protective measures that can be implemented to prevent exposure to the various routes of transmission.

Table 2. Protection for Routes of Infection

<table>
<thead>
<tr>
<th>Route of Exposure</th>
<th>Protective Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous Membranes: Exposure via the mucous membranes of the eyes, nose, mouth, or cuticles due to splash/splatter</td>
<td>Wearing a full-face shield and safety glasses</td>
</tr>
<tr>
<td></td>
<td>Working in a biosafety cabinet or behind a protective shield</td>
</tr>
<tr>
<td>Route of Exposure</td>
<td>Protective Measures</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
</tr>
</tbody>
</table>
| **Ingestion:** Mouth pipetting, eating, drinking, smoking in the lab | Following good microbiological practices  
Wearing gloves |
| **Inhalation:** Breathing in respirable sized aerosols (<5 mm), centrifuge leaks, spills, pipetting, etc. | Good microbiological practices, no mouth pipetting, no food, and drink in the lab.  
Use of the Biosafety Cabinet, sealed rotors or canisters for centrifuges, safety containment equipment, HEPA filtered respirator, and good microbiological practices. |
| **Percutaneous:** Through intact or non-intact skin via needlestick, puncture with contaminated sharp object, animal scratch or bite, through wounds, abrasions, or eczema | Substitute plastic for glass. Use extreme precautions with sharps, dispose of immediately in rigid leak-proof sharps container, use animal restraints, cut-resistant gloves, sleeve covers, waterproof bandages, and double gloves, good work practices. |
| **Contact (indirect transmission):** Via mucous membranes or non-intact skin from hands that have been in contact with a contaminated surface (i.e. benches, phones, computers, equipment, etc.) or by failure to wash hands after working. | Decontamination of work surfaces and hand washing. Good personal hygiene (avoid touching your face with glove or non-gloved hands), do not apply cosmetics within the laboratory. |

### 4.3. Biological Risk Assessments and Risk Management

Responsibility for biosafety exists at all levels and is shared throughout the University. The researchers, clinicians and technicians who perform work with biohazards are perhaps the most important component of the biosafety program, as they must incorporate the biosafety requirements and safety precautions into all facets of their work.

#### 4.3.1. Assessment of Risk.

The assessment of risk is an essential element of safety in the laboratory. It is incumbent upon the PI, who is ultimately responsible for safety within the laboratory, to perform a risk assessment prior to beginning any work with agents in the laboratory. Questions concerning the appropriate safety equipment, immunizations, training, and waste disposal need to be addressed as well as safe procedures and practices. One of the most helpful tools utilized for risk assessment is the risk group characterization of agents (see above). However, simply relying on the risk group classification of an agent for an assessment does not address the complete picture for laboratory risk. Additional factors that must be considered include:

- Pathogenicity of the agent and the infectious dose
- Potential outcome of an exposure incident (local and community wide)
• Natural route of infection (as well as the ability to infect by other routes)
• Stability of the agent in the environment
• Concentration and volume of the agent being manipulated
• Availability of a suitable host
• Information from other laboratory, clinical and animal studies with the agent
• Procedures planned in the laboratory for the agent
• Availability of appropriate engineering controls
• Any genetic manipulations of the agent that may alter its biology (including transmissibility, host range, therapeutic susceptibility, etc.)
• Availability of effective prophylaxis or post-exposure therapy

For most agents, guidelines, rules, and regulations have clearly defined the procedures and practices to be followed to achieve safety in the workplace. However, in cases of the newly isolated agents or toxins, or procedures not previously employed, further evaluation is needed due to the limited availability of information. Since individual judgment involves both personal and social values, opinions on what is “safe” vary significantly. To find a common ground for an acceptable risk assessment, the “rule of reason” needs to be applied. The following factors should be considered for the determination of what is reasonable:

1. **Custom of usage (or prevailing professional practice):** Many laboratory procedures involve the maintenance of sterility and cleanliness. These procedures are commonly considered safe since adverse effects would have been obvious over time. (Caution: because a procedure has been used for many years does not necessarily imply that it is a good practice. An example is mouth pipetting, which was used for centuries and finally considered very unsafe.)

2. **Best available practice, highest practicable protection, and lowest practicable exposure:** It should be common practice in the microbiological laboratory to use the best available procedures with the highest level of protection. This not only provides for a safe work environment but also fosters excellence in scientific conduct.

3. **Degree of necessity or benefit:** The common question to ask is, are the benefits worth the risk? For example, there is no need to use a human pathogen causing severe gastroenteritis when general microbiological practices can be taught with a noninfectious organism.

4. **No detectable adverse effects:** This can be a very weak criterion since it involves uncertainty and should be applied accordingly.

5. **Principal knowledge:** At times, existing procedures are modified, involving the same or similar toxic chemicals or agents. For that reason, similar safety procedures should be applied. If new agents are isolated, we need to ask what we know about the close relatives. Many agents of known etiologic character are already categorized in risk groups, allowing for the selection of the appropriate biosafety level. New isolates from infected animals or humans with known infectious relatives warrant, at a minimum, the same level of protection.

Taking the above-mentioned factors, as well as others, into consideration will allow for a reasonable approach to a new challenge. The BSO is available to assist in this process and should be contacted with questions. Once a risk assessment is completed, the results should be communicated to everyone involved in the process. If necessary, written standard operating procedures (SOPs) that are laboratory specific should be established.
4.3.2. Steps in Risk Assessment and Risk Management

Step 1:

Pathogen (Gather all information on the biohazard or agent)

- Obtain the Agent Summary Statement from the CDC/NIH BMBL if available.
- Understand the signs and symptoms of infection
- Learn preventive therapies (immunizations and post-exposure treatment)
- If a pathogen is antibiotic resistant or resistant to a commonly used therapy, know which treatments will be effective against the pathogen; this is especially important in the event of an exposure
- Know all clinical syndromes that can be caused by the agent
- Identify what medical conditions may make a person more susceptible or at higher risk of infection or serious disease
- Let staff know to seek counsel from the Occupational Health Provider prior to starting work with biohazards for a private consult regarding their health status
- Identify the incubation period, the infectious dose, the starting Risk Group
- Do a web search for laboratory-acquired infections with the proposed biohazard and identify how it was transmitted if known
- Learn and understand how the biohazard is transmitted in nature and in a lab setting
- Review unnatural exposure routes with staff, including aerosol deposition to lower lung, aerosol contamination of mucous membranes, aerosol contamination of surfaces, hand to face (eyes, nose, and mouth) transfer of pathogens, eye to nasal cavity to back of throat to gut transmission
- Find out how long the biohazard can survive on surfaces or the environment
- List the disinfectants that are effective at inactivating the biohazard and identify the concentration of the disinfectant and contact time required for kill
- Highlight pertinent risk assessment information and share with your staff (list at start of site-specific standard operating procedures)
- Post unique risk information on the lab door biohazard sign to ensure all visitors are informed of potential risks

Step 2:

Procedures (review all proposed procedures with the biohazard or agent)

- Identify and list all the procedures, equipment and supplies that will be used in your research or
- Ensure that every step is included, from removal of the biohazard from the freezer, transport to work areas, all protocol steps, through decontamination and disinfection and/or return to frozen storage
- Identify any steps performed outside your lab, such as in core facilities (i.e. specialized microscopy, flow cytometry) and any shared equipment locations
- Once all the steps, supplies and equipment have been documented and written down, identify all the risks and potential exposures that could possibly occur during the course of the work (splashes, splatter, spills, aerosols, cuts, lacerations, punctures, bites, scratches, etc.). Pay particular attention to punctures from contaminated sharps (if not working with animals – eliminate sharps and use plastic alternatives!)
- Any step with a liquid could generate a splash or splatter that might contact facial mucous membranes, skin or personal clothing and contact surrounding work areas
- Procedures that impart energy to a culture (basically all of them) from pipetting to vortexing to centrifugation and highlight these steps that may produce aerosols
For animal experiments consider the use of sharps for inoculation, bites and scratches from the animal, exposure to bedding contaminated from excretion of the biohazard

- Identify the potential for spills (dropped flasks, broken flasks in shakers, leaks in centrifuges, etc.)
- Write down all the steps and risks identified and detail the potential for exposure (this is your required written site-specific risk assessment).

PAUSE: Ask your Biosafety Officer to review your risk assessment at this point. Also ask your IACUC, IBC or IRB rep, where applicable, to review. Your Biosafety Officer will confirm your risk assessment and help identify any procedures with potential risk that may have been missed. Update your written assessment after this review.

Step 3:

Personnel Evaluation (review who will be handling biohazards)

- Do all staff have prior experience working with this biohazard or very similar biohazards? If not, an internship to gain hands-on experience with the agent can be arranged with another lab or within your lab
- Have all staff completed all required Biosafety and other applicable laboratory safety trainings prior to initiating work?
- Do staff have a positive safety attitude and a healthy amount of respect for the risks involved with the proposed biohazard?
- Have the proposed staff exhibited a strong safety record in the lab?
- Are all staff informed of the risks presented by this work and the proposed procedures?
  - Are any staff at greater risk due to their health status?
    - Have they met with and been cleared by Employee Health?
  - This discussion of likely elevated risks and review of proposed participation is critical
    - Do any staff have contraindications with any of the pre- or post-exposure treatment options?
    - Has a suitable treatment been identified for them if they are?
  - Have you documented the proficiency of the staff with the lab protocols and the associated biocontainment practices required to mitigate risks?

Invariably, your biosafety training will cover a lot of Risk Management. Risk Management includes:
The Biosafety Work Practices that will help to reduce or minimize the opportunity for exposure in the lab. The identification of Protective Equipment, which is a combination of:

- The personal protective clothing (lab coats or gowns, gloves—double gloves for higher risk work, face protection, safety glasses or goggles and a mask; and
- All Engineering Controls such as a biological safety cabinet, bench shields, sharps containers, vacuum system filters, biomedical waste containers, etc. that will be used to place a barrier between the biohazard and staff.

The final element of Risk Management is to review all the Places (lab spaces) where this research will be conducted. Verify that all air flows into these laboratories and not the opposite, that all surfaces are easily cleanable, and benches are resistant to the disinfectants and other chemicals that will be used. Examine the impact of foot traffic in these locations and select times where this will be at a minimum when scheduling the times biohazards will be used there.

4.3.3. Risk Assessment and Management Table

Table 3 – Risk Assessment and Management Table
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Risk Assessment</th>
<th>Risk Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Agent classification</td>
<td>• Registration</td>
<td></td>
</tr>
<tr>
<td>• Routes of infection</td>
<td>• Biosafety Office</td>
<td></td>
</tr>
<tr>
<td>• Infectious disease process</td>
<td>• Institutional Biosafety Committee (IBC)</td>
<td></td>
</tr>
<tr>
<td>• Virulence, pathogenicity, quantity, concentration, incidence in community, presence of vectors</td>
<td>• USDA – restricted agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CDC – select agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• FDA/NIH – human gene therapy</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Risk Assessment</th>
<th>Risk Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Aerosol risk: sonicating, centrifuging, homogenizing, blending, shaking, etc.</td>
<td>• Written set of standard operating procedures (SOPs) with safety practices incorporated</td>
<td></td>
</tr>
<tr>
<td>• Percutaneous risk: needles, syringes, glass Pasteur pipettes, scalpels, cryostat blade/knife, etc.</td>
<td>• Adherence to basic biosafety principles</td>
<td></td>
</tr>
<tr>
<td>• Splash/splatter risk: pipetting, microbial loop, etc.</td>
<td>• Label labs, areas, and equipment housing Risk Group 2 or higher agents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Conduct lab inspections to review practices and containment equipment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Use trial experiments with non-infectious material to test new procedures/equipment</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Personnel</th>
<th>Risk Assessment</th>
<th>Risk Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Host immunity</td>
<td>• Safety Training</td>
<td></td>
</tr>
<tr>
<td>• Neoplastic disease</td>
<td>• Prior work experience with biohazards</td>
<td></td>
</tr>
<tr>
<td>• Infection</td>
<td>• Demonstrated proficiency with techniques</td>
<td></td>
</tr>
<tr>
<td>• Immunosuppressive therapy</td>
<td>• Prompt reporting of all exposure incidents, near misses, as well as signs/symptoms of related disease to PI and Occupational Health Provider</td>
<td></td>
</tr>
<tr>
<td>• Age, race, sex, pregnancy</td>
<td>• Investigation/review of incidents/spills, etc. to prevent future occurrence</td>
<td></td>
</tr>
<tr>
<td>• Surgery (splenectomy, gastrectomy, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Diabetes, Lupus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Immunization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Post-exposure prophylaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Attitude towards safety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Comfort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Open wounds, non-intact skin, eczema, dermatitis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Protective Equipment

- **Protection (containment) for:**
  - Aerosols – respirable size particles <5mm
  - Droplets/splatter
  - Sharps

- **Personal protective equipment (PPE):**
  - Respirators – HEPA, N-95, etc.
  - Face (eye nose, mouth) protection – fluid-resistant surgical mask and safety glasses and chin-length face shield
  - Solid front gown or lab coat
  - Gloves
  - Biological safety cabinets
  - Centrifuge safety buckets/rotors

### Place – Laboratory facility

- **Risk group/biosafety level requirements**
  - Aerosol risk
  - Restricted access

- **Basic lab – door, sink, surfaces easily cleaned, eyewash, screens on windows that open**
  - Labels
  - Containment laboratory with directional airflow

---

There are numerous resources available to assist in Risk Assessment for biological hazards. Some are listed here:

- Association of Public Health Laboratories—[A Biosafety Checklist: Developing a Culture of Biosafety](#)
- Centers for Disease Control and Prevention—[Biosafety in Microbiological and Biomedical Laboratories (6th Edition)](#)
- Sandia National Laboratories—[Biorisk Assessment Methodologies](#)
- University of Washington—[Laboratory Risk Assessment Tool](#)

## 4.4. RISK GROUPS AND BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

### 4.4.1. Classification of infectious agents based on hazard (Risk Groups)

Globally, there are several systems in place to classify pathogens based on risk/hazard. In general, these classification systems are based upon the relative pathogenicity of the organism, that is, how hazardous that organism is to humans/animals. For the USA, the current classification system is found in the [American Biological Safety Association Risk Group Database](#). Biological agents are classified into one of four risk groups (RG; RG-1 through RG-4, with RG-4 being the highest hazard). Table 4 lists the basis for classification of these risk groups.

### Table 4. Biological Risk Groups

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Risk to the Individual and the Community</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Group 1 (RG-1)</td>
<td>A biological agent that is unlikely to cause disease in healthy workers or animals</td>
<td>E. coli K12 derivatives, Murine Leukemia Viruses</td>
</tr>
<tr>
<td>Risk Group 2 (RG-2)</td>
<td>Agents that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to</td>
<td>Adenovirus, Hepatitis B virus, Herpes Simplex Virus 1 and 2, Influenza virus,</td>
</tr>
<tr>
<td>Risk Group</td>
<td>Risk to the Individual and the Community</td>
<td>Examples</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Risk Group 3 (RG-3)</td>
<td>Laboratory workers, the community, livestock, or the environment and for which preventative or therapeutic interventions are often available</td>
<td>Listeria monocytogenes, Clostridium tetani, Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Risk Group 4 (RG-4)</td>
<td>Agents that are associated with serious or lethal human or animal disease for which preventative or therapeutic interventions may be available (high individual risk, low community risk)</td>
<td>HIV, HTLV-1, VSV, Prions, Rickettsia, Mycobacterium tuberculosis</td>
</tr>
</tbody>
</table>

4.4.2. Biosafety Levels

Biosafety levels describe the conditions (engineering controls, administrative controls, PPE, and SOPs) under which organisms are manipulated. The following is a brief description of the biosafety levels as defined in the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories; 6th Edition manual 2020 (BMBL). For more detailed information regarding the requirements for the different containment levels, contact the Biosafety Officer or refer to the BMBL (see Table 5 below for a summary of Biosafety 1-4 containment criteria).

4.4.3. Biosafety Level 1

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. Biosafety level 1 (BSL-1) practices, safety equipment, and facilities are appropriate for work that is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi,* and infectious canine hepatitis virus is representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Animal pathogens can infect other susceptible hosts, within same or different animal host species. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains. At BSL-1, basic PPE should be utilized, including lab coat, gloves and eye/face protection when anticipating splashes. Work is performed primarily at the laboratory bench.

4.4.4. Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment. Primary hazards to personnel working with BSL-2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of biohazardous materials. Extreme precaution with contaminated needles or sharp instruments must be
emphasized. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used as appropriate such as splash shields, face protection, gowns, and gloves. Biosafety level 2 (BSL-2) practices, safety equipment, and facilities are applicable for work which is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human or animal disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, the Salmonella, and Toxoplasma spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. This also applies to animal tissues or blood when the presence of an infectious agent is unknown. Personnel working with human-derived materials should refer to the Bloodborne Pathogens Exposure Control Plan for specific, required precautions. Secondary barriers such as hand washing, and waste decontamination facilities must be available to reduce potential environmental contamination. PPE to be utilized at BSL-2 includes lab coat (consider using disposable, waterproof infectious disease gowns), gloves and eye/face protection when anticipating splashes.

4.4.5. Biosafety Level 2+

BSL2+ is not specifically described in written guidance documents, but refers to work done at BSL2, but with enhanced practices. These practices include, but are not limited to: Use of a double-door entry into the laboratory; use of disposable wrap-around gowns instead of lab coats; use of double-gloving of hands; all centrifugation should be conducted in closed containers using sealed buckets; laboratory should have negative pressure with respect to the hallway; sharps use is eliminated (including use of glass); and all waste is autoclaved prior to disposal.

4.4.6. Biosafety Level 3

Biosafety level 3 (BSL-3) practices, safety equipment, and facilities are applicable for work which is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory. PPE for BSL-3 is based on risk assessment, but can include the use of double gloves, disposable Tyvek suits and N-95 respirators or PAPRs.

4.4.7. Biosafety Level 4

Biosafety level 4 (BSL-4) practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents which 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. Additionally,
agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4. Agents in BSL-4 require very specific facilities only available at certain institutions. There are two models for BSL-4 laboratories: 1. A Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC; and 2. A Suit Laboratory—Personnel must wear a positive pressure supplied air protective suit.

Table 5. Summary of Biosafety Levels

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Primary Barriers and Safety Equipment</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause disease in healthy adults</td>
<td>Standard Microbiological Practices</td>
<td>None required</td>
<td>Laboratory bench and sink required</td>
</tr>
<tr>
<td>2</td>
<td>Agents associated with human disease, not transmitted by aerosols in nature</td>
<td>BSL-1 practices plus: Limited access; Biohazard warning signs; Sharps precautions; Lab-specific Biosafety manual required, including defining any needed waste decontamination or medical surveillance policies</td>
<td>Class II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials</td>
<td>BSL-1 plus: Eye wash station; Method for decontaminating waste available</td>
</tr>
<tr>
<td>3</td>
<td>Indigenous or exotic agents with the potential for aerosol transmission</td>
<td>BSL-2 practices plus: Controlled access; Decontamination of all waste</td>
<td>Class II BSCs required for manipulation of agents</td>
<td>BSL-2 plus: Physical separation from access corridors; Self-closing, double-door access; Exhaust air not recirculated; Negative airflow into laboratory</td>
</tr>
</tbody>
</table>

Disease may have serious morbidity and/or mortality
### Table 1: BSL Requirements

<table>
<thead>
<tr>
<th>BSL</th>
<th>Agents</th>
<th>Practices</th>
<th>Primary Barriers and Safety Equipment</th>
<th>Facilities (Secondary Barriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Dangerous/exotic agents which pose high risk of life-threatening disease</td>
<td>BSL-3 practices plus: Clothing change before entering; Shower on exit; All material; decontaminated on exit from facility</td>
<td>Primary barriers: All procedures conducted in Class III BSCs or Class II BSCs in combination with full-body, air-supplied, positive pressure personnel suit</td>
<td>BSL-3 plus: Separate building or isolated zone; Dedicated supply and exhaust, vacuum, and decontamination systems</td>
</tr>
</tbody>
</table>

Determining the actual Biosafety Level that should be utilized for experiments is part of the overall risk assessment (see below) undertaken initially by the PI. For example, HIV is an RG-3 agent, but in normal experimentation, HIV can be handled under BSL-2 levels (if large quantities (>10 liters) of virus are not prepared in the lab).

## 5. ANIMAL BIOSAFETY

### 5.1. BIOSAFETY AND ANIMALS-INFECTIONOUS DISEASE WORK WITH VERTEBRATES

Laboratory facilities must provide containment for laboratory animals exposed to or harboring infectious agents. The containment provided and the biosafety level must be appropriate to the risk level of the infectious agents involved. In addition to facility requirements, special equipment (e.g. filter cages, partial or isolation caging systems) may be used (refer to Table 6 below).

Laboratory animal facilities are simply a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) that is recommended for working with infectious agents *in vivo* and *in vitro* is comparable. However, the animal rooms can present some unique problems. In the microbiological laboratory, hazardous conditions are caused by personnel or by the equipment being used. In the animal room, the activities of the animals themselves can present new hazards. Animals may generate aerosols; they may bite, and scratch and they may be infected with a zoonotic disease.

FAU will follow the animal biosafety guidelines outlined in CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual. 2007 (BMBL). For more detailed information regarding requirements contact your Biosafety Officer or refer to the BMBL. Table 6 summarizes the requirements of Animal Biosafety Levels (ABSL) 1-4.
The BMBL presupposes that laboratory animal facilities, operational practices, and quality of animal care meet applicable standards and regulations (e.g. Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations) and that appropriate species have been selected for animal experiments. In addition, the BMBL assumes that the institution has in place an occupational health and safety program and references the recent publication of Institute of Medicine, **Occupational Health and Safety in the Care of Research Animals**.

All animal work shall be reviewed and approved by the FAU IACUC prior to work beginning. In addition to IACUC approval, all animal work involving biohazardous materials, infectious agents, or acute toxins, that is directed by FAU researchers must be reviewed and approved by the Biosafety Officer and/or the IBC. Other policies and procedures may come into play when doing animal research. Please contact the IACUC Office for more information.

**Table 6. Animal Biosafety Levels**

<table>
<thead>
<tr>
<th>ABSL</th>
<th>AGENTS</th>
<th>PRACTICES</th>
<th>SAFETY EQUIPMENT (PRIMARY BARRIERS)</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
</tr>
</thead>
</table>
| 1    | Not known to consistently cause disease in healthy human adults | Standard animal care and management practices, including appropriate medical surveillance programs | As required for normal care of each species | Standard animal facility  
- No recirculation of exhaust air  
- Directional air flow recommended  
- Hand washing sink is available |
| 2    | Associated with human disease  
- Hazard: percutaneous exposure, ingestion, mucous membrane exposure | ABSL-1 practices plus:  
- Limited access  
- Biohazard warning signs  
- Sharps precautions  
- Biosafety manual  
- Decontamination of all infectious waste and of animal cages prior to washing | ABSL-1 equipment plus:  
- Containment equipment appropriate for animal species  
- Class I, II, III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols  
- PPE:  
  - Laboratory coat, gloves, face, and  | ABSL-1 facility plus:  
- Autoclave available  
- Hand washing sink available  
- Mechanical cage washer recommended |
<table>
<thead>
<tr>
<th>ABSL</th>
<th>AGENTS</th>
<th>PRACTICES</th>
<th>SAFETY EQUIPMENT (PRIMARY BARRIERS)</th>
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</thead>
</table>
| 3    | Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects | ABSL-2 practices plus:  
• Controlled access  
• Decontamination of clothing before laundering  
• Cages decontaminated before bedding removed  
• Disinfectant foot bath as needed | ABSL-2 equipment plus:  
• Containment equipment for housing animals and cage dumping activities  
• 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 |
| 4    | Dangerous/exotic agents that pose high risk of life-threatening disease; aerosol transmission, or related agents with unknown transmission | ABSL-3 practices plus:  
• Entrance through change room where personal clothing is removed, and laboratory clothing is donned  
• Shower on exiting  
• All wastes are decontaminated before removal from facility | ABSL-3 equipment plus:  
• Maximum containment equipment (i.e. Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure suit) used for all procedures and activities | ABSL-3 facilities plus:  
• Separate building or isolated zone  
• Dedicantes supply and exhaust, vacuum, and decontamination systems  
• Other requirements outlined in text |

5.2. Preventing Transmission of Zoonotic Diseases

5.2.1. Risks for Those Who Handle Animals and Their Tissues

Hazards associated with the handling of animals fall into three basic categories:
1. Physical injuries can occur from bites or scratches (rodents, rabbits, dogs, cats, swine, nonhuman primates and others), kicks or other direct injuries. The key to prevention of these types of injuries is proper animal handling training of personnel by the animal care staff or other qualified individuals.

2. Allergic hazards can be associated with breathing or contacting allergens found in animal dander or urine. Though some persons are much more susceptible than others, all employees can reduce their risk by wearing protective clothing (such as safety glasses, respirators, gloves, and a lab jacket) when handling animals. Additional precautions may be posted on the animal room door.

3. There is the potential for transmission of zoonotic diseases between animals and humans. Although zoonotic diseases are not common in modern laboratory facilities, the prevention, detection, and eradication of zoonotic diseases from the animal facility is a primary concern of the entire animal care staff. The risk for zoonotic diseases may be increased in farm situations. Remember that infected tissues, body fluids/secretion/excretion as well as the living animals can frequently be a source for exposure to zoonotic diseases.

5.3. Overview of zoonotic diseases

Humans can be susceptible to infectious diseases that affect animals. Infections of animals may sometimes produce severe disease in humans even when the animals themselves show little, if any, signs of illness. A pathogen in the normal flora of a healthy animal may cause a serious disorder in a person exposed to it because the animal has developed resistance to these microorganisms, whereas humans with no previous exposure to the agent lack this protective immunity. Therefore, one should always be aware of possible consequences when working with each species of animal and take precautions to minimize the risk of infection. If an employee becomes ill with a fever or some other sign of infection, it is important to let the physician know that he/she works with animals.

The FAU Comparative Medicine Department can provide general information regarding work with laboratory animals. Additionally, EH&S publishes an Animal Research Health and Safety Plan and oversees the Medical Monitoring Program for animal researchers. Please visit these websites for additional details.

5.3.1. Special Considerations for Pregnant Employees

Employees who become pregnant (and who work with animals) should contact the FAU Occupational Health Medical Provider as soon as possible for a consultation.

Toxoplasma is an infectious agent found primarily in cat feces and infected meat. It can infect the unborn fetus in women exposed during pregnancy who do not have immunity to the agent. Asymptomatic toxoplasma infection is common before childbearing years and many women have elevated antibody levels indicative of immunity. To help assess the level of immunity against this agent, serum samples can be tested prior to pregnancy. Cat feces should be avoided, and gloves should be worn when working in areas potentially contaminated with cat feces. Thorough hand washing after handling any potential source of infection is also necessary.

Listeriosis, a bacterial disease, can occur in small laboratory animals, farm animals and humans. Stress or pre-existing illness can contribute to susceptibility. Infection can cause acute febrile illness in pregnant women, followed by abortion, stillbirth, or seriously ill premature infants. It can be acquired by
coming in contact with infected fetal membranes and feces, or ingesting milk, especially of stressed animals. It is primarily prevalent in farm animals, including sheep and goats.

5.4. Working with nonhuman primates, their tissues and blood

Nonhuman primates present several unique challenges in biosafety. Because of their size and agility, persons working with them are at a higher risk of exposures through biting, scratching, and contact with bodily fluids. Nonhuman primates also have species-specific viruses that, while being benign to the nonhuman primate, can have significant morbidity and mortality effects on humans. Additionally, depending upon the species, nonhuman primates may be susceptible to infection with several human pathogens, thus can act as a transmitter to persons working with these animals.

5.4.1. Herpes B Virus

Although there are several nonhuman primate agents that can cause disease in humans, Herpes B virus (Macacine alphaherpesvirus 1) is the pathogen of most concern to persons working with macaque species or their tissues.

Herpes B virus is a neurotropic herpes virus indigenous to the macaque species (incl. rhesus, cynomolgus, pig tailed, and stump tailed). B virus infection in macaques is like Herpes simplex virus infection in humans—it can be associated with lesions or, in most cases, is sub-clinical. However, there is no correlation between presentation of lesions and active shedding of virus—macaques without lesions will also shed virus. In humans, herpes B virus presents as an acute ascending myelitis and encephalitis and is usually fatal without early identification and treatment. The greatest risk for infection with B virus is through bites or scratches from macaques. However, it is now clear that mucous membrane exposure to bodily fluids can also result in transmission. Additionally, those working with fresh tissues (especially neural-derived tissues) and cells must also be vigilant in preventing exposures.

5.4.1.1. General Laboratory Requirements

Universal precautions as well as strict adherence to ABSL-2 and BSL-2 practices (at a minimum, depending upon the biohazardous agent being researched) and the use of appropriate PPE are necessary when handling nonhuman primates and their tissues, etc.

5.4.1.2. First Aid Following a Potential Exposure from a Nonhuman Primate

An exposure is defined as: a bite or scratch by a nonhuman primate, laceration or puncture wound caused by potentially contaminated equipment, mucous membrane exposure to potentially contaminated tissues, cells, and OPIM derived from nonhuman primates. It is extremely important that all employees working with nonhuman primates, and materials derived there from, be educated on the proper procedures to follow in the event of an exposure. Following a potential exposure, the most important first step is to immediately wash the site with soap and water for 15 minutes. If you receive an exposure to the eye, immediately go to an eye wash station and flush the eyes for 15 minutes with water. Report the incident to your supervisor and to Occupational/Employee Health as well as the Biosafety Office.

5.5. Work with dogs or cats
Dogs and cats used in long-term studies may be vaccinated against rabies. Check with the Attending Veterinarian. Rabies vaccinations are provided to employees upon recommendation of the Occupational Health medical provider.

Some dog and cat parasites are a potential risk to those handling infected animals. Examples include some roundworms, tapeworms, hookworms, and mange mites. Ringworm, a fungal disease of dogs and cats, is also readily transmitted to humans. Cat Scratch disease is a zoonotic infection characterized by regional lymph node infection that can follow a scratch, bite or primary lesion caused by a cat. The agent involved is a *Bartonella* sp. While the prognosis is usually excellent and the disease in most cases is self-limiting, employees must report an infection or possible infection.

5.6. **Work with farm animals (e.g., sheep, swine)**

Q fever, a potentially serious human disease caused by the rickettsia, *Coxiella burnetti*, was formerly quite common in those drinking unpasteurized milk and in slaughterhouse workers exposed to freshly slaughtered ruminants (cattle, sheep, and goats). It is known that the organism is shed from the placental membranes of sheep and goats. It can also be acquired by ingesting milk from infected animals. This route of exposure has been the cause of Q fever pneumonia and other associated symptoms in laboratory workers. Unless known to be free of the rickettsia, you should assume sheep to be infected and all personnel working where exposure is possible should take suitable precautions. Gloves, safety glasses, a mask and protective clothing are required for individuals working with pregnant sheep and goats. Infected persons can be effectively treated.

Erysipelas in swine can be transmitted to humans causing a severe local skin infection. Therefore, swine showing diagnostic "diamond back" lesions should be handled with care. Swine are also hosts for influenza that can be transmitted to humans (Swine flu).

Similar in appearance, though less severe than erysipelas, skin lesions are also seen on the hands after contact with sheep and goats infected with contagious ichthyoma and vesicular stomatitis virus. Rabies can also be a threat from any unvaccinated cat or dog, or food animal, especially those on pasture or exposed to feral animals.

Cattle from commercial farms may be asymptomatic carriers of salmonella, campylobacter, toxigenic *E. coli* (O157:H7), and Cryptosporidia. These organisms are present in feces and some may also be shed in the milk. Calves with diarrhea may be shedding some of these organisms in high numbers.

Commercial swine may also carry salmonella and campylobacter. Aborted fetuses from swine and cattle, sheep and goats may be associated with several zoonotic pathogens such as Brucellosis, Leptospirosis, or Q fever. Aborted fetuses should be handled with extreme care and appropriate PPE (boots, mask, Tyvek coveralls, and gloves). *Baylisascaris procyonis* (raccoon large roundworm) is found wherever raccoons are found. This roundworm causes a highly pathogenic visceral larval migrans that is untreatable. Avoid contact with raccoon feces. If a raccoon latrine is found in a barn (haystack), use extreme caution. Use respirator, gloves, Tyvek suit, and boots to remove feces from area and burn it. Heat is the only way to kill the eggs.

5.7. **Work with rodents**

Contact with rodents requires precautions against such diseases as tapeworm infection, lymphocytic choriomeningitis virus (LCMV), salmonellosis and ringworm fungal skin infections. Additional concerns for
investigators using some rodents are leptospirosis and bubonic plague. Specific attention should also be focused on the possibility of allergic reactions to rodent dander and urine. Care should be taken to limit exposure to soiled bedding as these can contain excreted chemical or biological hazards that have been given to the rodents and may be excreted in feces and urine (be sure to review the IACUC hazard forms on file for the specific study). Use of laminar flow, HEPA-filtered dump stations for bedding disposal is a first line of engineering control that should be utilized. Gloves, safety glasses and respirators not only limit exposure to soiled bedding, but also help prevent transmission of diseases through handling of rodents.

6. EXPOSURE CONTROL MEASURES

6.1. The Hierarchy of Controls.

Lab supervisors and primary supervisors are responsible for ensuring that control measures are in place to reduce employee exposure to biohazards. The hierarchy of control defines, in a preferred order, an approach to selecting exposure controls. It considers the reliability of various control approaches and the principles of good occupational hygiene practice. The hierarchy of controls is illustrated in the figure below:

![Hierarchy of Controls Diagram]

In summary, the hierarchy of control indicates that total elimination of the hazard is the preferred option, followed by substitution with a less hazardous material. The hierarchy goes on to list various engineering-, procedural- and PPE-based control solutions in order of reliability, the general principle being to achieve control of hazards at the source in preference to measures closer to the worker, such as PPE. Numerous, slightly different versions of the hierarchy exist, but all follow the same basic principles. A common element is the inclusion of the STOP principle (Substitute the substance or process, Technical controls, Organizational measures, Personal protective equipment - PPE). It is unusual that a single control measure will be practical and effective, and a range of controls used in combination usually offers the best solution.

There is a tendency to adopt strategies which rely heavily on PPE when controlling dermal exposure. This is incorrect. The risk management strategy for dermal exposure should follow the same philosophy as that for inhalation exposure. The hierarchy of control applies equally to all exposure routes.

6.2. Engineering controls
Engineering controls can be described as a physical modification to a process, or process equipment, or the installation of additional equipment with the goal of preventing the release of contaminants into the workplace. Examples include biological safety cabinets, fume hoods, glove boxes, and local exhaust/ventilation. Engineering controls are the preferred primary control measure and should be utilized first to control exposures to hazards in the lab. Listed below are some engineering controls routinely used for biological hazards in the lab:

6.2.1. Ventilation

Ventilation Controls are engineering controls intended to minimize employee exposure to infectious agents, hazardous chemicals, or toxic substances by removing air contaminants from the work site. There are two main types of ventilation controls:

A. General (Dilution) Exhaust is where you have a room or building-wide system which supplies air from the outside and removes it at the same rate. Laboratory air is to be continually replaced, at a rate that prevents the concentration of toxic substances. General exhaust systems alone are inadequate for RG-3 agents or BSL-3 work.

B. Local Exhaust or Filtration: a ventilated, enclosed workspace intended to capture, contain, and exhaust or filter harmful or dangerous fumes, vapors, and particulate matter. In the case of hazardous chemicals this includes a fume hood. In the case of infectious agents, biosafety cabinets should be used.

Other ventilation controls include the use of single-pass air, increased number of air changes, and the maintenance of negative pressure inside laboratories relative to outside corridors. All these controls provide for the mitigation of agent escape from the laboratory.

6.2.2. Biological Safety Cabinet (BSC)

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. BSCs provide primary containment when working with biohazardous agents. Three kinds of biological safety cabinets (BSCs), designated as Class I, II and III have been developed to meet various research and clinical needs. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems and are intended to be used when handling infectious, toxic, or sensitizing materials.

BSCs should not be confused with other laminar flow devices or "clean benches"; in particular, horizontal flow cabinets, which direct air towards the operator. These benches protect the product but do not protect the operator. Laboratory personnel should be trained in the correct use and maintenance of biological safety cabinets to ensure that personnel and product protection (where applicable) is maintained.

When properly used in research involving the manipulation of biohazardous agents, biological safety cabinets are effective in containing and controlling particulates and aerosols and complement good laboratory practices and procedures. The correct location, installation, and certification of the biological safety cabinet are critical to containing infectious aerosols.
All BSCs shall be inspected annually and certified by trained and accredited service personnel according to the NSF (National Sanitation Foundation) Standard 49, Annex F. Inspection and re-certification is required if the cabinet is relocated or after major repairs, filter changes, etc.

For general guidance on the safe and effective use of BSCs refer to the CDC\NIH document Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets available in Appendix A of the BMBL 6th edition

NOTE: Before selecting any BSC for purchase, contact the Biosafety Officer for assistance with work-specific assessment and selection criteria.

A brief description of the different types of biosafety cabinets is as follows:

6.2.2.1. CLASS I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface and personnel protection are provided by this inward airflow. With the product protection provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures, or homogenizing tissues) with a potential to generate aerosols.

6.2.2.2. Class II BSC

The Class II BSC provides personnel, environmental and product protection. Airflow 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. HEPA filtering of exhaust air provides environmental protection.

The Class II cabinet has five designs (A1, A2, B1, B2 and C) that differ in the amount of air that is recirculated and/or exhausted, and whether the BSC is hard ducted to the ventilation system.

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. Class II cabinets also provide the microbe-free work environment necessary for cell culture propagation. Certain types of BSC may also be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs. Care must be exercised when selecting the correct Class II cabinet design for these purposes. (While hard-ducted biosafety cabinets (B1 and B2) can be used for small amounts of toxic chemicals, they cannot be used in lieu of a chemical fume hood). The Biosafety Officer should be consulted to aid in the selection of the correct biosafety cabinet.

6.2.2.3. Class III BSC

The Class III BSC is designed for work with Risk Group 4 microbiological agents in a BSL-4 containment lab and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing that
process allows for safe removal of materials from the cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (usually about 0.5 inches of water pressure).

6.2.2.4. Biological Safety Cabinet Function and Operation

Biological safety cabinets (BSCs), when used properly, provide a clean work environment for research. Biological safety cabinets offer personnel, product, and environmental protection. The BSC provides primary containment for infectious materials. The efficacy of BSCs depends upon the behavior of the operator and the orientation of the unit in the facility.

The BSC isolates biohazards from personnel by confining the biohazardous material in the unit. The BSC removes aerosolized biohazardous material by moving air through high efficiency particulate air (HEPA) filters. The intake air is filtered through a HEPA filter before entering the BSC work area. Exhaust air also passes through a HEPA filter. Thus, aerosols generated in the work area of the BSC are contained within the BSC.

Operating Procedures for Class II Biological Safety Cabinet:

- If used, turn off UV light; turn on fluorescent light and blower.
- Let blower run for at least 5 minutes.
- Disinfect all interior surfaces with a suitable disinfectant, followed by 70% ethanol to avoid damaging the stainless steel.
- Gather items needed for your work and place them into cabinet; do not obstruct grills.
- Keep materials at least 4 inches inside work area.
- Work should proceed in a linear flow from clean to contaminated areas.
- After procedure, allow cabinet to run 2-3 minutes before removing all materials.
- Wipe down all work surfaces with a suitable disinfectant, followed by 70% ethanol.
- Turn off fluorescent light and blower if desired.

Many BSCs are equipped with germicidal ultraviolet (UV) lamps. Time of exposure, distance, presence of dust or debris and UV lamp intensity significantly affect the germicidal effect of the UV lamp. The visible blue-violet glow of the UV lamp does not necessarily indicate there is germicidal effect. The UV lamp needs to be cleaned periodically to remove dust. UV lamps may damage eyes, skin, and laboratory equipment. UV lamps should be turned off while the room is occupied.

**The Biosafety Office discourages the use of UV lamps due to the potential damage resulting from UV lamp use and the unpredictability of sufficient energy emitted from the UV light to have a disinfecting effect.**

6.2.2.5. Bunsen Burners and Loop Sterilizers in the BSC

**Bunsen burners are not permitted for use inside Biosafety Cabinets.** Continuous flame gas burners shall not be used in BSCs. These burners can produce turbulence, which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter. Sterilization of inoculating loops or needles in an open...
flame generates small particle aerosols, which may contain viable microorganisms. The use of a shielded electric incinerator (micro-sterilizer or Bacti-cinerator) or hot bead sterilizers minimizes aerosol production during loop sterilization. These can be safely utilized in a BSC. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

6.2.3. Chemical Fume Hoods

Chemical Fume Hoods are an important engineering control used to prevent exposure to hazardous materials. In conjunction with sound laboratory techniques, a chemical fume hood serves as an effective means for capturing toxic, carcinogenic, offensive, or flammable vapors or other airborne contaminants that would otherwise be released to the general laboratory atmosphere. Chemical fume hoods should not be used with biological materials as they do not afford the same protection as a BSC. Similarly, a BSC should not be used as a chemical fume hood substitute.

6.2.4. Other Safety Equipment

6.2.4.1. Safety showers

Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are outlined in the Chemical Hygiene Plan.

6.2.4.2. Eyewash stations

Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially biohazardous materials. Standards for location, design and maintenance of emergency eyewash facilities are outlined in the Chemical Hygiene Plan.

6.2.4.3. Handwashing sinks

Laboratory workers should have access to handwashing facilities for hygiene purposes. While gloves should always be used when working in the labs, it is important that workers frequently wash their hands during the day. Hands must be washed when gloves are removed and after any potential exposure. Hands should also be washed prior to leaving the laboratory.

6.3. Administrative controls

Administrative Controls are methods of controlling employee exposures to infectious agents by adherence to appropriate work practices and by written procedures or policies. Examples include standard operating procedures or programs, training, signage, manuals and guidance documents.

At times unique programs, standard operating procedures or guidelines are required to address situations or achieve regulatory compliance. Programs like Waste Disposal, Bloodborne Pathogens Exposure Control Plan, Institutional Biosafety Committee review, and CDC Select Agents are examples of such programs.

6.3.1. Laboratory Biosafety Manual
Laboratories conducting research utilizing Risk Group 2 agents or higher are required to assemble a laboratory biosafety manual that is specific for their laboratory. The 5th edition of the BMBL requires that the following be included in a laboratory biosafety manual:

- Lab-specific biosafety policies (e.g. hand washing, food storage, restricted access, daily surface decontamination procedures, emergency spill and clean-up procedures, use of PPE, etc.).
- Project-specific safety SOPs
- Fact sheets on organization safety policies (e.g. Lab sharps policies, Disposal policies, etc.)
- Exposure control plans
- Relevant sections of the BMBL regarding agents utilized in the laboratory
- Documentation of training for all laboratory personnel
- Copies of IBC permits and Inspection Results

6.3.2. Training

Training requirements are important administrative controls instituted to minimize or prevent exposures to biohazards. Section 1.7.7 above outlines the training requirements for working with potentially biohazardous agents. Training must be completed prior to working in the laboratory and some training requires annual updates.

6.3.3. Waste Handling and Disposal

Labs can generate biohazardous waste and hazardous chemical waste that requires decontamination and/or disposal. All waste disposal should be coordinated through FAU’s Environmental Health and Safety Office. Procedures for waste handling and disposal are contained elsewhere in this biosafety manual (see section 7.1, Decontamination and Disposal Methods).

6.3.4. Signs and Labels

Signs and labels are to be posted where biohazardous materials requiring containment at BSL2 or higher are used and/or stored. The information on the sign or label will vary depending on the use of the sign or label.

6.3.5. General Labeling and Signage Requirements – Hazard Communication

The following guidance is to be used when determining where biohazard signs and labels are to be used in laboratory and/or storage areas:

1. All laboratories where biohazards are stored or used are clearly labeled using the laboratory safety plaques (front door signs).

2. All other rooms and storage areas are clearly labeled with either the new laboratory safety plaques or other biohazard signage.

3. All biosafety cabinets where biohazards are used are clearly labeled.
4. All refrigerators, freezers, centrifuges, and incubators where biohazards are used are labeled.

5. All other pieces of equipment are evaluated by the laboratory occupants and assessed for risk. Items will be labeled with the biohazard symbol if they are at risk of being contaminated during laboratory activities and are either:
   A. Being moved from the work area without adequate disinfection or decontamination, and/or.
   B. Being serviced in-place by Institutional or third-party serviceperson.

6. Pieces of equipment that do not fit these categories or risks do not need to be labeled.

7. Any equipment items that leave the laboratory for service or disposal must be handled according to the institutional policy on biohazard/decontamination tagging.

6.3.5.1. Biohazard Warning Sign

The design specifications of the universal biohazard symbol were established in 1966 and the same specifications are still used today. The colors used on biohazard labels can vary by country; in the United States, the biohazard label has an orange background with the biohazard symbol and the word “Biohazard” printed in black.

A biohazard label is required for all areas or equipment in which RG-2 or RG-3 agents are handled or stored, or where BSL-2 or BSL-3 procedures are performed. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, on equipment such as refrigerators/freezers that contain hazards, incubators, and transport containers. See below for an example of the biohazard symbol:

![Biohazard Symbol]

6.3.5.2. Entryway Signs

Door Signs

An EHS door sign must be posted at the entryway to all laboratories. Door signs provide the following information to those entering the lab, including emergency responders such as police and fire personnel:

- Building and room number
- Name and phone number of all PIs using the room
- Name and phone number of research personnel knowledgeable about the materials used in the space
- Areas where biohazards requiring BSL2 or higher containment are used and/or stored
- Symbols indicating types of chemicals used in the space
• Symbols indicating radioactive materials are used in the space (if appropriate)
• PPE requirements to enter the lab
• A list of emergency contact personnel

6.3.5.3. Animal Rooms

The entrance to individual animal rooms must be posted with a Biohazard Sign when a study involving infectious/biohazardous substances is in progress. The sign should contain information about what infectious substance(s) is(are) being utilized in the room. Door signs can be formatted and printed from the SciShield platform.

6.3.5.4. Labeling and Storage of Biological Materials

The contents of all laboratory containers shall be properly identified. One of the overriding goals of prudent practice in the labeling and identification of materials is to avoid orphaned containers of unknown materials. The labels should be understandable to laboratory workers, members of emergency response teams, and others.

Sample Container Labeling and Storage Requirements:

1. All containers and/or racks are to be clearly labeled to identify the contents. Secondary containment should be considered where appropriate. If there is a large quantity of smaller containers of the same agent, labeling of the storage container, tray or cupboard will suffice.

2. If volatile materials are used (flammable, explosive potentials), they must be stored in equipment that is designed for this purpose (e.g. lab safe refrigerators).

3. Personal items, e.g., food and beverages, are ABSOLUTELY prohibited in lab refrigerators, cold rooms, freezers, or incubators.

6.3.5.5. Labeling Equipment Sent Out for Repair or Disposal

Contaminated and potentially contaminated equipment sent out for repair or disposal must be decontaminated as thoroughly as possible. Affix a Decontamination Tag (shown in Appendix B) to the equipment indicating when the equipment was decontaminated, what disinfectant was used, and the name of the person who performed the decontamination. Thorough decontamination of highly technical or sensitive equipment or equipment with limited access to contaminated areas may not be possible. Decontaminate the equipment to the degree possible (flushing lines and/or wiping down the exterior) and affix a Biosafety Notice to the equipment, be sure to indicate which area(s) of the equipment could not be decontaminated. Place a biohazard label on the equipment indicating the name of the biohazardous material that remains and the location of the contaminated area. The label must convey this information to all affected workers (service representatives, manufacturer, etc.).

6.3.6. Laboratory Practices

6.3.6.1. Human Factors and Attitudes in Relation to Laboratory Accidents
For the purposes of safety, an attitude can be defined as an accumulation of information and experience that predisposes an individual to certain behavior. Human factors and attitudes result in tendencies on the part of the individual to react in a positive or negative fashion to a situation, a person, or an objective. Laboratory supervisors and Principal Investigators should understand the importance of attitudes and human factors in their own efforts to control biohazards in their laboratory. Some observations that may be of help to supervisors are listed below:

- The lack of accident perception ability is often a significant factor in laboratory accidents.
- Inflexibility of work habits, that tend to preclude last minute modification when an accident situation is recognized, plays a part in the causation of some laboratory accidents.
- Working at an abnormal rate of speed is a significant causal factor.
- Intentional violations of regulations are a frequent cause of accidents. This is termed excessive risk taking.
- The performance of routine procedures such as diluting and plating cultures is the most frequent task being performed at the time of laboratory accidents.
- Working when one is very tired is more likely to create a higher potential for accidents.
- Working at a well-organized and uncrowded laboratory bench will help in the prevention of lab accidents. Each employee working with biohazardous agents must be consistently aware of the importance of the proper attitude in preventing accidents in the laboratory.

The following are basic procedures that should be followed in laboratories with the indicated Biosafety Levels:

**Biosafety Level 1**

- Keep laboratory door closed when experiments are in progress.
- Use procedures that minimize aerosols.
- Do not smoke, eat, drink or store food in BSL1 areas.
- Wear laboratory gowns or coats when appropriate.
- Do not mouth pipette. Use mechanical pipetting devices.
- Avoid using hypodermic needles.
- Wash hands after completing experimental procedures and before leaving laboratory.
- Disinfect work surfaces daily and immediately after a spill.
- Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
- For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
- Control insect and rodent infestations.
- Keep areas neat and clean.

**Biosafety Level 2**

- Keep laboratory door closed.
- Post a universal biohazard label on equipment where infectious agents are used/stored.
- Allow only persons informed of the research to enter BSL2 areas.
• Keep animals not used in BSL2 experiment out of the laboratory.
• Do not smoke, eat, drink, store food or apply cosmetics in BSL2 areas.
• Wear PPE (laboratory gowns or coats, gloves, and full-face protection) when appropriate; do not wear PPE outside of the laboratory.
• Wash hands after removing PPE as well as before leaving laboratory.
• Change PPE when soiled or compromised.
• Do not mouth pipette. Use mechanical pipetting devices.
• Use procedures that minimize aerosol formation.
• Avoid using hypodermic needles.
• Substitute plastic for glass where feasible.
• Use biological safety cabinets to contain aerosol-producing equipment.
• Wash hands after completing experimental procedures and before leaving laboratory.
• Disinfect work surfaces daily and immediately after a spill.
• Maintain a biological spill kit within the laboratory.
• Report spills, accidents, near misses and disease symptoms related to laboratory acquired infection to the PI.
• Ensure that all biomedical waste containers are labeled with the biohazard symbol.
• Decontaminate all biological wastes before discard. Decontaminate other contaminated materials before washing, reuse, or discard.
• For off-site decontamination, package contaminated materials in closed, durable, leakproof containers.
• Control insect and rodent infestations.
• Keep areas neat and clean.

Biosafety Level 2+

Biosafety level 2+ (BSL2+) is the designation utilized for those biohazard experiments that require practices that are more stringent than standard BSL2 procedures. Generally, BSL3 practices are mandated in a space designed for BSL2 work. It is preferred that the BSL2 laboratory be self-contained with all equipment required for the experiment located within the laboratory. A biohazard door sign listing the agent in use, emergency contact, and entry requirements is posted on the door while BSL2+ work is in progress and access is restricted to those involved in the experiment. When work is completed and equipment has been decontaminated, the sign is removed, and the laboratory is returned to standard BSL2 use.

All manipulations of BSL2+ materials are conducted in a class II biological safety cabinet and secondary containment is utilized for centrifugation and other potential aerosol generating procedures.

Please consult the Biosafety Office prior to initiating any work at BSL2+.

6.3.6.2. Guidance for Common Practices

The following information is provided as general guidance for common practices and procedures occurring in biological laboratories:

A. Cell Culture:
   • Wear a lab coat and gloves when working in the biosafety cabinet.
• Glassware and other contaminated items should be disinfected or autoclaved before washing, reuse or disposal.
• Cell culture wastes must be decontaminated.
• Maintain a clean lab coat reserved solely for cell culture work.
• Avoid talking during culture manipulations as aerosols may be drawn into the work area.
• Place pipettes on a rack to avoid disrupting airflow when removed.
• Keep open tubes parallel to the airflow.
• After transferring inoculum always recap vials.
• Do not place tubes on work surface.
• Discard empty tubes immediately.
• Work with one specimen at a time; recap before going to the next.
• Autoclave verification should be performed routinely.

B. Transport of Biological Samples on Campus

The transportation of biological or clinical samples on campus must utilize at least primary and secondary containment devices to ensure safety. Please refer to the Guidelines for Transport of Biological Samples, Appendix C in this document, for detailed guidance on sample transport.

C. General Microbiological Practices

When pipetting, use the following precautions:

• Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.
• Use disposable plastic pipettes instead of glass pipettes.
• Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. If pipetting is done on the open bench, use absorbent pads or paper on the bench.
• Respiratory protection may need to be considered depending on the agent in use.
• Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
• Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette, which create aerosols.
• Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
• Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
• Avoid accidentally dropping hazardous material from the pipette onto the work surface. Place a disinfectant dampened towel or other absorbent material on the work surface, and autoclave before discard or reuse. Plastic backed bench paper is suitable for this purpose.
• Place discard pans for used pipettes within the biosafety cabinet.
• Contaminated pipettes should be placed horizontally into a pan or tray containing enough suitable disinfectant, such as hypochlorite, to allow complete immersion of the pipettes. Pipettes should not be placed vertically in a cylinder that, because of its height, must be placed on the floor outside the biosafety cabinet. Removing contaminated pipettes from the biosafety cabinet and placing them vertically in a cylinder provides
opportunity for dripping from the pipette onto the floor, or the rim of the cylinder, thereby creating an aerosol, and the top of the pipettes often protrude above the level of disinfectant.

- After suitable contact time, excess disinfectant can be carefully poured down the sink. The pan and pipettes can be autoclaved together and replaced by a clean pan with fresh disinfectant.

D. Syringes and Needles

The hypodermic needle is a dangerous instrument. To lessen the chance of accidental injection, aerosol generation, or spills, the use of syringes should be avoided when alternate methods are available. For example, use a blunt needle or cannula on the syringe for oral or intranasal inoculations and never use a syringe and needle as a substitute for a pipette in making dilutions.

The following practices are recommended for hypodermic needles and syringes when used for parenteral injections:

- Use the syringe and needle in a biological safety cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
- Examine glass syringes for chips and cracks, and needles for barbs and plugs. This should be done prior to sterilization before use. Use needle-locking syringes only and be sure that the needle is locked securely into the barrel. Replace glass syringes with plastic disposable syringes whenever possible.
- Whenever possible use safety-engineered needle systems.
- Wear latex gloves for all manipulations with needles and syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Expel excess air, liquid, and bubbles from a syringe vertically into a cotton pledget moistened with an appropriate disinfectant, or into a small bottle of sterile cotton.
- Do not use the syringe to forcefully expel a stream of infectious fluid into an open vial for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the syringe is held below the surface of the fluid in the tube.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in the transfer of infectious material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with an appropriate disinfectant. If there is concern of the disinfectant contaminating sensitive experimental materials, a sterile pledget may be used and immediately discarded into a biohazard bag.
- When injecting materials into animals, position the hand that is holding the animal “behind” the needle or use a pair of forceps to hold the animal to avoid puncture wounds.
- Be sure the animal is properly restrained prior to the inoculation and be on the alert for unexpected movements of the animal.
- Before and after injection of an animal, swab the injection site with an appropriate antiseptic.
- Discard syringes into appropriate sharps container. **DO NOT** bend, shear, recap or otherwise manipulate the needle. If recapping is unavoidable, use a one-handed scoop method or use a mechanical device. **DO NOT** discard syringes into a biohazard bag.
E. Sharps usage

In addition to needles/syringes, other sharps are often utilized in the laboratory, such as scalpels, blades, Pasteur pipets, etc. Laboratory personnel, along with PIs, should make every effort to find suitable substitutes for sharps instruments. For example, instead of using scalpels to generate single cell suspensions from tissues, one can use a tissue dissociator. It is important to minimize (and, if possible, eliminate) sharps usage, or employ the usage of safety-engineered sharps devices, when working with biohazardous agents.

F. Culture Plates, Tubes and Bottles

In the absence of definite accidents or obvious spillage, it is not certain that the opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents some infections have occurred by this means. Care is required when opening plates, tubes, or bottles containing fungi, for this operation may release many spores. Such cultures should be manipulated in a biological safety cabinet.

To assure a homogenous suspension that will provide a representative sample, liquid cultures are agitated before a sample is taken. Vigorous shaking will create a heavy aerosol. A swirling action will generate homogenous suspension with a minimum of aerosol. When a liquid culture is re-suspended, a few minutes should elapse prior to opening the container to reduce the aerosol.

The insertion of a sterile, hot wire loop or needle into a liquid or slant culture can cause spattering and release of an aerosol. To minimize the aerosol production, the loop should be allowed to cool in the air or be cooled by touching it to the inside of the container or to the agar surface where no growth is evident prior to contact with the culture of colony. Following use of inoculating loop or needle, it is preferable to sterilize the instrument in an electric or gas incinerator specifically designed for this purpose rather than heating in an open flame. These small incinerators have a shield to contain any material that may spatter from the loop or needle. Disposable inoculating loops are available commercially. Rather than decontaminating them immediately after use with heat, they are discarded first into a disinfectant solution.

The practice of streaking an inoculum on rough agar results in aerosol production created by the vibrating loop or needle. This generally does not occur if the operation is performed on smooth agar. It is good safety practice to discard all rough agar poured plates that are intended for streaking purposes with a wire loop.

Water of syneresis in Petri dish cultures usually contains viable microorganisms and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when opening the plate breaks this film. Vented plastic Petri dishes, where the lid touches the rim at only three points, are less likely to offer this hazard. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent dispersal. If plates are obviously wet, they should be opened in the biological safety cabinet.

Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of contaminated liquid, which may collect between the rim and the liner, is broken during removal of the closure. The practice of removing cotton plugs or
other closures from flasks, bottles, centrifuge tubes, etc., immediately following shaking or centrifugation can generate aerosols and cause environmental contamination. The technique of shaking tissue cultures with glass beads to release viruses can create a virus-laden aerosol. Removal of wet closures, which can occur if the flask or centrifuge tube is not held in an upright position, is also hazardous. In addition, when using the centrifuge, there may be a small amount of foaming and the closures may become slightly moistened.

Because of these possibilities, it is good safety practice to open all liquid cultures of infectious or hazardous material in a biological safety cabinet wearing gloves and a long-sleeved laboratory garment. Dried, infectious culture material may also collect at or near the rim or neck of culture tubes/flasks and may be dispersed into the air when disturbed. Containers of dry powdered hazardous materials should be opened in a biological safety cabinet.

G. Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened an aerosol may be created.

Aerosol creation should be prevented or minimized; opening of ampoules should be done in biological safety cabinets. When recovering the contents of an ampoule, care should be taken not to cut the gloves or hands or disperse broken glass into eyes, face, or laboratory environment. In addition, the biological product itself should not be contaminated with foreign organisms or with disinfectants. To accomplish this, work in a biological safety cabinet and wear gloves. Nick the ampoule with a file near the neck. Wrap the ampoule in disinfectant wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, at the file mark on the neck of the ampoule, apply a hot wire or rod to develop a crack. Then wrap the ampoule in disinfected wetted cotton and snap it open. Discard cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling and withdraw the contents into a fresh container. Some researchers may desire to use commercially available ampoules pre-scored for easy opening. However, there is the possibility to consider that this may weaken the ampoule and cause it to break during handling and storage. Ampoules of liquid cultures are opened in a similar way.

Ensure that all hazardous fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, unbreakable leakproof secondary containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel. The secondary container must be labeled with a biohazard label bearing the name of the infectious material.

7. PERSONAL PROTECTIVE EQUIPMENT (PPE)

Multidisciplinary research conducted in FAU laboratories requires that personal protective equipment (PPE; protective clothing and safety apparatus/equipment) be used to protect the researcher from contact with infectious, toxic, and corrosive agents, excessive heat, cold, fire and other physical hazards. Suitable PPE also helps to protect the experiment/material from contamination by the researcher. The extent and kind of clothing and equipment to be selected for any activity depends upon the research operations and levels of risk associated with the research. While PPE is an important component of any biological safety program, it is used
with the understanding that PPE serves as a second line of defense. Good laboratory techniques, procedures and appropriate laboratory equipment are the primary barriers against potential exposure to hazardous agents.

For additional information you are urged to consult the Biosafety Office. We can offer guidance on selection of PPE and help with locating specific PPE items of interest for the laboratory.

7.1. Laboratory Clothing

Laboratory clothing serves to protect the wearer, the experiment, and environment against contamination. If proper precautions are not taken, contaminated clothing may carry infectious materials outside the laboratory and into other work areas, cafeterias, or the home. Infectious agents can remain viable on cotton and wool fabrics and be disseminated from these fabrics.

It is important that lab workers also wear laboratory-friendly street clothing. No shorts, short skirts, or open-toed shoes should be worn in the lab.

Both reusable and disposable clothing is available. Whichever is used, it must be durable, designed to provide protection and prevent exposure of the skin to harmful agents, as well as be compatible with the methods of decontamination employed.

Some additional points:

- Overt exposure to agents at all levels of risk should be followed by immediate decontamination of the PPE (if reusable) or disposal, and then a change into clean PPE to protect the worker, the experiments, and the environment.
- Provisions should be made for PPE to be provided to visitors and maintenance or security personnel, if applicable.
- PPE worn within the laboratory should not be worn outside the facility to the library, cafeteria, or other places accessible to the public.
- Personnel should be encouraged to use disposable facial tissues instead of personal handkerchiefs.
- PPE should be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.
- All reusable PPE should be decontaminated before being sent to the laundry. Treat contaminated areas of PPE with an appropriate disinfectant. Lab coats with extensive contamination may be placed in a biohazard bag and autoclaved.
- DO NOT take PPE home to launder; select a laundry service that follows universal precautions.
- Change PPE as soon as feasible whenever it is compromised, soiled, or torn.
- Wear appropriate sizes and keep an adequate supply of PPE available in the laboratory.
- Wash hands whenever PPE is removed.
- Do not touch door handles, elevator buttons, telephones, computers or other clean surfaces or items with gloved hands.
- Wear closed-toe shoes and long pants to guard against skin contamination or chemical exposure. Do not wear sandals or shorts in the laboratory.
7.2. Gloves

Gloves should be comfortable and of sufficient length to prevent exposure of the wrist and forearm. Depending upon intended use, the composition and design of the glove may vary to provide the desired level of flexibility, strength, impermeability, and resistance to penetration by sharp objects, as well as protection against heat and cold. Quality assurance is an important consideration.

No one glove can be expected to be satisfactory for all intended uses. Gloves may be fabricated of cloth, leather, natural and synthetic rubbers, or plastics. New formulations of synthetic rubber and plastic continue to be developed as research makes varied and changing demands on the protective capabilities of gloves. Changing applications lead to improved capabilities of impermeability, strength, flexibility, tactile sense, and control. Within even the modest laboratory, the glove applications may be such that no less than four or five types of protective gloves need to be stocked and used.

Disposable (single use) gloves provide a barrier between infectious agents and the skin. Glove use is a basic precept of preventing infectious agent transmission. Breaks in the skin barrier of the hand (damaged cuticles, scrapes, micro-cuts, dermatitis, etc.) are common.

Gloves shall be removed, and hands washed before exiting the laboratory. Use the one glove method, or an appropriate secondary container, when transporting materials through common use areas.

The Biosafety Office can provide information on gloves needed for various tasks, such as working with animals, dry ice, heat, acids, etc. Consult OEHS with details of your work to receive further information about the type and availability of gloves that will best meet your requirements.

Considerations for the selection and use of gloves:

- Gloves are not 100% leakproof; change gloves periodically and when soiled and always wash hands after removing gloves or other PPE.
- Gloves will not prevent needle sticks or other puncture injuries.
- Check gloves for visible tears before use.
- Avoid wetting examination gloves as water or disinfectants will encourage wicking and leaking
- Do not reuse examination gloves; discard contaminated gloves in a biohazard bag immediately after use.
- Double glove or use household utility gloves when cleaning spills. Household utility gloves may be decontaminated and reused (replace when compromised.)

Procedure for removing gloves:

Grip the outside of one glove at wrist with the other gloved hand, pull glove off and gather in palm of gloved hand. Place index or middle finger of the ungloved hand on wrist of gloved hand, slide finger under the glove opening and pull glove off inside out.

When removing PPE, remove lab coat or solid front gown first, then remove gloves (aseptically), remove face protection last to avoid touching your face with contaminated hands. If wearing double gloves, remove outer gloves before removing lab coat or solid front gown.

7.3. Shoes

Shoes worn in the laboratory must be closed toe. Protective shoes are required for certain work activities.
When working with infectious agents it can be advantageous to wear disposable shoe covers over street shoes. For work in tissue culture laboratories it may be necessary to change from street shoes to specific laboratory shoes for protection of cultures from contamination.

7.4. Gowns, Lab Coats, Jumpsuits, Aprons, and Other Protective Clothing

Gowns, lab coats and jumpsuits protect the wearer’s clothing and skin from contamination. As with all PPE, the type of clothing needed is dependent upon a proper risk assessment and of the task being performed and the degree of potential exposure anticipated.

Solid front wrap-around clothing (ex. Disposable infectious disease gowns) offers better protection than pull-over type clothing or clothing with front closures, such as lab coats. Lab coats are not 100% leakproof; one should always change PPE when soiled, and always wash your hands after removing any PPE. Lab coats or other protective clothing will not prevent needle sticks or other punctures. Many workers prefer not to button up front closing jackets, which leaves street clothing exposed. If front closing jackets must be worn, strict measures shall be implemented to assure the clothing is closed at all times when performing procedures or tasks that may cause exposure.

Long sleeved garments with snug fitting cuffs are preferred over open or short sleeves. Snug fitting cuffs prevent splashes, splatters, and aerosols from contacting exposed skin on the lower arms. Longer single-use gloves can be pulled over snug fitting cuffs to seal out any infectious materials.

Plastic, vinyl, or rubber aprons are usually worn over other protective clothing when extra protection is desired. Aprons are necessary for protection against liquids spilling or splashing on clothing. It is recommended that appropriate aprons be worn to protect against the potential harmful effects of liquid waste. Aprons may also be used to provide protection from steam and hot water in locations such as animal handling facilities, autoclave rooms and laboratory glass washing rooms.

7.5. Face and Eye Protection

Protection of the face and eyes is of prime importance in laboratories due to the potential for foreign material, both liquid and solid, to splash on the head, face and eyes or contact lenses. A variety of face shields, head covers/hoods, protective goggles, and lenses are available from safety supply houses. The selection is dependent upon materials of construction, fit, comfort, and compatibility with the work and the overall facial area requiring protection.

Some of the considerations for selection and use of face and eye protection are indicated below:

- Face shields and hoods protect the face and the neck from flying particles and sprays of hazardous material; however, they do not provide basic eye protection against impacting objects.
- Shields should cover the entire face, permit tilting back to clean the face if desired, and be easily removed in the event of an accident.
- If an eye hazard exists in a particular operation or experiment, the soundest safety policy would be to require that eye or face protection, or both, be worn at all times by all persons entering or working in the laboratory.
- Contact lenses do not provide eye protection. It is recommended that contact lenses not be worn when working around chemicals, fumes, and other hazardous material and dust particles since these items may become trapped in the space between the contact lens and the cornea. When contact lenses are worn, eye protection, such as tight-fitting goggles, must be worn.
7.6. Respiratory Protection

Protection of the respiratory system is a major concern of any biological safety program because infectious organisms can readily enter the human body through the respiratory tract. The possibility of this occurring depends on the type and infectious dose of the particular organism. For some, as few as one to ten organisms, when inhaled, may cause infection. Particles with an effective aerodynamic diameter of between 0.5 and 5.0 µm (the respirable fraction) are most effective at penetration and retention in the deep pulmonary spaces. Particles larger than 5 micrometers are generally trapped in the upper respiratory tract and eventually cleared or swallowed.

Engineering controls, such as the use of biological safety cabinets, should always be considered as a first line of defense against respiratory infection when working with infectious organisms. Respirators should only be considered as a second line of defense after feasible engineering controls have been put into place and additional controls are still needed.

Respirators vary in design, application, and protective capability. Respirators can be placed into two categories:

♦ air purifying
♦ supplied air

By far, the most commonly used respirators in laboratories are air purifying respirators. These protect by purifying the existing breathing air through a filter (for particulates) or cartridge (for gases and vapors). Dust masks that have been approved by NIOSH are also considered to be air purifying respirators. These are ranked by their filtering efficiencies and by whether they can be used in an environment containing oil aerosols.

Approved dust masks will have one of the following designations – N95, N99, N100, R95, R99, R100, P95, P99, or P100. Proper selection of cartridges and respirators is very important and should not be made without input from the Biosafety Office and Occupational/Employee Health. New regulations concerning respirators require initial and annual training and fit-testing, and well as medical surveillance of all respirator wearers. Please make sure that the Biosafety Office and Occupational/Employee Health is notified whenever the use of a respirator is being considered. The Occupational/Employee Health Office can assist in evaluating the procedure, selecting the proper respirator, and provide the required training and fit testing. The Employee Health Office must also be notified so that medical surveillance and clearance can be issued prior to wearing the respirator. Powered air purifying respirators (PAPR) can also be used instead of N95-type masks. Please contact the Biosafety Office if considering these for use. Medical clearance for PAPR use also needs to be obtained.

7.7. Selection of PPE

The Biosafety Office is your first contact regarding the use of PPE. We can provide recommendations for ordering the correct PPE to use in your laboratory. Table 6 below also provides some guidance on proper selection of PPE.

7.7.1. Use the following PPE to minimize exposure via mucous membrane or non-intact skin:
♦ For face protection, wear safety glasses and a mask, or a chin length face shield whenever splashing, splattering or droplets may be anticipated (any work with liquids on the open bench). An impact resistant face shield should be used when operating the autoclave. Impact resistant face shields will protect the user’s face against splatters of hot liquids or broken glass fragments.

♦ Gloves and a lab coat are worn to protect the skin and clothing from contact with potentially infectious materials. Wear gloves that are long enough to extend over the sleeves of the lab coat and cover wrists. Consider double gloving when working with cultures of infectious agents or handling spills. Thicker household utility gloves can be worn for cleaning blood or BL2 spills. Utility gloves can be decontaminated and reused until the integrity of the glove is compromised. Temperature resistant gloves should be worn to protect hands from physical damage when working with very hot (autoclave) or cold (liquid nitrogen tank, -70°C freezer) materials.

♦ Sleeve covers are worn over lab coat and gown sleeves to provide protection to the sleeves and wrists from contamination when working in the biological safety cabinet. Disposable sleeve covers have tight fitting grips at both ends.

♦ Waterproof bandages are worn to cover any wounds or non-intact skin before gloving. It is preferred to double glove when skin is damaged or non-intact. Inform your supervisor of any severe skin conditions or wounds. Avoid working with BL2, BL3 or other potentially infectious materials if non-intact skin cannot be adequately covered.

♦ Solid front gowns provide more protection to clothing and skin than lab coats. Solid front gowns are worn for high hazard infectious agent work. The tight-fitting cuffs of the gown help to minimize wrist contamination.

♦ Impervious lab coats, gowns or aprons are worn when heavy contamination or soiling is likely.

♦ Head covers are worn to protect the hair and scalp from splatter or droplets when working with heavy contamination or when contact with the head is likely. When choosing a head cover make sure it is impervious to liquids (some head covers are not impervious).

♦ Shoe covers are worn over the shoes to protect shoes from contamination when working in heavily contaminated areas (such as large spills, crime scenes, morgues, cadaver dissection areas, surgical operation areas).

♦ Gowns, head and shoe covers also help keep contaminants from entering the sterile area in clean rooms and surgical suites.

7.7.2. Use the following PPE to minimize exposure via cuts, slices, or scratches:

Kevlar gloves and sleeves are cut resistant and will help guard against slices, scratches, or cuts, but will not prevent direct puncture or needlestick injuries. Steel mesh gloves also protect against slices, cuts, and scratches but will not eliminate punctures. Neoprene and other abrasive resistant gloves are cut resistant, but significantly reduce dexterity.

7.7.3. Use the following PPE to minimize exposure via aerosols:
HEPA filtered respirators (air purifying or powered air purifying) are worn to prevent exposure to potentially infectious aerosols when cleaning spills of concentrated infectious material or responding to centrifuge incidents. Employees who wear a respirator must enroll in the Occupational/Employee Health program prior to using these respirators.

Table 7. Proper Selection of PPE Based Upon Biosafety Level

<table>
<thead>
<tr>
<th>PPE</th>
<th>Biosafety Level 1</th>
<th>Biosafety Level 2</th>
<th>Biosafety Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloves</td>
<td>Required</td>
<td>Required</td>
<td>Double Gloves Required</td>
</tr>
<tr>
<td>Lab Coat</td>
<td>Required</td>
<td>Required—Solid front protective gown recommended</td>
<td>Solid front protective clothing such as back fastening gown with tight fitting cuffs must be worn to protect street clothing and skin from contact with infectious agents</td>
</tr>
<tr>
<td>Face Protection</td>
<td>As needed when anticipating any splashes or splatters</td>
<td>Wear protective eyewear and surgical mask or chin length face shield whenever splashing, splattering, or spraying is anticipated to prevent contact with mucous membranes of eyes, nose, and mouth. Researchers may choose to augment eye protection by performing experiments behind a protective splash shield</td>
<td>Face protection is not required when performing all work inside a biological safety cabinet. However, if there is a potential for splashing or splattering (such as during container transport, face and eye protection must be worn.</td>
</tr>
<tr>
<td>Respiratory Protection</td>
<td>As needed when anticipating any splashes or splatters</td>
<td>Based on risk assessment</td>
<td>The use of respiratory protection such as a PAPR or N95 mask will be recommended or required by the Biosafety Officer on a case by case basis. PIs should do a primary risk assessment to gauge the need for such protection. The use of a PAPR is required for response and cleanup of a BSL3 spill. All those persons that wear a respirator must be enrolled in the Employee Health program, be medically cleared, and be certified for use.</td>
</tr>
<tr>
<td>Other</td>
<td>Other PPE such as Tyvek coveralls, booties, sleeve guards, plastic aprons and household rubber gloves can be recommended on a case by case basis. As a general</td>
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<td>Biosafety Level 1</td>
<td>Biosafety Level 2</td>
<td>Biosafety Level 3</td>
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<td></td>
<td>rule, persons should use additional protective clothing when performing procedures that have the potential for generating splashes, splatters, or sprays of infectious material.</td>
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8. OCCUPATIONAL HEALTH

A Medical Monitoring Program for FAU is overseen by EH&S. All persons utilizing research animals are required to enroll in the program. Additionally, persons having indirect contact with animals, persons requiring respiratory protection for work, persons potentially coming into contact with Bloodborne Pathogens, HazMat workers, and scientific divers are required to enroll in the Medical Monitoring Program. The purpose of the Medical Monitoring Program is to:

- Evaluate personnel for medical issues related to performing research duties
- Recommend appropriate medical precautions to be followed, and
- Do periodic reassessment of employees

The extent of medical surveillance for a given employee will vary greatly and be dependent upon:

- The nature of the research project in which involved,
- The biological agents to which directly or potentially exposed, and
- Certain additional factors relating to the current or previous health status of the individual.

For additional information and a detailed description of the Medical Monitoring Program, please refer to the FAU Occupational Health Program.

Medical surveillance is provided without charge for any employee of Florida Atlantic University whose job may result in potential exposure. For more information about this program, contact EH&S.

9. LABORATORY EQUIPMENT

9.1. Procedures for Centrifugation

All centrifugation of biohazardous agents (Risk Group 2 and above) shall be done using centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. If a small centrifuge (microfuge) is used and centrifuge safety cups are not available, the centrifuge should be operated in the biological safety cabinet.

Each person operating a centrifuge should be trained on proper operating procedures.
Keep a logbook detailing operation records for centrifuges and rotors to assist in determining service requirements.

The following procedures for centrifugation are recommended:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles within the biological safety cabinet. Wipe outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakage may occur when tubes are filled to capacity. The maximum for centrifuge tubes is 3/4 full.
- Always cap tubes before spinning.
- Place all tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Correct rough walls caused by erosion or adhering of matter and remove debris from the rubber cushions.
- Wipe exterior of tubes or bottles with disinfectant prior to loading into rotor or safety bucket.
- Never exceed safe rotor speed.
- Stop the centrifuge immediately if an unusual condition (noise or vibration) begins.
- Wait five minutes after the run before opening the centrifuge. This will allow aerosols to settle in the event of a breakdown in containment.
- Decontaminate safety carriers or rotors and centrifuge interior after each use.
- Open safety buckets or rotors in a biological safety cabinet. If the rotor does not fit in the biological safety cabinet, use the fume hood.
- If construction of the centrifuge permits, the centrifuge chamber is to be connected to a vacuum pump with a HEPA filter installed between the centrifuge and the vacuum pump.

9.2. Vacuum Line Chemical Traps and Filters
Vacuum line chemical traps and filters prevent suction of infectious and non-infectious materials into the vacuum lines. A typical setup is illustrated below:

![Diagram of vacuum line chemical traps and filters]
Considerations and Limitations of Vacuum Line Chemical Traps and Filters:

♦ Add full strength chemical disinfectant to chemical trap flasks. Allow the aspirated fluids to complete the dilution. (For example: Start with 100-ml household chlorine bleach, aspirate 900-ml fluids and discard.)

♦ Vacuum line filters shall be examined and replaced if clogged or if liquid contacts the filter. Used filters shall be discarded in the biohazardous waste stream.

♦ Vacuum trap lines should be contained within the Biological Safety Cabinet. However, if space is at a premium, they may be kept outside of the hood, contained within a large tray that would be able to hold the contents of the flasks should they spill. The flasks must contain disinfectant.

9.3. Blenders, Mixers, Sonicators, and Cell Disruption Equipment

Hazardous aerosols are created by most laboratory operations involving blending, mixing, stirring, grinding, or disrupting biohazardous materials. Even the use of a mortar and pestle can be a hazardous operation. Other devices that may produce aerosols include ball mills, colloid mills, jet mills, tissue grinders, magnetic mixers, stirrers, sonic cleaning devices, ultrasonic cell disintegrators, and shakers.

Adequate decontamination is essential prior to sonic cleaning due to possible aerosol generation. Wherever sonicators are used in the cleaning process, such as in dishwashers, animal cage washers, etc.; all items should be disinfected prior to cleaning.

The laboratory practices generally required when using equipment that may generate aerosols with biohazardous materials are as follows:

♦ Operate blending, cell disruption, and grinding equipment in a biological safety cabinet.

♦ Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl. In the absence of a leakproof rotor, inspect the rotor for leakage prior to operation. A preliminary test run with sterile water, saline or methylene blue solution is recommended prior to use.

♦ If the blender is used with infectious material place a towel moistened with an appropriate disinfectant over the top of the blender. Sterilize the device and residual contents promptly after use.

♦ Glass blender bowls are undesirable for use with infectious material because of the potential for glass bowls to break.

♦ Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal effects on the product.

♦ Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

♦ Grinding of infected tissues or materials with any open device is best done within a biological safety cabinet.

9.4. Microtome/Cryostat
Due to the very sharp blade and the nature of the materials used with the microtome/cryostat, training is essential in the use of the equipment and in the hazards of the materials used with the equipment. Users should be informed of the need to prevent cuts and scrapes as well as protect the eyes, nose, mouth, and skin from exposure to the materials being used.

New personnel must be trained in the proper use and maintenance of the equipment and demonstrate proficiency prior to use.

If using human or nonhuman primate tissue, microtome/cryostat users are required to take Bloodborne Pathogens training. Fixatives take time to fully penetrate tissue, thus the fixatives may not inactivate pathogens deep in the tissue. Freezing and drying do not inactivate most pathogens, especially within the context of tissue samples. So, as with fixative use, the pathogens that may be present in the tissue should be considered capable of causing infection.

When purchasing new microtome/cryostat units the available safety features should be taken into consideration prior to deciding on a manufacturer or model. Some available safety features are:

- Auto-decontamination cycle.
- Easy blade release for installing and changing blades.
- Retractable knife/blade to permit safe entry into chamber for cleaning, retrieving specimens, etc.
- Disposable blades.

10. EMERGENCY PROCEDURES (Biological Exposures)

Refer to FAU Emergency Procedures for more information on chemical and radiological spills and fire (all through the EH&S website); and evacuations and tornadoes (through the Emergency Management website). Also, refer to the Spill Response section in this document (section 9).

Biohazard exposures include the following situations:

- Cuts or lacerations from contaminated objects, delivering contaminants directly into the blood stream of the researcher.
- Contact of biohazards with non-intact skin, such as through cuts, rashes, acne, or other areas of abraded or unsealed skin surfaces.
- Introduction of biohazards into the body through facial mucous membranes (eyes, nose, mouth) through splashes, splatter, droplets, or accidental self-inoculation from contaminated hands to these areas.
- Accidental ingestion of biohazards from contaminated hands touching food, or eating, drinking, and smoking in laboratories.
- Inhalation of biohazardous aerosols that are created or released outside of primary containment devices.

Researchers must be aware of all the routes of exposure, in addition to the specific exposure routes for the biohazard used in their laboratory. As a general rule, all routes of exposure should be blocked when handling biohazards. Many biohazards handled in the laboratory are in higher concentrations than normally observed in
nature; this can facilitate exposures through routes not normally associated with the specific biohazard. The goal is to keep biohazards out of the body to prevent the agents from potentially traveling to an area of the body for which it is tropic. The following practices will help minimize the potential for exposure:

- Never eat, drink, or smoke in the laboratory. These activities should only be conducted in designated non-lab areas after protective clothing has been removed and hands have been suitably washed with soap and water.
- Always wear protective clothing and equipment when working in the laboratory. If working outside of a biosafety cabinet and there is a chance of splash or splatter of biohazards, wear full face protection to minimize exposure to your eyes, nose, or mouth. Safety glasses and a mask, or a full-face shield, are adequate for facial mucous membrane protection.
- Avoid the use of sharps in cell culture and non-animal lab areas. Use plastic and non-glass alternatives for all manipulations of biohazards. Work safely with sharps when required, such as with the inoculation of biohazards into animals or the sampling of infected animals.
- Don’t work with biohazards if you have non-intact skin that cannot be adequately covered with tightly adhered waterproof bandages and double gloving.
- Confine all aerosol-generating procedures to inside a biosafety cabinet or other primary containment device.

There are four basic steps to exposure response: 1) Immediate care; 2) Reporting; 3) Medical attention; and 4) Follow-up.

10.1. Immediate Care

An exposure is defined as a specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of a person’s duties. A person who sustains a known or potential exposure incident must immediately stop work, remove gloves, and treat the affected area immediately as indicated below:

10.1.1. Percutaneous Injury
If you experience a needle stick or cut (scalpel, scissors, razor blade, etc.) while working with biohazardous agents, immediately stop your work, remove your gloves, and wash the affected area for 15 minutes with soap and water.

10.1.2. Splash to the Face
If you are splashed in the face with a potential biohazard, proceed to the eyewash and flush the affected area for 15 minutes.

10.1.3. Aerosol Exposure
If you experience an aerosol exposure (such as from a large spill), hold your breath and immediately leave the room. Remove your PPE carefully and make sure to turn potentially exposed areas of the PPE inward. Post a spill sign on the entrance to the lab and inform others of the spill—the lab should be evacuated for at least 30 minutes. Wash hands well with soap and water and wash exposed area(s)
well also with soap and water (unless the exposed areas are the eyes—then flush the eyes at the eyewash).

In all exposure cases, any clothing that is exposed should be removed and discarded as biohazardous waste. Personal items that can be decontaminated should be removed and decontaminated appropriately before taking home. The person experiencing the exposure must report to FAU Medical Provider after the primary response for a proper medical evaluation.

10.2. Incident Reporting

The person experiencing the exposure must report the incident immediately to his/her supervisor. An incident report (Appendix F) also needs to be filed with the Biosafety Officer (bso@fau.edu) within 24 hours. If the exposure incident involves work with research animals, it must also be reported to the FAU IACUC. The Biosafety Officer may initiate an investigation, in cooperation with the PI, based upon the incident report.

10.3. Medical Attention

Employees experiencing exposures are required to access medical care by first contacting Amerisys. Students should go to Student Health Services during working hours, or go to the Emergency Room.

It should be emphasized that the reporting of accidents to the principal investigator or laboratory supervisor is the responsibility of the employee who has the accident. Please also report incidents that did not result in an exposure (near miss) to the Biosafety Office. Evaluation of near misses can lead to alternative work practices and implementation of engineering controls to minimize future incidents.

Whenever an injury involves a sharp and human material (body fluid, tissue, cell line, etc.) the Biosafety Office must perform an investigation to determine if a safe sharps device is available to prevent future occurrences of the injury. If safe sharps devices are available, they must be evaluated by the Biosafety Officer in conjunction with the Laboratory or Department.

10.4. Follow-up

The person experiencing the exposure may need follow-up contact with the medical professional for additional treatment or testing. In addition, the Biosafety Officer will follow-up with the person to fully investigate the exposure and provide any assistance in ensuring that such an exposure does not occur again.

11. DECONTAMINATION AND DISPOSAL

11.1. Decontamination Methods
Decontamination is an important aspect for consideration in laboratory biosafety. It is first important to understand the principles of sterilization and disinfection. An item is sterile when it is completely free of all living microorganisms and viruses—something is either sterile or it is not. A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Disinfection is, in general, a less effective process than sterilization. It eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Physical and chemical means of decontamination are available to researchers. These include three main categories: heat; liquid decontaminants; and vapors and gasses. It is important for the PI to perform a risk assessment regarding the use of specific decontaminants, especially liquids, as these can vary in effectiveness, depending upon the biohazardous agent in use.

11.1.1. Heat

The application of heat, either moist or dry, is considered the most effective method of decontamination/sterilization.

11.1.1.1. Autoclaves.

Steam at 121°C, under pressure (autoclaving) is the most convenient method of rapidly achieving sterility under ordinary circumstances. Sterility can be achieved in as little as 30 minutes (depending upon load size).

11.1.1.2. Dry Heat Sterilizers.

Dry heat, at 160°C to 170°C for two to four hours is suitable for sterilization of impermeable non-organic materials such as glass but is not reliable in even shallow layers of organic or inorganic material that could act as insulation.

Finally, incineration is also a method of heat decontamination. Incineration provides a complete combustion of waste to render it nonpathogenic. This is an ideal method for human and animal pathological wastes.

The primary hazard with the use of heat in decontamination/sterilization is the potential for serious burns when handling hot solids and liquids. Autoclaves are especially hazardous due to the presence of steam, which can be forcefully ejected if the equipment is improperly used.

11.1.2. Liquid Decontaminants

For the most part, the usefulness of liquid decontaminants is limited to surface decontamination and as decontaminants of liquid wastes prior to disposal in the sanitary sewer system.

There are a great number of liquid decontaminants on the market, under several different names. However, they can be placed into the following categories: Halogens; Acids and Alkalis; Heavy Metal Salts; Quaternary Ammonium Compounds; Phenols; Aldehydes; Ketones; Alcohols; and Amines. It is important to note that all compounds do not have the same effects on all agents and that these compounds can fail to disinfect. Thus, liquid disinfectants must be properly evaluated for efficacy. The following considerations should be considered when choosing a disinfectant: Target organism;
Temperature of effectiveness; Contact time; pH; Humidity; Concentration; and penetrability and reactivity of organic material at the site. Small variations in these factors may make large differences in the effectiveness of some liquid decontaminants. An additional consideration to consider is that the more active the decontaminant is, usually the more corrosive it is.

It is important to handle liquid decontaminants, especially concentrated solutions, with care. These have the potential to induce significant damage to exposed areas.

The charts on the next two pages provide some guidance regarding choosing an appropriate liquid disinfectant(s) for use in the laboratory. The first chart lists several liquid disinfectants and their effectiveness against various microorganisms. The second chart lists characteristics of various liquid disinfectants.
## The Antimicrobial Spectrum of Disinfectants

**Note:** Removal of organic material must always precede the use of any disinfectant.

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<td><strong>most susceptible</strong></td>
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<td>mycoplasmas</td>
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<td>gram-positive bacteria</td>
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<td>picornaviruses (i.e. FMD)</td>
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<td>parvoviruses</td>
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<td>acid-fast bacteria</td>
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<td>bacterial spores</td>
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<td>coccidia</td>
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</table>

**Legend:**
- ++ = highly effective
- + = effective
- 0 = no activity
- - = information not available

**Notes:**
- a – varies with composition
- b – peracetic acid is sporidial
- c – ammonium hydroxide
- d – some have activity against coccidia
### Characteristics of Selected Disinfectants

<table>
<thead>
<tr>
<th>Disinfectant Category</th>
<th>Alcohols</th>
<th>Aldehydes</th>
<th>Biguanides</th>
<th>Halogens: Hypochlorites</th>
<th>Halogens: Iodine Compounds</th>
<th>Oxidizing Agents</th>
<th>Phenols</th>
<th>Quaternary Ammonium Compounds (QAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Trade Names</strong></td>
<td>Ethanol, Isopropyl alcohol</td>
<td>Formaldehyde, Glutaraldehyde</td>
<td>Chlorhexidine, Nalvacin, Viroxin</td>
<td>Bleach, Biocidin, Protexone</td>
<td>Hydrogen peroxide, Procion acid, Virkon 5</td>
<td>Ox-Sept 335, Tek-Tol</td>
<td>One-Strobe Environ, Primo-Lys, Tish Tek-Tol</td>
<td>Roccal, Quat-26</td>
</tr>
<tr>
<td><strong>Mechanism of Action</strong></td>
<td>Precipitates proteins, Denatures proteins, Denatures lipids</td>
<td>Denatures proteins, Alkylates nucleic acids</td>
<td>Denatures proteins</td>
<td>Denatures proteins, Alters membrane permeability</td>
<td>Denatures proteins, Alters cell wall permeability</td>
<td>Denatures proteins, Alters cell membrane</td>
<td>Denatures proteins, Damage cell membranes</td>
<td></td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Fast acting, Leaves no residue</td>
<td>Broad spectrum</td>
<td>Broad spectrum</td>
<td>Broad spectrum, Short contact time</td>
<td>Stable in storage, Relatively safe</td>
<td>Good efficacy with organic material, Non-corrosive</td>
<td>Stable in storage, Non-irritating to skin, Effective at high temperatures and high pH</td>
<td></td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Rapid evaporation, Flammable</td>
<td>Carcinogenic, Mucous membranes and tissue irritation, Only use in well ventilated areas</td>
<td>Only functions in limited pH range (5–7)</td>
<td>Inactivated by sunlight, Requires frequent application</td>
<td>Inactivated by QACs, Requires frequent application</td>
<td>Inactivated by QACs, Requires frequent application</td>
<td>Can cause skin and eye irritation</td>
<td></td>
</tr>
<tr>
<td><strong>Precautions</strong></td>
<td>Flammable</td>
<td>Carcinogenic</td>
<td>Never mix with acids, toxic chlorine gas will be released</td>
<td>May be toxic to animals, especially cats and pigs</td>
<td></td>
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</tbody>
</table>

**Vegetative Bacteria**
- Effective

**Mycoplasma**
- Effective

**Enveloped Viruses**
- Effective

**Non-enveloped Viruses**
- Variable

**Spores**
- Not Effective

**Fungi**
- Effective

**Efficacy with Organic Matter**
- Reduced

**Efficacy with Hard Water**
- Reduced

**Efficacy with Soap/ Detergents**
- Reduced

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Disclaimer: The use of trade names does not in any way signify endorsement of a particular product. For additional product names, please consult the most recent Compendium of Veterinary Products.

11.1.3. Vapors and Gases

For many years, formaldehyde gas was the gold standard for large-scale decontamination (Biological Safety Cabinets, rooms, etc.). Now, there are several alternatives to formaldehyde, which has been recently designated a carcinogen. Ethylene oxide has been used as an alternative for several years and is primarily utilized in surgical and clinical areas. However, mutagenic potential has been noted for this compound as well. More recently, vaporized hydrogen peroxide (VHP) and chlorine dioxide gas (CD) have been increasingly used for large-scale decontaminations.

Formaldehyde gas (achieved by heating paraformaldehyde flakes in a pan; 0.3 g/cubic foot) is still utilized for small space decontaminations (such as for a BSC). It does require humidity and temperature control (relative humidity of 80% is optimal; temperature of at least 20°C). These procedures usually take from four hours to overnight to complete dependent upon the size of the area to be decontaminated. The formaldehyde gas is neutralized with ammonium carbonate. Formaldehyde is low-cost, but the disadvantages include that it is a carcinogen and it can polymerize on surfaces due to changes in temperature, humidity, or concentration. **Formaldehyde gas is not approved for decontamination purposes at FAU.**

Vaporized Hydrogen Peroxide (VHP; 0.5 – 10 mg/liter effective concentration) is a powerful oxidizing agent and is sporicidal. It can be used for BSCs, rooms, isolators, ambulances and in sterilizers for medical and dental equipment. It requires less time than formaldehyde, but still has a requirement for temperature and humidity (15-25°C, from 25-70% humidity, depending on the system utilized). Advantages include the shorter time for decontamination, no harmful breakdown products (result is water and oxygen), it is compatible with electronics, and it is not carcinogenic. Disadvantages include that it is toxic, it is a vapor—so it cannot get everywhere like a gas can and can be trapped in porous materials such as wood.

Chlorine Dioxide Gas (1-5 mg/liter) is another alternative to formaldehyde for instrument and space decontamination. It is toxic, but it is non-carcinogenic and is not a reproductive hazard. It will effectively penetrate water. And because Chlorine Dioxide is a gas, it will penetrate places that a vapor cannot. It is approved by the NSF for BSC decontamination. Disadvantages include that it must be generated onsite; it is an oxidizer and can be incompatible with uncoated ferrous metals and latex rubbers; and it is rapidly broken down by light.

Both VHP and Chlorine Dioxide Gas have high initial capital equipment costs associated with the instrumentation purchase. However, there are companies that use these technologies and provide decontamination services on a fee-for-service basis.

11.2. Autoclave Procedure

Moist heat causes the denaturation of proteins at lower temperatures and shorter times than dry heat. One of the most effective physical decontamination controls is steam sterilization (autoclave), which generates moisture and high temperature pressurized steam within a sealed chamber. Autoclaves can sterilize all items that are heat stable. In gravity autoclaves, a cycle of 250°F (121°C) at 15 to 18 pounds per square inch (psi) of pressure for one hour may be required for
decontamination. In the newer vacuum autoclaves, decontamination may require a cycle of 270°F (132°C) at 27 to 30 psi for 45 minutes. Be sure to check with your autoclave vendor for specific instructions on how to operate your autoclave. The following safety guidelines must be followed:

PPE

- Always use personal protective equipment (PPE) when using an autoclave
- Wear a lab coat, heat-resistant gloves, and safety glasses.
- Be sure arms are covered by a lab coat and longer heat-resistant gloves to prevent burns from heat and steam.

Prepare the Autoclave

- Inspect the door gasket (seal) for any cracks or bulges. The gasket should be smooth and pliable.
- Clean the drain screen of debris if necessary.
- If any problems are found, contact the responsible person before using the autoclave.
- Turn the autoclave on and allow time for the jacket to reach sufficient temperature and pressure.

Prepare Items

- Do not autoclave flammable, combustible, reactive, corrosive, toxic or radioactive materials (this includes bleach solutions). Contact EH&S for disposal of hazardous materials.
- Check that plastics are compatible with the autoclave. Not all plastics can be autoclaved.
- Inspect glassware for cracks. Do not autoclave cracked or compromised glassware.
- For liquids, leave caps loose or cover with foil to allow steam penetration and prevent explosion.
- For bagged items, loosely tape or tie close. Leave an opening for steam to penetrate the bag.

Load

- Inspect for spills or debris inside the autoclave, check door gasket for cracks or bulges.
- Ensure that the jacket has reached sufficient pressure to start a cycle.
- Place items in an autoclave-safe tub on the rack. Never place items directly on the autoclave bottom or floor.
- Do not overload the autoclave. Allow sufficient space between items for steam.
- Add water if needed (100 ml of water can be added to the autoclave pan to ensure even heating of liquids).
- Always use secondary containment in case of spillover.

Operate

- Follow the manufacturer’s user manual and laboratory SOP for operating the autoclave.
- Close and lock the door. Ensure the door is secure before starting a cycle.
- Select appropriate cycle (e.g. dry heat, sterilize media, sterilize biohazardous waste).
- Record run on log sheet.
• Check about 20 minutes into the cycle to verify the autoclave has reached sterilization temperature (121°C).
• Do not open the autoclave door during a cycle. If necessary, abort the cycle and wait until the chamber depressurizes.
• If the cycle fails, notify the person responsible for the autoclave. Items may not be sufficiently decontaminated if the cycle did not complete.

Unload

• When the cycle is complete, verify that chamber temperature has dropped, and pressure is zero.
• Wear appropriate PPE to protect yourself from heat and steam (e.g. heat-resistant glove, lab coat, safety glasses).
• Slowly open the door to allow steam to escape gradually. Keep your face away from the door.
• Allow items to stand in the autoclave for 10 minutes.
• Cautiously remove items and place in a safe area to cool. Do not agitate containers as boiling or superheated liquids can explode if moved too quickly.
• Record cycle information on autoclave log sheet or logbook.

Train and Maintain

• Designate a responsible person for the autoclave. The responsible person will train users and inform them if the autoclave is out of service.
• Train all autoclave users and keep documented training records on the autoclave training log.
• Implement a regular maintenance schedule to ensure safe operation. Keep contact information for maintenance technician available.

Accidents and near misses

• Post the Exposure Response Poster (Appendix D) near the autoclave. In the event of an accident, immediately provide first aid and get help according to the instructions on the poster.
• Report any accidents or near misses to EH&S via an Incident Report Form (Appendix F), so that they can be investigated and hopefully prevented in the future.

Other considerations for autoclaves:

• **FAU autoclaves may not be used for disinfection of biohazardous waste.** None of the autoclaves on campus have been appropriately certified for use according to FL Department of Health Guidelines. Thus, autoclaves should only be used for disinfection of instruments/equipment and treatment of media.
• All autoclaves should be associated with an autoclave log. A log that can be printed and used can be found in Appendix E.
• Autoclaves should be checked **at least monthly** for sterilization capability, especially if being used to sterilize instruments. An appropriate biological indicator should be included in an autoclave run to test the autoclave. The biological indicator should be placed approximately
in the middle of the load or wrapped as an instrument pack is wrapped for sterilization. Results of the biological indicator should be included in the autoclave log for that run.

- When purchasing a new autoclave, please consult with the Biosafety Officer on which makes and models to consider.

11.3. Disposal of biohazardous waste.

11.3.1. Solid waste.

All laboratories should have an appropriate number of biohazardous waste receptacles. It is required that these receptacles have covers to prevent possible aerosol exposure when waste is disposed. Red biohazard bags should be placed in the receptacles for the collection of solid waste materials. When the bags are ¾ full, they should be removed from the receptacles and tied loosely. The bag should then be placed into a second red biohazard bag and that bag similarly tied. Please consult the FAU Biological Waste Manual for detailed information.

Contractor disposal. FAU contracts with Stericycle to haul away and incinerate medical and biohazardous waste. After the waste is collected in the laboratory, a request can be made online for EHS to collect the waste. The biohazard waste bags are placed into cardboard boxes for Stericycle to collect for incineration. This option is much less taxing on the laboratory worker as no validations need to be performed.

11.3.2. Liquid waste.

Any biohazardous liquid waste should be decontaminated by laboratory personnel. An appropriate disinfectant should be chosen by the laboratory, based upon the agent being utilized (please see the charts earlier in this section for aid in determining an appropriate disinfectant, or contact the Biosafety Office for assistance). The Biosafety Office usually recommends household bleach as the disinfectant of choice for most agents. In this case, a final concentration of 10% bleach is used for treating liquid waste (example—100 mls of undiluted bleach is added to 900 mls of liquid waste to give a 10% final concentration). The bleach should be allowed to work for at least 30 minutes, then the mixture can be disposed of down the sanitary sewer. If using a different disinfectant, incubation times may vary before disposing.

11.3.3. Pathological waste.

Pathological waste is considered to be composed of recognizable human derived tissues, organs and body parts, as well as vertebrate animal-derived tissues, organs and body parts used in research activities. Pathological wastes are treated differently than other biological wastes. They must be separately segregated and properly identified so they can be shuttled to the correct waste stream. Ultimately pathological wastes are collected by Stericycle and subjected to incineration, where other biological wastes are autoclaved.
12. SPILL RESPONSE

This guide outlines the basic procedures for dealing with some of the biological spills that you may encounter in your research laboratory. All lab personnel should refer to the relevant spill response within their laboratory-specific Biosafety Manual procedures before initiating their experiments.

12.1. Composition of a Basic Spill Kit
Microbiological and biomedical research laboratories should prepare and maintain a biological spill kit. A spill kit is an essential safety item for labs working with microbiological agents classified for work at Biosafety Level 2 or higher and for groups working with large volumes (> 1 liter) of material at Biosafety Level 1. A basic spill kit should include:

- Concentrated household bleach
- A spray bottle for making 10% bleach solutions
- Forceps, broom and dustpan, or other mechanical device for handling sharps
- Paper towels or other suitable absorbent
- Biohazard autoclave bags for the collection of contaminated spill clean-up items
- Utility gloves and medical examination gloves
- Face protection (eye wear and mask, or full-face shield)

Additional personal protective equipment, such as Tyvek jump suits and powered air-purifying respirators (PAPR’s), may be required for response to spills in Biosafety Level 3 laboratories.

12.2. Biosafety Level 1 (BSL1) Spill Cleanup
- Notify others in the area, to prevent contamination of additional personnel and environment.
- Remove any contaminated clothing and wash exposed skin with soap and water.
- Wearing gloves, lab coat, and face protection, cover spill with paper towels, pour concentrated disinfectant around the spill allowing it to mix with spilled material. Allow suitable contact time.
- Pick up any pieces of broken glass with forceps and place in a sharps container.
- Discard all disposable materials used to clean up the spill into a biohazard autoclave bag.
- Wash hands with soap and water.

12.3. Biosafety Level 2 (BSL2) Spill
- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with a warning sign.
- Remove contaminated clothing, turning exposed areas inward, and place in a biohazard bag.
- Wash all exposed skin with soap and water.
- Inform Supervisor, and, if assistance is needed, consult the Biosafety Officer.

12.3.1. Clean-up of BSL2 spill
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (disinfectant, paper towels, biohazard bags, and forceps).
• Put on protective clothing (lab coat, face protection, utility gloves, and booties if necessary). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask.
• Cover the area with disinfectant-soaked towels, and then carefully pour disinfectant around the spill. Avoid enlarging the contaminated area. Use more concentrated disinfectant as it is diluted by the spill. Allow at least a 30-minute contact time.
• Pick up any sharp objects with forceps and discard in a sharps container. Soak up the disinfectant and spill using mechanical means, such as an autoclavable broom and dustpan, since there may be sharps under the paper towels, and place the materials into a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held with forceps. If no sharps were involved in the spill discard the materials into an autoclave bag.
• Wipe surrounding areas (where the spill may have splashed) with disinfectant.
• Soak up the disinfectant and spill and place the materials into a biohazard bag.
• Spray the area with 10% household bleach solution and allow to air-dry (or wipe down with disinfectant-soaked towels after a 10-minute contact time). Place all contaminated paper towels and any contaminated protective clothing into a biohazard bag and autoclave.
• Wash hands and exposed skin areas with disinfectant or antiseptic soap and water.

12.3.2. Blood spills
For blood or other material with a high organic content and low concentration of infectious microorganisms:
• Wear gloves, eye/face protection, and a lab coat.
• Absorb blood with paper towels and place in a biohazard bag. Collect any sharp objects with forceps or other mechanical device and place in a sharps container.
• Using a detergent solution, clean the spill site of all visible blood.
• Spray the spill site with 10% household bleach and allow to work for 30 minutes.
• After the 30-minute contact time, wipe the area down with disinfectant-soaked paper towels.
• Discard all disposable materials used to decontaminate the spill and any contaminated personal protective equipment into a biohazard bag.
• Wash your hands.

12.3.3. Spill in a Biosafety Cabinet
• Leave the biosafety cabinet turned on and begin cleanup immediately.
• While wearing PPE (gown and gloves) cover the spill area with paper towels or disinfectant-soaked towels. Do not place your head inside the cabinet to clean the spill. Keep your face behind the front sash. If necessary, flood the work surface, as well as drain pans and catch basins below the work surface with disinfectant.
• Spray or wipe cabinet walls, work surfaces, and inside the front sash with disinfectant.
• Soak up all disinfectant and spill and drain catch basins into a container after an appropriate contact time (based upon disinfectant instructions; for bleach, allow 30 minutes).
• Lift the front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or soiled debris are blown into the area below the grill.
• Dispose of all clean up materials in biohazardous waste. Wash hands and exposed skin areas with soap and water.
• Notify your PI and supervisor.
• File an incident report (See Appendix F) with the Biosafety Officer.

12.3.4. Centrifuge Spill
• When centrifuging infectious agents, utilize sealed tubes and either a sealed rotor or safety buckets for containment. Ensure that all O-rings or gaskets are in place and in good condition.
• Wait five minutes before opening the centrifuge following the end of a run with potentially hazardous biological material. If a spill is identified after the lid is opened, carefully close the lid, and evacuate the laboratory for at least 30 minutes. Post a sign at the lab door indicating “Biohazard Spill: Do Not Enter.”
• In the event of a spill during centrifugation, turn off the centrifuge, leave the lid closed, and evacuate the laboratory and allow aerosols to settle for at least 30 minutes. Post a sign at the lab door indicating “Biohazard Spill: Do Not Enter.”
• Remove any contaminated protective clothing and place in a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.
• Notify your supervisor and the Biosafety Officer.
• After 30 minutes, enter the lab with PPE and spill cleanup materials. Full face protection, a lab coat and utility gloves should be worn.
• Transfer rotors and buckets to a biological safety cabinet. Immerse in 70% ethanol or a non-corrosive disinfectant against the agent in use and allow at least 10 minutes of contact time. Uncapped or unbroken tubes may be wiped down with disinfectant after the soak and placed in a new container. Handle any broken glass with forceps and place in a sharps container.
• Carefully retrieve any broken glass from inside the centrifuge with forceps and place in a sharps container. Smaller pieces of glass may be collected with cotton or paper towels held between forceps. Carefully wipe the inside of the centrifuge with disinfectant. Spray the inside of the centrifuge with disinfectant and allow to air dry. If bleach is used, follow by wiping with 70% ethanol to remove any corrosive residues.
• Place contaminated items and disposable PPE in a biohazard bag and dispose of as biohazardous waste.
• Wash hands with soap and water.

13. SHIPPING BIOLOGICAL MATERIALS

Infectious agents, biological materials and other dangerous goods must be transported according to the applicable regulations. The shipment of biological materials and infectious agents is regulated by the International Air Transport Association (IATA), the US Department of Transportation (DOT), the US Postal Service (USPS) and other national and international agencies. Carrying dangerous goods on one’s person, in luggage or in private automobiles is strictly prohibited. Those who violate the regulations are subject to significant fines and criminal prosecution.

13.1. Training Requirements

Federal regulations require that individuals shipping biological materials or dry ice must first have appropriate training. Depending upon the individual’s role in the shipping process, certain training
requirements will need to be fulfilled from among these: General Awareness; Function-Specific; Safety; and Security Awareness. For example, a worker in a shipping department would only need General Awareness, but a worker in a lab packing materials for a shipment would require all areas of training. Training is required to be repeated at least every two years. EH&S staff members are trained on shipping requirements and can assist PIs with shipping.

There are four specific steps required for shipping biological materials. These are briefly described below:

13.1.1. Identification/Classification

The first step is correctly identifying and classifying the materials that are to be shipped. Biological materials will fall into one of four categories for classification: Category A, Category B, Exempt or Not Regulated. Category A materials are those infectious biological substances that likely will result in permanent disability, life-threatening or fatal disease in otherwise healthy humans. Category B materials are infectious substances that do not meet the criteria for Category A. Exempt specimens are those for which there is minimal likelihood that pathogens are present.

13.1.2. Packaging

The next step is packaging. This requires assembling all the correct containers for the shipment and ensuring that the biological material is secure. All biological shipments from Category A to Exempt Specimens require triple packaging: Primary, Secondary and Outer containers.

13.1.3. Marking and Labelling

All packages must be marked and labeled according to the material that is being shipped. There are specific guidelines for each classification as to what needs to be marked and labeled on the outer package.

13.1.4. Documentation

For all shipments, proper documentation must accompany the package. All packages require a shipping airway bill and an itemized list of contents. Category A shipments also require a Shippers Declaration for Dangerous Goods.

This is only an overview of shipping requirements, shippers must take one of the courses provided on the EH&S Training website (http://www.fau.edu/ehs/training/): Either the DOT or IATA training course. Additionally any lab shipping biological materials for the first time should involve the BSO as oversight during the process.

14. IMPORTING AND EXPORTING BIOLOGICAL AGENTS

Receiving/sending animals, biological/infectious agents, and Genetically Modified Organisms from/to outside the United States may require the approval of federal agencies such as the CDC, USDA and the
US Fish and Wildlife Services (USFWS). These regulatory agencies govern the transfer of such materials to minimize and eliminate the possible threats to public health and agriculture.

For importation of agents infectious to humans, human and animal specimens with a suspected human pathogen, hosts and vectors of human disease and anything to do with nonhuman primates the permit application and instructions are on the CDC website: https://www.cdc.gov/cpr/ipp/applications/index.htm. The USDA permit is required to import or domestically transfer regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms. Information on the USDA process can be found at this website: https://www.aphis.usda.gov/aphis/resources/permits. For transporting fish, wildlife, or endangered species, the USFWS website has information and application procedures can be found at this website: https://fwsepermits.servicenowservices.com/fws.

An export license may be required from the Department of Commerce when sending specimens abroad, including infectious agents of human, plant, or animal diseases. Information and instructions can be found at this website: https://www.bis.doc.gov/.

15. MINORS IN THE LABORATORY

According to federal and state laws, children under the age of 18 must not work in laboratories that use hazardous materials such as infectious agents, chemicals, and radioactive substances. However, minors are allowed to work or conduct research in laboratories if the following requirements are met in full:

- The minor is supervised by the Principal Investigator/Sponsor or his or her designee at all times while in the laboratory and never left alone.
- The minor complies with all safety training and medical monitoring requirements required for the laboratory and/or fieldwork to be performed.
- The FAU EH&S Policy titled; Minors in Research Laboratories and Animal Facilities has been read and understood. The Parent/Legal Guardian Informed Consent form package has been reviewed and signed by the parent/legal guardian and minor and returned to EH&S by mail or by fax.
- A Minors in Research Proposal Registration Form is submitted to and approved by EH&S and, if necessary, by the FAU Institutional Biosafety Committee and the Institutional Animal Care and Use Committee, if animals are involved.
- Hazard specific safety training is completed by the Principal Investigator/Sponsor with the minor as approved by EH&S. All volunteers must complete laboratory safety training prior to work in laboratories.
- Personal protective equipment, specific to the hazard, is provided to the minor with instructions for use and disposal. At a minimum, and without exception, minors must wear safety glasses, lab coat, long pants/slacks, and closed-toed shoes at all times while in a laboratory. Gloves must be worn when handling hazardous materials.
- The laboratory is in full compliance with all applicable FAU safety programs and regulations.

16. WILDLIFE AND FIELD STUDIES
When conducting field studies, it is most important to know what environmental hazards may be encountered, including, but not limited to, heat stroke, venomous insects or reptiles, zoonotic diseases, and to understand what preventative measures and personal protection should be taken to minimize the hazard exposure. Please consult FAU's Animal Research Health and Safety Plan for more detailed information.
Appendix A

PI Requirements for Biosafety Compliance
### Appendix A. PI Requirements for Biosafety Compliance

<table>
<thead>
<tr>
<th>If using items below requirements indicated with an X</th>
<th>Required Registration</th>
<th>Required Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAU IBC registration</td>
<td>FAU IBC Full Review</td>
<td>FAU IRB</td>
</tr>
<tr>
<td>Recombinant DNA work (exempt)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Recombinant DNA work</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Human Materials (Cells, Tissues, Cell Lines, etc.)</td>
<td>X</td>
<td>X**</td>
</tr>
<tr>
<td>Infectious Agents, BSL-2</td>
<td>X</td>
<td>X*</td>
</tr>
<tr>
<td>Animal Use with Recombinant DNA</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Animal Use with Human Materials</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Animal Use with BSL-2 Agents</td>
<td>X</td>
<td>X*</td>
</tr>
<tr>
<td>Human Gene Therapy</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Infectious Agents, Dry Ice Shipping</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix B

Equipment Decontamination Form
Equipment Decontamination Form

Equipment that has been used with hazardous materials must be decontaminated before it can be discarded, moved, repaired, or recycled. Directions: complete the below form and return to the FAU Biosafety Officer via email (fnovembre@fau.edu). If you have any questions about decontaminating your equipment, contact the Biological Safety Officer at: (561)297-3129. The Biosafety Officer will review proper decontamination and disposal recommendations and return the signed form to you. Once you receive the signed Equipment Decontamination Form from the Office of Biosafety, please ensure the equipment is appropriately decontaminated, post this form on the decontaminated equipment, and save an additional copy for your records. Use a separate form for EACH piece of equipment.

### Equipment Location and Type

<table>
<thead>
<tr>
<th>Building/Room</th>
<th>Equipment Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Click here to enter text.</td>
<td>Click here to enter text.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer and Model #</th>
<th>Serial #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Click here to enter text.</td>
<td>Click here to enter text.</td>
</tr>
</tbody>
</table>

### Equipment Disposition and Hazardous Materials Usage

This Equipment is being:

- [ ] Discarded
- [ ] Repaired
- [ ] Relocated
- [ ] Returned
- [ ] Other (Specify): Click here to enter text.

If this equipment is being discarded, indicate how it will be disposed of:

Click here to enter text.

Indicate who will be processing this equipment:

Click here to enter text.

This equipment:

- [ ] Has never been used with radiological, chemical, or biological agents. Date cleaned: Click here to enter text.
- [ ] Has been used with the following materials:
  - [ ] Chemical (List chemicals used): Click here to enter text.
  - [ ] Biological (List biological agents used): Click here to enter text.
  - [ ] Radiological (List radioisotopes used): Click here to enter text.

### Equipment Decontamination Process and Personnel

This equipment has been cleaned with (describe the process/agent which is suitable for deactivating/removing/disinfecting the hazardous materials):

Click here to enter text.

Name and Title of Person Performing the Cleaning:

Click here to enter text.

Signature: __________________________ Date: ____________

### Biosafety Officer Only—Biosafety Review

Comments: ___________________________________________ Incident #: ____________ Date: ____________

Office of Biosafety Reviewer Name: ___________________ Title: __________________ Signature: __________________
Appendix C

Guidelines: Transport of Hazardous Research and Clinical Biological Samples

The research and clinical enterprise occasionally involve the transport of biological materials from one location to another. In general, movement or ground transport of regulated materials is covered by the DOT Hazardous Materials Regulation (HMR) only when they are considered to be “in commerce” and transported over public highways. Biological materials transported in an FAU-owned vehicle for use in FAU activities (projects, research, etc.) is generally not considered to be “in commerce.” These biological materials are also considered “Materials of Trade” in that they are required for work to be done at the institution. For such transportation, FAU vehicles are to be used.

- **HAND CARRY**: Safely hand-carry biological materials within the FAU system and between labs/buildings/clinics through public areas (includes the use of FAU golf carts).
- **TRANSPORT IN FAU MOTOR VEHICLE**: Safely transport non-infectious biological materials and non-pathogenic cultures (e.g., exempt patient specimens, human/animal blood, tissues, body fluids, body parts, cells, cell lines, DNA, plasmids, etc.) in a private (FAU-owned) motor vehicle that is used exclusively for that purpose during the transport. These biological materials must fall under the exception category of 49CFR 173.134 Class 6, Division 6.2—Definitions and exceptions, and do not meet the DOT division 6.2 definition of Infectious Substance. Biological materials meeting the definition of hazardous materials, Infectious Substances, division 6.2, Category A or B, shall not be transported in an FAU vehicle and should be transported by an appropriate courier or shipping company.

**Procedure for hand carrying biological materials**

<table>
<thead>
<tr>
<th>Type</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Requirements for Primary Container</strong></td>
<td>• Place material in a primary (specimen) container that is leak-proof and secured with a tight-fitting cap, parafilm, or lab tape.</td>
</tr>
</tbody>
</table>
| **Requirements for Secondary Container** | • All biological materials that are hand carried from lab to lab, hospital to lab, etc. must also be packaged in secondary containment.  
  • Place the primary containers in a secondary transport container that is also sealed. Secondary containment should be of a nature such that if sample broke or spilled, the secondary container would contain the spilled material. The secondary container may be a plastic box with tight fitting lid, cooler with sealed lid, etc.  
  • Place absorbent material (diapers, absorbent towels, pads) around the primary containers for transport of liquids.  
  • These materials may be moved on a cart or other device between rooms or buildings. |
| **Requirement for Labeling** | • The outer secondary transport container shall be labeled with a biohazard symbol if carrying specimens of human/non-human primate origin or any infectious agent risk group 2 or higher.  
  • The outer container should also be labeled with the name and location of the sender and recipient of the material, along with respective phone numbers, in case of loss of package.  
  • Specimen data forms, indicating what the material is, should be included between the inner and outer packaging. |
<p>| <strong>Personal Protective Equipment</strong> | • Personal Protective Equipment (PPE), gloves, shoe covers, etc., shall NOT be worn in public corridors or areas. Personnel should take additional clean gloves and other PPE (as necessary) with them. |
| <strong>Security and Safety</strong> | • Travel directly from the pick-up location to the drop off point. Do not make additional stops. |</p>
<table>
<thead>
<tr>
<th>Type</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Make use of service elevators whenever possible. Use less-frequently traveled paths to avoid crowds.</td>
<td></td>
</tr>
<tr>
<td>Do not leave packages or containers unattended. Personally deliver the package to the personnel in the lab. Lab personnel should check for leaks on delivery.</td>
<td></td>
</tr>
<tr>
<td>Personnel involved in transport should be trained on how to clean up biological spills.</td>
<td></td>
</tr>
</tbody>
</table>

### Receiving Packages or Specimens

- Before opening a package, shipment should be examined for the following: Proper paperwork and labeling, Package integrity
- Leaking packages: report any leaking packages to the sending department and the PI. Contact Biosafety if necessary, to assist in cleanup.
- Package Delivery: deliver package directly to the designated person
- Do not leave packages or containers unattended. Personally deliver the package to the personnel in the lab. Lab personnel should check for leaks on delivery.
- Opening the package: Open package in lab with appropriate safety equipment (Personal Protective Equipment, Biosafety cabinet). Universal precautions (gloves, lab coat/gown, face protection/face shield) shall be employed in the clinical/research area. This includes washing hands after package is delivered.

### Procedure for transport of biological materials in an FAU vehicle

- The FAU Office of Environmental Health & Safety specifies the use of FAU-owned vehicles rather than personal vehicles when transporting biological materials off grounds to or from another FAU facility or collaborating institution. Accidents during movement or transportation of any of these materials can result in serious harm to persons and property. Release and spills of these materials may involve police and HazMat responders including clean-up and cost of recovery.

- Under NO circumstances may public transportation (e.g. buses, trolleys, private taxis/rideshare vehicles (Uber, Lyft, etc), FAU Shuttle, etc.) or personal vehicles be used for transport of work-related Biological Materials.

### Type | Requirement
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>FAU Transport Vehicle</td>
<td>Personnel shall have a valid driver’s license issued by the state where they permanently reside that is not currently suspended or revoked.</td>
</tr>
<tr>
<td>Personnel must carry auto liability insurance that meets the minimum requirements in their state of residence.</td>
<td></td>
</tr>
<tr>
<td>During transport of biological materials, the vehicle shall only be used for that purpose.</td>
<td></td>
</tr>
<tr>
<td>No other persons shall be in the vehicle when used for transport of biological material, except if there to assist in the transport.</td>
<td></td>
</tr>
<tr>
<td>Travel directly from the pick-up location to the drop off point.</td>
<td></td>
</tr>
</tbody>
</table>

### Requirements for Specimen and Transport Container

- Triple packaging MUST be employed.
- Primary specimen containers should be watertight and leakproof. If the specimen container is a tube, ensure it is tightly capped and placed in a rack to maintain an upright position. The caps on tubes can be wrapped with parafilm to ensure that there is no leakage.
- Secondary container: Single tubes can be placed in a Ziploc bag with the biohazard label on the bag. Absorbent (paper towels, Kimwipes, diaper pads, etc.) should be placed inside the bag or in the transport box.
- When transporting multiple primary containers, package them in a manner that will prevent damage to the containers. For example, if you are preparing to transport several vacutainers, place these in a rack or tube holder that will prevent contact between the tubes. If possible, place rack inside a large Ziploc bag.
- Triple packaging: Place specimen containers and racks in robust, leak-proof plastic or metal transport boxes with secure, tight fitting covers. For additional
<table>
<thead>
<tr>
<th>Type</th>
<th>Requirement</th>
</tr>
</thead>
</table>
| **Type** containment, the transport box, which may be a cooler, can be placed inside a rigid plastic type box with lid. Place absorbent inside the container.  
  - Specimen data forms, identification data or list of contents (scientific name) and quantity (amount) should accompany each transport box.  
  - Secure the transport boxes in the transport vehicle.  |                                                                                                                                              |
| **Labeling**  
  - Label each transport box appropriately, consistent with its contents. Label the transport container with a biohazard symbol if transporting human or non-human primate material. Infectious agents shall not be transported in private motor vehicle.  
  - The name and telephone number of an emergency contact person, and the receiver's name, address, and telephone number. Specimen data forms and identification data should accompany each transport box, if applicable.  
  - A list of biological material should accompany the transport container.  |                                                                                                                                              |
| **Personal Protective Equipment**  
  - Personal Protective Equipment (PPE), gloves, shoe covers, etc., shall NOT be worn in public corridors or areas. Personnel should take extra clean gloves and other PPE (as necessary) with them.  |                                                                                                                                              |
| **Security and Safety**  
  - Travel directly from the pick-up location to the drop off point. Do not make additional stops.  
  - Do not transport other persons, unless required for package handling.  
  - The container(s) should be placed into the trunk of the car (or the very back if it is an SUV-type vehicle) and secured from movement as much as possible.  
  - A Biological Spill Kit should be present in the vehicle during transport in case of leaks or spills of the material.  
  - During transport, vehicle should only be used for that purpose.  
  - Do not leave packages unattended. Deliver the package directly to the personnel in the lab.  
  - Personnel involved the transport process should be trained on how to clean up biological spills.  |                                                                                                                                              |
| **Receiving Packages or Specimens**  
  - Before opening a package, shipment should be examined for the following: Proper paperwork and labeling, Package integrity  
  Leaking packages: report any leaking packages to the sending department and the PI. Contact Biosafety if necessary, to assist in cleanup.  
  - Package Delivery: deliver package directly to the designated person  
  - Do not leave packages or containers unattended. Personally deliver the package to the personnel in the lab. Lab personnel should check for leaks on delivery.  
  - Opening the package: Open package in lab with appropriate safety equipment (Personal Protective Equipment, Biosafety cabinet). Universal precautions (gloves, lab coat/gown, face protection/face shield) shall be employed in the clinical/research area. This includes washing hands after package is delivered.  |                                                                                                                                              |
APPENDIX D

Exposure Response Poster
# EXPOSURE RESPONSE

For biological, chemical, or radiological exposures

**CALL 911 FOR ANY LIFE-THREATENING EMERGENCY**

## 1. PERFORM FIRST AID

<table>
<thead>
<tr>
<th>Exposure Type</th>
<th>First Aid Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needlestick, puncture or sharps injury, or animal/bite scratch</td>
<td>Wash thoroughly for 15 minutes with warm water and sudsing soap.</td>
</tr>
<tr>
<td>Eye exposure</td>
<td>Use emergency eyewash station to flush eyes for 15 minutes while holding eyes open.</td>
</tr>
</tbody>
</table>
| Skin exposure | • **Radioactive**: Survey skin and wash until the count rate cannot be reduced further. Stop if skin becomes irritated.  
• **Chemical**: Wash with tepid water for 15 minutes.  
• **Hydrofluoric acid**: Wash for 5 minutes, then apply calcium gluconate gel to skin.  
• **Biological**: Wash with sudsing soap and water for 15 minutes. |
| Inhalation or ingestion | • Move out of the contaminated area and seek fresh air.  
• Do not induce vomiting unless instructed to do so.  
• **Radioactive**: Blow nose into clean tissue and survey for contamination. |

## 2. GET MEDICAL HELP

For radiological exposure or emergency:  
- Call Radiation Safety at:  
- Call 911 if office is closed.  
- Provide the radionuclide, estimated amount and time since exposure.

For chemical exposure or emergency:  
- Call 911 and follow the instructions given.  
- Provide the chemical name, concentration, time since exposure and Safety Data Sheet (SDS).

For biological and all other exposures:  
- Call our Occupational Medical Provider, Concentra at:  
- If closed, call 911 and follow the instructions given  
- **Biological**: Wash with sudsing soap and water for 15 minutes.

For all exposures:  
- Notify your supervisor  
- Secure the area before leaving

## 3. REPORT THE INCIDENT

For hospitalization, fatality or recombinant nucleic acid exposure:  
- Notify EH&S immediately after performing first aid and getting medical help:  
- Call the EH&S main phone line at: 561-297-3129

All incidents and near misses:  
- Submit an incident report to EH&S. The incident report form can be found here:
APPENDIX E

Autoclave Log
# AUTOCLAVE LOG SHEET

All loads containing biohazardous waste must be autoclaved at 121°C for a minimum of 30 minutes.

<table>
<thead>
<tr>
<th>Autoclave make/model:</th>
<th>Location (building/room number):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab/Facility name:</td>
<td>Principal Investigator/Supervisor name:</td>
</tr>
<tr>
<td>Person responsible for autoclave:</td>
<td>Phone Number:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Contents</th>
<th>Cycle Number</th>
<th>Cycle Type</th>
<th>Sterilization Time (min)</th>
<th>Pressure (psi)</th>
<th>Max Temp Reached (°C)</th>
<th>Tape Results (pass/fail)</th>
<th>Chemical Integrator Result (pass/fail)</th>
<th>Biological Indicator Used? (Y/N)</th>
<th>Operator</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
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APPENDIX F

Biosafety Incident Report Form
Biosafety Incident Report Form

This is not a worker’s compensation report.

If this is an injury to a paid employee, have you filled out a worker’s compensation form?

☐ Yes  ☐ No

<table>
<thead>
<tr>
<th>Personal Information (affected party)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date:</td>
<td>Z Number:</td>
</tr>
<tr>
<td>First Name:</td>
<td>Last Name:</td>
</tr>
<tr>
<td>Email:</td>
<td>Cell Phone Number:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact Information (if person completing form is different from affected party):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First Name:</td>
<td>Last Name:</td>
</tr>
<tr>
<td>Email:</td>
<td>Cell Phone Number:</td>
</tr>
</tbody>
</table>

Incident Information

Indicate material involved by checking below as appropriate:

☐ Infectious Agent  ☐ Recombinant/Synthetic Nucleic Acids  ☐ Poisonous Plants

☐ Human Derived Materials  ☐ Biological Toxins

☐ Animal Derived Materials  ☐ Venomous Animals

<table>
<thead>
<tr>
<th>Location: (building, room):</th>
<th>Time of Incident:</th>
</tr>
</thead>
</table>

Incident Type (exposure, physical injury, etc.):

Incident Description (provide as much detail as possible and list external events that may have contributed to the incident):

What conditions or actions contributed to the incident?
**Method and Location of Injury/Exposure (check all that apply):**

**Method:**
- Needlestick
- Blood or Body Fluids
- Biological Spill
- Aerosol
- Animal Bite/Scratch
- Sharps
- Container Loss of Containment
- Other (describe)

**Note:**
- Needlestick
- Blood or Body Fluids
- Biological Spill
- Aerosol
- Animal Bite/Scratch
- Sharps
- Container Loss of Containment
- Other (describe)

**Action(s) taken to control risk of future incidents (e.g., hand washing, spill clean-up, etc.):**

**Personal Protective Equipment (PPE) worn at time of injury/exposure:**

<table>
<thead>
<tr>
<th>Scrubs</th>
<th>Surgical Gown</th>
<th>Lab Coat</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-95 respirator mask</td>
<td>Gloves</td>
<td>PAPR</td>
</tr>
<tr>
<td>Tyvek</td>
<td></td>
<td>Face Shield</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goggles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shoes</td>
</tr>
</tbody>
</table>

**What additional PPE may have mitigated the incident?**

**Explain:**

In the event of an injury, exposure, or near miss complete this form and email it to [EHS@fau.edu](mailto:EHS@fau.edu).

If you need assistance, contact the Biological Safety Officer at 561-297-3129.