

TITLE:

**EHS-402, BIOSAFETY MANUAL**

## Table of Contents

<b>1.0</b>	<b>Introduction.....</b>	<b>4</b>
1.1	Purpose .....	4
1.2	Scope .....	4
1.3	Prerequisites.....	4
1.4	Management.....	4
	Biosafety Officer (BSO).....	5
	Institutional Biosafety Committee / Research Health and Safety Committee (IBC/RHSC) Chair .....	5
	Institutional Biosafety Committee / Research Health and Safety Committee (IBC/RHSC) Members.....	5
1.5	Definitions.....	5
1.6	Responsibilities.....	6
	Biosafety Committee (IBC/RHSC) .....	6
	Biosafety Officer (BSO).....	7
	Executive Management .....	7
	Principal Investigator (PI) and Primary Supervisors.....	7
	Employees and Laboratory Workers.....	8
	Occupational Injury Management .....	9
1.7	Training Requirements .....	9
1.8	Recordkeeping Requirements .....	9
1.9	Review/Revision .....	9
<b>2.0</b>	<b>Emergency Procedures .....</b>	<b>9</b>
2.1	Biological Spills.....	10
2.2	Basic Biological Spill Kit .....	10
2.3	Bio-Spill Clean Up Procedures .....	10
	Spill Inside the Biological Safety Cabinet.....	11
	Spill Inside of a Centrifuge .....	11
	Spill Outside of the Laboratory (example – during transport).....	11
<b>3.0</b>	<b>Biosafety .....</b>	<b>12</b>
3.1	General Principles .....	12
3.2	Biosafety Levels for Biological Materials .....	13
	Biosafety Level 1 .....	13
	Biosafety Level 2 .....	13
	Biosafety Level 3 .....	14
	Biosafety Level 4 .....	14
	Table 1.0 Summary of Biosafety Levels for Infectious Agents (BSL 1-4).....	14
3.3	Classification of Biological Agents Based on Hazards (Risk Groups).....	15
	Table 2.0 Basis for the Classification of Biological Agents by Risk Group.....	16
3.4	Routes of Infections.....	16
3.5	Biological Risk Assessments (RA).....	17
3.6	General Considerations for Work with Biological Toxins .....	18
3.7	Work with Plants.....	18

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

<b>4.0</b>	<b>Animal Biosafety .....</b>	<b>19</b>
4.1	Biosafety and Animals-Infectious Disease Work with Vertebrates.....	19
	Table 3.0 Animal Biosafety Levels.....	19
4.2	Preventing Transmission of Zoonotic Diseases.....	21
	Risks for Those Who Handle Animals and Their Tissues .....	21
	Overview of Zoonotic Diseases .....	21
	Special Considerations for Pregnant Employees.....	21
4.3	Herpes B Virus .....	22
	Working with Non-human Primates, Their Tissues and Blood .....	22
	First Aid Following Potential Exposure.....	22
	Herpes B Virus Training.....	23
4.4	General Laboratory Requirements for Working with Animals .....	23
	Working with Dogs or Cats .....	23
	Work with Farm Animals .....	23
	Work with Birds, Rabbits, Fish and Snails .....	24
	Work with Rodents (e.g. Gerbils, Guinea Pigs, Hamsters, Mice or Rats) .....	24
4.5	Work with Arthropods .....	25
<b>5.0</b>	<b>Exposure Control Methods.....</b>	<b>25</b>
5.1	Engineering Controls .....	25
	Ventilation .....	25
	Biological Safety Cabinets (BSCs).....	25
	Bunsen Burners and Loop Sterilizers inside the BSC .....	27
	Chemical Fume Hoods .....	27
	Safety Equipment .....	27
5.2	Administrative Controls.....	28
	Medical Surveillance.....	28
	Hazard Communication .....	29
	Animal Rooms .....	30
	Labeling and Storage of Biological Materials .....	30
	Figure 1.0 Examples of Biohazard Signs.....	31
	Safety Standard Operating Procedures (SOP) .....	31
5.3	Recommended Work Practices .....	31
	Pipettes and Pipetting Aids .....	31
	Syringes and Needles and Other Sharps.....	32
	Cryostats.....	33
	Centrifuge Equipment .....	33
	Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers .....	34
	Ampules.....	35
	UV Lights .....	35
	Vacuum Lines .....	35
	Figure 2.0 Vacuum Set-Up for Biohazard Waste .....	35
	Housekeeping.....	36
	Packaging and Transportation of Biological Materials On and Off Site.....	36
5.4	Work-Specific Guidelines .....	37
	Guidelines for Working with Recombinant and Synthetic Nucleic Acid Molecules....	37

TITLE:

**EHS-402, BIOSAFETY MANUAL**

Guidelines for Working with Tissue Culture/Cell Lines .....	37
Working with Human or Non-Human Primate Cells and Tissues .....	37
Guidelines for Preventing the Transmission of Tuberculosis .....	38
Guidelines for Clinical Laboratories .....	38
Biological Agent Reference Sheet (BARS) .....	39
Biological Materials Registration .....	39
Bloodborne Pathogens Program and Exposure Control Plan .....	39
CDC and USDA Permits .....	40
<b>6.0 Biohazardous Waste Management .....</b>	<b>40</b>
<b>7.0 Non-Contaminated Broken Glass .....</b>	<b>41</b>
<b>8.0 Personal Protective Equipment.....</b>	<b>41</b>
8.1 Face Protection .....	41
8.2 Laboratory Clothing .....	41
8.3 Gloves .....	42
8.4 Respirators .....	42
<b>9.0 Methods of Decontamination .....</b>	<b>42</b>
9.1 Heat.....	43
9.2 Liquid Chemicals Used as Disinfectants.....	43
Table 4.0 Increasing Resistance to Chemical Disinfectants .....	43
Alcohols .....	44
Formalin.....	44
Glutaraldehyde .....	44
Phenol and Phenol Derivatives.....	44
Quaternary Ammonium Compounds (Quats).....	44
Halogens (Chlorine and Iodine) .....	44
9.3 Vapors and Gases.....	44
Table 5.0 Comparison of the Most Common Vapors and Gases Used for Decontamination .....	45
9.4 Radiation .....	45
<b>10.0 Use of Laser-Containing Sources .....</b>	<b>45</b>
<b>11.0 References .....</b>	<b>45</b>
<b>Appendix A: Spill Kit Essentials and Steps to Clean-Up Spill .....</b>	<b>48</b>
<b>Appendix B Safety Checklist for Operating a Small Cryostat or Microtome .....</b>	<b>50</b>
<b>Appendix C Safety Checklist for Using Lyophilizers.....</b>	<b>51</b>
<b>Appendix D. Characteristics of Selected Disinfectants .....</b>	<b>53</b>

TITLE:

**EHS-402, BIOSAFETY MANUAL**

## 1.0 Introduction

### 1.1 Purpose

This Biosafety Manual has been developed as part of the overall Emory University Biosafety Program. The manual was established to provide guidance in accomplishing the following goals:

- Protect personnel from exposure to infectious agents;
- Prevent environmental contamination;
- Provide an environment for high quality research while maintaining a safe work place, and;
- Comply with applicable federal, state and local requirements.

The primary objective of the Biosafety Program is to provide guidance to employees assigned to work with, or in the vicinity of, potentially infectious or otherwise hazardous materials derived from plant, animal or human sources.

In general, the handling and manipulation of biohazardous materials requires the use of various precautionary measures depending on the material(s) involved. Biohazardous materials will henceforth refer to all infectious agents, toxins, as well as recombinant and synthetic nucleic acids. This manual will aid in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary. The Biosafety Officer (BSO) and the Institutional Biosafety Committee (IBC) are available to assist in matters related to the use of recombinant or synthetic nucleic acids; the BSO and the Research Health and Safety Committee (RHSC) will assist in all non-recombinant-related research.

The program and manual follow the guidance of the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual, 2009 (BMBL). When the BMBL does not adequately address the hazards associated with an agent or process, other University-recognized biosafety guidance documents shall be used. References on Section 11 for a list of biosafety resources.

### 1.2 Scope

This Biosafety Program applies to all Emory University personnel whose occupational tasks or responsibilities include the handling and manipulation of biohazardous materials. This includes occupations with non-routine exposure.

### 1.3 Prerequisites

It is the employee's right to have access to information about the known physical and health hazards of potentially infectious and hazardous materials in his/her work areas and to receive adequate training to work safely with or around these substances.

The Biosafety Manual will be readily available to employees through their Principal Investigator (PI) or Primary Supervisor and is accessible from the Emory University's Environmental Health and Safety Office (EHSO) Web Site: [www.ehso.emory.edu](http://www.ehso.emory.edu).

### 1.4 Management

The Biosafety Program is a cooperative effort between Emory University and its employees.

## TITLE:

EHS-402, BIOSAFETY MANUAL

The IBC/RHSC, the Biosafety Officer, PIs and laboratory personnel must work in concert to minimize the risk of injury and illness associated with research involving potentially biohazardous materials.

The Biosafety Program is managed through oversight provided by the IBC/RHSC and the BSO. The IBC/RHSC is responsible for implementation of biosafety policies throughout the University.

Following is a list of individuals and/or organizations and their assigned responsibilities that will ensure the Biosafety Program is effectively implemented:

***Biosafety Officer (BSO)***

It is recommended that the BSO be experienced in the control and safe handling of laboratory biosafety hazards and the regulations which govern and provide guidance to biosafety issues.

***Institutional Biosafety Committee / Research Health and Safety Committee (IBC/RHSC) Chair***

Chair shall be a senior researcher with extensive knowledge in working with potentially infectious materials and/or toxins.

***Institutional Biosafety Committee / Research Health and Safety Committee (IBC/RHSC) Members***

The function of each member is to assist the Chair and the BSO in all matters relating to Biosafety. Members are appointed by management on the recommendation of the IBC/RHSC for a 2 -3-year (renewable) term. They should be selected from the scientific community according to the NIH guidelines based on their past or current experience in working with biohazardous materials, or because of their need to be closely aligned to the Biosafety Program.

**1.5 Definitions**

**Biohazardous Agents.** Infectious or etiologic (disease causing) agents, potentially infectious materials, certain toxins and other hazardous biological materials are included in the definition of a biohazard. Biohazardous materials may include but are not limited to: bacteria, fungi, viruses, rickettsia, chlamydia, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain viroids, prions and other infectious agents as outlined in laws, regulations, or guidelines.

**Biohazardous Materials.** Materials containing biological agents that are potential sources of transmission of such agents to healthy (i.e., non-immunocompromised) humans, animals, or plants (e.g., human blood) and/or that can produce an unfavorable environmental impact outside of the facility.

**Biological Waste.** Solid and liquid waste generated in the research laboratory which may contain biological materials or remnants of biological materials. Biological waste contaminated with radioisotopes or hazardous chemicals should be disposed of as mixed waste according to Standard Operating Procedures (SOP) reviewed by the Biosafety Office.

TITLE:

**EHS-402, BIOSAFETY MANUAL**

**Diagnostic Specimen.** Any human material including, but not limited to, excreta, blood and its components, tissue and tissue fluid being shipped for purposes of diagnosis.

**Exposure Incident.** An accident resulting in the inoculation, inhalation or mucous membrane exposure to a biological agent or biohazardous material. This may include specific eye, mouth, other mucous membrane, nonintact skin, or parenteral contact.

**Household Bleach.** An aqueous solution of 5.25% sodium hypochlorite with an approximate concentration of 50,000 parts per million (ppm) of available chlorine. A 10% dilution in water (1-part bleach; 9-parts water) yielding an approximate concentration of 5,000 ppm of available chlorine should be prepared regularly for the purpose of disinfection.

**Infectious Substance.** A substance containing a viable microorganism, or its toxin, that is known or is suspected to cause disease in animals or humans.

**Medical Waste.** According to the EPA, Medical waste is defined as: "... a subset of wastes generated at health care facilities, such as hospitals, physicians' offices, dental practices, blood banks, and veterinary hospitals/clinics, as well as medical research facilities and laboratories. Generally, medical waste is healthcare waste that that may be contaminated by blood, body fluids or other potentially infectious materials and is often referred to as regulated medical waste."

**Off-Site Transportation.** Transportation that goes beyond the confines of an operating facility on campus (e.g., requires travel on a public road).

**On-Site Transportation.** Transportation within the confines of Emory University, including main campus and off-site locations, including travel on a public road.

## 1.6 Responsibilities

Each personnel or lab involved in the use of biohazardous materials has a defined degree of responsibility for implementation of the Biosafety Program. Failure of any personnel to recognize this responsibility or to comply with established procedures is cause for disciplinary action.

### ***Biosafety Committee (IBC/RHSC)***

**NOTE:** For the purposes of this document, we refer to the IBC/RHSC as the Biosafety Committee. IBC is an institutional committee created under the NIH Guidelines to review research involving the use of recombinant and synthetic nucleic acids.

- Develop policy and procedures, which provide guidance for activities involving potentially biohazardous materials.
- Ensure that our biosafety policies, practices and facilities meet regulatory requirements and follow University-accepted practice.
- Ensure that an inventory of potentially biohazardous materials and toxins is maintained.
- Review and/or approve risk assessments for specific biohazardous agents. When warranted, ask whether the scientific aims of the proposed research cannot be sought by using materials of lower biohazard potential, and when

**TITLE:****EHS-402, BIOSAFETY MANUAL**

appropriate, bring to executive management's attention the risks associated with an experiment.

- Review biological registrations submitted through Electronic platform, EHSO's electronic management platform. The biological registration includes the use of non-hazardous, potentially biohazardous materials, and or toxins.

***Biosafety Officer (BSO)***

The BSO shall:

- Review activities and facilities for proper biohazard control, apply relevant laws, standards and guidelines, and be aware of community concerns and health and environmental considerations.
- Take measures necessary to ensure that all biohazardous activities comply with the policies and practices established by the IBC/RHSC.
- Report any significant problems, trends and/or violations of regulations or policies and practices to the IBC/RHSC and appropriate support and management.
- Assist the PI and laboratory staff in identifying hazardous operations. establishing safe work practices and selecting protective equipment and other exposure controls.
- Interact with the PI to evaluate and correct deficiencies in the Biosafety Program.
- Support follow-up to accidents and incidents and assist the PI with accident investigation.
- Advise the IBC/RHSC, PIs and workers on biosecurity, biosafety and technical compliance questions.

***Executive Management***

- Management is responsible for maintaining University safety and compliance with the Biosafety Program.
- They have the responsibility to support the BSO, IBC/RHSC, and the PIs in implementing the provisions of the Biosafety Program within their respective departments.

***Principal Investigator (PI) and Primary Supervisors***

The PIs/Primary Supervisors are responsible for biosafety in their laboratory. They shall:

- Register the use of biological, chemical and radioactive materials with EHSO.
- Ensure that all work is conducted in accordance with established policies and guidelines described in this document.
- Ensure that all employees under his/her supervision are adequately trained in good microbiological techniques and have received required biosafety training.
- Develop, review and approve laboratory-specific and/or protocol-specific procedures, consulting with the BSO when necessary.
- Provide training/information to all employees under his/her supervision regarding laboratory-specific or protocol-specific hazards and document such training.
- Ensure employee participate in Occupation Health Program as needed.
- Ensure that all at-risk employees have been informed of risk assessments and/or provisions for any recommended precautionary medical practices, such

**TITLE:****EHS-402, BIOSAFETY MANUAL**

as vaccinations and any special health or handling requirements regarding potentially biohazardous materials or toxins used or stored in the laboratory or work area.

- Ensure prompt reporting of any job-related injuries, exposures or illnesses via PeopleSoft.
- Inform the PI or Primary Supervisor and BSO of any serious, or potentially serious, accidents/incidents or situations involving exposure to biohazardous materials. This would include any accidental releases, illnesses or diseases to workers, plants or animals involved in or potentially exposed to the activity, and any possible adverse personnel exposure.
- Act upon requests and/or directives from the IBC/RHSC and/or BSO and correct any unsafe laboratory conditions.
- Ensure that appropriate containment devices and other engineering controls are in place and appear to be operating correctly, are current with certifications (where applicable) and are used according to established procedures.
- Conduct regular laboratory safety inspections and participate in audits and evaluations as necessary.
- Ensure that appropriate personal protective equipment (PPE) is available, used and that staff is adequately trained on the use and limitations of PPE equipment.
- Keep self and staff informed of new criteria, guidelines, directives or procedures that may be developed or which become applicable to activities in which they are engaged.
- Ensure proper decontamination of the laboratory or animal facility and equipment necessary to ensure safety during any needed inspection, calibration certification, disposal or termination of use.
- Ensure proper disposal of all infectious material or toxins.
- Keep required inventory and use of specific agents requiring such documentation.
- Provide adequate storage of materials and security based on risk categorization.
- Maintain proper biohazard labeling of premise under their control.

***Employees and Laboratory Workers***

All employees performing work with biohazardous materials must accept a shared responsibility for operating in a safe manner. Ultimately, everyone is responsible for his/her own safety. They also shall:

- Ensure that all work is conducted in accordance with established policies and guidelines described in this document or specific laboratory SOPs.
- Report all hazardous conditions to the PI and /or BSO.
- Promptly report any job-related injuries, exposures or illnesses to the PI and/or BSO and seek medical treatment immediately.
- Refrain from operating any equipment or instrument without proper instruction.
- Request information and training when unsure how to handle potentially hazardous materials.
- Wear and maintain personal protective equipment necessary to perform each task.
- Use engineering controls properly, e.g. biosafety cabinet.
- Practice good microbiological techniques.
- Participate in all required training programs.



TITLE:

**EHS-402, BIOSAFETY MANUAL*****Occupational Injury Management***

- Coordinate follow-up to injuries, illnesses and incidents, including medical consultation and/or examination.
- Assist in developing surveillance programs for work involving specific biohazardous agents. Programs such as vaccination should be included.

**1.7 Training Requirements**

Good microbiological and laboratory practices are essential for a safe work environment. All personnel working with Risk Group 2 or 3 agents (RG-2 or 3) or at BSL-2 or 3 should receive adequate laboratory specific training from the PI or primary supervisor. Training should include, at a minimum:

- Good laboratory and animal practices as applicable
- Specific information on risks, hazards, and procedures
- Laboratory or environment specific BSL-2 or 3 procedures as applicable
- All personnel working with biological materials in research spaces shall complete training in person or online using the electronic platform:
- Research Laboratory Safety - annually
- Bloodborne pathogens training (if working with materials of human source) - annually
- Biosafety - every three years
- Additional training may be necessary for high consequence pathogens based on risk assessment.

**1.8 Recordkeeping Requirements**

This Biosafety Manual shall be retained in accordance with the Rules, Regulations, and Guidelines listed on Section 11. This manual shall be maintained by EHSO.

**1.9 Review/Revision**

- The Biosafety Manual shall be reviewed and updated periodically and whenever necessary to reflect new or modified tasks and procedures.
- All change requests to this manual should be submitted to the BSO.

**2.0 Emergency Procedures**

- Report ALL incidents and accidents. Individuals who are injured while conducting research activities must promptly notify their supervisor and report to Employee Health according to Emory University Policy 4.93 Workplace Health and Safety.
- It is the expectation that ALL work-related injuries or illnesses be reported immediately or as soon as reasonably possible
- What to do if an exposure occurs?
  1. Remove PPE
  2. Provide immediate care to exposure site, for example wash wound with soap and water (if possible) for 15 minutes
    - Blood or Body Fluids Exposure —Including Needle Sticks: Wash exposed area with soap and water for 15 minutes.
    - (mucus membrane) exposures: Flush the area (eyes, mouth or nares) with water for 15 minutes.
  3. Seek medical attention as necessary
  4. Notify supervisor

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- Refer to Emory University Emergency Response and Evacuation Procedures for more information on chemical, biological and radiological spills, fire, evacuations and tornadoes.
- Exposures are reported in PeopleSoft (<https://hrprod.emory.edu>)  
Emory HR website > Self-Service > Workplace Health> HOME portal  
OIM: 7:30 am- 4pm 404-686-8587  
After Hours, Weekends, Holidays: NP On Call: 404-686-5500 PIC# 50464
- Yerkes National Primate Center Accident/Injury Reporting  
Yerkes Environmental Health and Safety Office  
3rd floor Main Center room # 3147 or 2nd floor room # 2109  
Office: 404-727-8012  
Cell: 404-275-0963
- Additional information can be found at the Emory University Accident/Injury Reporting webpage.
- To report a spill, contact the EHSO Spill Response Team at 404-727-2888 – this is a 24/7 number, someone will contact you to get additional details.
- Undergraduate students conducting research activities in the laboratory must promptly notify their supervisor and contact Emory University Student Health Services if there is an accident, incident or near-miss.
  - Link to Student Health Services:  
[http://studenthealth.emory.edu/hs/about/patient\\_portal.html](http://studenthealth.emory.edu/hs/about/patient_portal.html)

## 2.1 Biological Spills

The hazard associated with a biological spill is a function of the volume of the spill, the pathogenicity of the agent, and its concentration within the spilled material. When a spill occurs, the appropriate response should consider the protection of employees, preventing release of viable biological agents outside of the BSL-2 area, and cleanup/decontamination of the area. A minor biological spill is one that the laboratory staff is capable of handling safely without the assistance of the spill response team. All other biological spills are considered major and Spill Team should be called at 404-727-2888.

Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards should always have a basic biological spill kit ready to use. For most instances the basic kit can be assembled with materials already used in the laboratory. Although it is preferable to have the content of the spill kit in one location, if the materials are easily accessible to everyone in the lab, prior assembly might not be necessary.

## 2.2 Basic Biological Spill Kit

- A basic spill kit should contain the following, but is not limited to:
  - Disinfectant (e.g., bleach);
  - Absorbent Material (e.g., paper towels);
  - Waste Container (e.g., biohazard bags, sharps containers);
  - Personal Protective Equipment (e.g., lab coat, gloves, eye and face protection);
  - Mechanical Tools (e.g., forceps or tongs, dustpan and broom).
- Instructions for cleaning a spill in the laboratory can be found in Appendix A.

## 2.3 Bio-Spill Clean Up Procedures

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

In addition to the procedures on the Emergency Response Guidelines located in all laboratories, the following procedures are provided as a guideline to biohazardous spill cleanup and will need to be modified for specific situations. As with any emergency situation, stay calm.

If the spill requires assistance from the spill team, especially if the spill outgrows the resources in the laboratory, call the EHSO Spill Team at 404-727-2888. This is available 24 hours a day, 7 days a week.

***Spill Inside the Biological Safety Cabinet***

- Have a complete biological spill kit ready to go before starting clean-up procedures.
- Wear a lab coat, safety goggles, gloves, and respiratory protection (as appropriate).
- Allow cabinet to run during clean up.
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.
- Wipe up spillage and disinfectant with disposable paper towels.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags or heat resistant pans or containers with lids before autoclaving and further clean up.
- Expose non-autoclavable materials to disinfectant with at least 10 minutes contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for further processing by laundry.
- Run cabinet at least 10 minutes after cleaning up and before resuming work.
- Inform all users of the BSC as well as the laboratory supervisor about the spill and successful clean up as soon as possible.

***Spill Inside of a Centrifuge***

- Have a complete biological spill kit ready to go before starting the cleanup procedures.
- Clear area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up the spill.
- Wear a lab coat, safety goggles, appropriate respiratory protection and gloves during clean up.
- Remove rotors and buckets to the nearest biological safety cabinet. The rotors and buckets will also need to be properly decontaminated.
- Thoroughly disinfect inside of centrifuge.
- Remove contaminated debris after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal.

***Spill Outside of the Laboratory (example – during transport)***

- Should a spill of biohazardous material occur outside the laboratory in a public area, the Spill Response Team should be contacted, dial 404-727-2888.

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- Do not attempt to clean up the spill without the proper personal protective equipment and spill clean-up materials.
- Always transport biohazardous materials in accordance with packaging and transportation of biological materials on and off site as noted in the packaging and transportation of biological materials on and off site heading on Section 5.3.

## 3.0 Biosafety

### 3.1 General Principles

**NOTE:** In recognition of the growing number of microbiological and biomedical laboratories working with toxins of biological origin, guidelines for working with these materials can be found in the Guidelines for Working with Toxins of Biological Origin section.

Biological safety or biosafety is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or other biohazards. Biosafety defines the containment conditions under which these materials 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 biohazardous agents. It can be accomplished through the following means:

- Primary Containment - is the protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.
- Secondary Containment - is the protection of the environment external to the laboratory from exposure to biohazardous materials 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, which is not available at Emory University. Since most of the research at Emory University is conducted at BSL-2 with a few research experiments at BSL-3, this manual will mainly focus on those Biosafety Levels. For more information on Biosafety Level 4 requirements, refer to the appropriate literature or contact the Biological Safety Officer. A summary of the biosafety levels for infectious agent (BSL-1, 2 and 3) can be found in Table 1.

The recommended biosafety level(s) for an organism or toxin represents the conditions under which the agent can ordinarily be safely handled. The PI is specifically and primarily responsible for assessing risks and for appropriately applying the recommended biosafety level(s) (see Assessments on section 3.5).

The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. Everybody working with infectious agents or potentially infected materials must be aware of the potential 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 PI or person in charge of the laboratory

TITLE:

**EHS-402, BIOSAFETY MANUAL**

to provide and/or arrange for appropriate training of personnel in their laboratory.

- Personnel shall not eat, drink, apply cosmetics or lip balm, smoke or handle contact lenses in work areas where there is a reasonable likelihood of exposure to biological materials.
- In addition, food and drink shall not be stored in refrigerators, freezers or cabinets where biological materials are present or other areas of possible contamination, such as counter tops.
- See the Emory University Guidelines for the Consumption and Storage of Food and Beverages in Laboratory Areas.

### 3.2 Biosafety Levels for Biological Materials

The following is a brief description of the biosafety levels as defined in the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition 2009 (BMBL). For more detailed information regarding the requirements for the different containment levels, contact the BSO.

#### ***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 are representative of the 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 animal host species or different. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

#### ***Biosafety Level 2***

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. 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, *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

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

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 located at [www.ehso.emory.edu](http://www.ehso.emory.edu) for specific, required precautions.

Secondary barriers such as hand washing and waste decontamination facilities must be available to reduce potential environmental contamination.

***Biosafety Level 3***

Biosafety level 3 (BSL-3) practices, safety equipment, and facilities are applicable 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.

***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.

***Research involving Risk Group 4 agents or those agents requiring BSL-4 containment is strictly prohibited at Emory University.***

**Table 1.0 Summary of Biosafety Levels for Infectious Agents (BSL 1-4)**

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Laboratory bench and sink required
2	Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of	BSL-1 plus: • Autoclave available

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
	membrane exposure	<ul style="list-style-type: none"> <li>Biosafety manual defining any needed water decontamination or medical surveillance policies</li> </ul>	agents that cause splashes or aerosols of infectious materials PPE*: <ul style="list-style-type: none"> <li>Laboratory coats; gloves; face protection as needed</li> </ul>	
3	<ul style="list-style-type: none"> <li>Indigenous or exotic agents with potential for aerosol transmission</li> <li>Disease may have serious or lethal consequences</li> </ul>	BSL-2 practice plus: <ul style="list-style-type: none"> <li>Controlled access</li> <li>Decontamination of all waste</li> </ul>	<ul style="list-style-type: none"> <li>Primary barriers:</li> <li>Class I or II BSCs or other physical containment devices used for all open manipulation of agents</li> <li>PPE:                             <ul style="list-style-type: none"> <li>Protective laboratory clothing; gloves; respiratory protection as needed, and lab coat is decontaminated before laundering</li> <li>Baseline serum</li> </ul> </li> </ul>	BSL-2 plus: <ul style="list-style-type: none"> <li>Physical separation from access corridors</li> <li>Self-closing, double-door access</li> <li>Exhaust air not recirculated</li> <li>Negative airflow into laboratory</li> </ul>
4	<ul style="list-style-type: none"> <li>Dangerous/exotic agents which pose high risk of life-threatening disease</li> <li>Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission</li> </ul>	BSL-3 practices plus: <ul style="list-style-type: none"> <li>Clothing change before entering</li> <li>Shower on exit</li> <li>All material decontaminated on exit from facility</li> </ul>	Primary barriers: <ul style="list-style-type: none"> <li>All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit</li> </ul>	BSL-3 plus: <ul style="list-style-type: none"> <li>Separate building or isolated zone</li> <li>Dedicated supply and exhaust, vacuum, and decontamination systems</li> <li>Other requirements outlined in the text</li> </ul>

\*Animal Biosafety Levels (ABSL) can be found in Table 3.0

### 3.3 Classification of Biological Agents Based on Hazards (Risk Groups)

Worldwide there are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ from each other, they all are based on the notion that some microorganisms are more hazardous than others are. In general, the pathogenicity of the

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

organism, mode of transmission, host range, availability of effective preventive measures and/or effective treatment is some of the criteria taken into consideration when classifying infectious agents. In the U.S., the most current classification is found in the CDC 5th BMBL. The human etiologic agents addressed in these guidelines are classified into four risk groups with Risk Group 1 (RG-1) of low or no hazard and Risk Group 4 (RG-4) representing highly infectious agents (Table 2.0).

Determining the risk of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level for work practices and containment. In general, RG-2 agents are handled at BSL-2, and RG-3 agents at BSL-3. However, certain RG-2 agents depending upon the operation may require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2 under certain conditions. HIV is an example of a RG-2 agent that depending on the task being performed will be handled at BSL-2 or BSL-3.

For more information, refer to the Biological Risk Assessments (RA) in section 3.5 or contact the BSO.

***Table 2.0 Basis for the Classification of Biological Agents by Risk Group***

<b>RISK GROUP</b>	<b>RISK TO THE INDIVIDUAL AND THE COMMUNITY</b>
<b>Risk Group 1 (RG-1)</b>	A biological agent that is unlikely to cause disease in healthy workers or animals.
<b>Risk Group 2 (RG-2)</b>	A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment and for which preventive or therapeutic interventions are often available.
<b>Risk Group 3 (RG-3)</b>	Agents that are associated with serious or lethal human or animal disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
<b>Risk Group 4 (RG-4)</b>	Agents that are likely to cause serious or lethal human or animal disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

### 3.4 Routes of Infections

When working in a biological research environment, it is not unreasonable to expect that a laboratory person working with infectious materials are more likely to become infected than members of the general community are. 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:

- Through the mouth
  - Eating, drinking and smoking in the laboratory.
  - Mouth pipetting.
  - Transfer of microorganisms to mouth by contaminated fingers or articles.
- Through the skin
  - Accidental inoculation with a hypodermic needle, other sharp instruments or glass.
  - Abraded skin through cuts, scratches, skin rashes, etc.
  - Animal bites, scratches, scrapes, kicks, etc.



## TITLE:

## EHS-402, BIOSAFETY MANUAL

- Through the eye
  - Splashes of infectious material into the eye.
  - Transfer of microorganisms to eyes by contaminated fingers.
- Through the lungs
  - Inhalation of airborne microorganisms.

Most of the laboratory-acquired infections (LAIs) reported in the literature point to spills, splashes and accidents involving needles or other sharp objects. The general laboratory procedures outlined in this manual address those issues and provide guidance in handling infectious or potentially infectious materials.

### 3.5 Biological Risk Assessments (RA)

The assessment of risk is an essential element of safety in the laboratory. The RA should include information related to the microbe, appropriate safety equipment, occupational health requirements, training, decontamination, waste management, and safety Standard Operating Procedures (SOPs). For certain high-risk agents, Biological Agent Reference Sheets (BARS) are available to employees. The BARS outline recommendations for working with these agents within Emory University research laboratories.

Also, other excellent resources are the Centers for Disease Control and Prevention ([www.cdc.gov](http://www.cdc.gov)) and Canada Biological Agent Material Safety Data Sheets available. These apply to both human and animal pathogens. A current list of BARS and a link to the CDC and Public Health Agency of Canada are located on the EHSO web site.

For most situations, guidelines, rules and regulations have clearly defined the procedures and practices to be followed in order to achieve safety in the work place. However, in cases of newly isolated agents (emerging agents), toxins, or procedures not previously employed, further evaluation is needed. Since individual judgment involves both personal and social values, opinions on what is “safe” vary significantly. In order 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:

- **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: just 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.)
- **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 provides for a safe work environment and fosters excellence in scientific conduct.
- **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.
- **No detectable adverse effects:** This can be a very weak criterion since it

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

involves uncertainty and should be applied accordingly.

- **Existing/current 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, safety practices of close relatives should be consulted. 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 phylogenetic background warrant, at a minimum, the same level of protection.

Taking the above-mentioned factors and external resources 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, laboratory-specific SOPs should be established.

### 3.6 General Considerations for Work with Biological Toxins

Biological toxins are substances that can be produced by bacteria, fungi, protozoa, insects, animals, or plants. Biological toxins can often cause death or severe incapacitation at relatively low exposure levels. Biological toxins are non-replicative, and non-infectious.

Appendix I of the CDC 5th ed BMBL presents guidelines for work with toxins of biological origin. Here are the main considerations:

- The main laboratory risks are associated with accidental exposure by direct contamination of mouth, eyes, or mucous membranes, inadvertent aerosol generation or needle-stick.
- Personnel handling biological toxins should have completed Research Lab Safety training
- Personnel should be familiar with the Emory Chemical Hygiene Plan.
- An inventory system should be in place to account for toxin usage and disposition.
- Research space where work with biological toxins is conducted should be clearly posted "Toxins in Use".
- All work with toxins is recommended to be conducted inside a Chemical Fume Hood or BSC.
- PPE should include lab coat, disposable gloves, eye protection.
- Only workers trained and experienced in handling animals should be permitted to conduct activities involving injection of biological toxins.
- Discard sharps in a puncture-resistance sharps container.
- Most toxins are only inactivated by use of chemicals disinfectants such as sodium hypochlorite, sodium hydroxide or a combination of the two products.
- Contact the BSO to discuss the use of biological toxins.

### 3.7 Work with Plants

- All research activities conducted at Emory University involving Plants and Plant-pathogens must be registered through the electronic platform.
- Contact the Biosafety Office to initiate the registration process and prepare the corresponding SOPs.

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

- Plant Protection and Quarantine (PPQ) regulates the importation of plants and plant products under the authority of the Plant Protection Act. Ensure that all regulatory permits (USDA APHIS) are uploaded to the electronic platform.

## 4.0 Animal Biosafety

### 4.1 Biosafety and Animals-Infectious Disease Work with Vertebrates

Laboratory facilities must provide containment for laboratory animals exposed to or harboring infectious agents. The containment provided, 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-top cages, partial or isolation caging systems) may be used (Table 3.0).

Animal facilities are considered a special type of laboratory. Generally, the biosafety level of a given microorganism (including facilities, practices, and operational requirements) is comparable in both in vitro and in vivo models. Animal rooms present unique challenges, as the presence of an animal introduces new challenges including potential aerosolization or skin abrasions on the handlers.

Emory University will follow the animal biosafety guidelines outlined in CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual. 2009 (BMBL). For more detailed information regarding requirements contact your BSO or refer to the BMBL. Table 3.0 summarizes the requirements of Animal Biosafety Levels 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 Emory University IACUC prior to work beginning. In addition to IACUC approval, all animal work involving infectious agents or acute toxins shall be reviewed and approved by the BSO or designee of the Biosafety Committee. Contact the IACUC Office for more information.

**Table 3.0 Animal Biosafety Levels**

ABSL	AGENTS	PRACTICES	SAFETY EQUIPMENT (PRIMARY BARRIERS)	FACILITIES (SECONDARY BARRIERS)
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 <ul style="list-style-type: none"> <li>No recirculation of exhaust air</li> <li>Directional air flow recommended</li> <li>Hand washing</li> </ul>

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

ABSL	AGENTS	PRACTICES	SAFETY EQUIPMENT (PRIMARY BARRIERS)	FACILITIES (SECONDARY BARRIERS)
				sink is available
2	Associated with human disease Hazard: Percutaneous exposure, ingestion, mucous membrane exposure	ABSL-1 practices plus: <ul style="list-style-type: none"> <li>Limited access</li> <li>Biohazard warning signs</li> <li>Sharps precautions</li> <li>Biosafety manual</li> <li>Decontamination of all infectious wastes and of animal cages prior to washing</li> </ul>	ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> <li>Containment equipment appropriate for animal species</li> <li>PPEs1:                             <ul style="list-style-type: none"> <li>Laboratory coats, gloves, face and respiratory protection as needed</li> </ul> </li> </ul>	ABSL-1 facility plus: <ul style="list-style-type: none"> <li>Autoclave available</li> <li>Hand washing sink available</li> <li>Mechanical cage washer recommended</li> </ul>
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.	ABSL-2 practices plus: <ul style="list-style-type: none"> <li>Controlled access</li> <li>Decontamination of clothing before laundering</li> <li>Cages decontaminated before bedding removed</li> <li>Disinfectant foot bath as needed</li> </ul>	ABSL-2 equipment plus: <ul style="list-style-type: none"> <li>Containment equipment for housing animals and cage dumping activities</li> <li>Class I, II, III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols</li> <li>PPE: appropriate respiratory protection</li> </ul>	ABSL-2 facility plus: <ul style="list-style-type: none"> <li>Physical separation from access corridors</li> <li>Self-closing, double-door access</li> <li>Sealed penetrations</li> <li>Sealed windows</li> <li>Autoclave available in facility</li> </ul>
4	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of transmission.	ABSL-3 practices plus: <ul style="list-style-type: none"> <li>Entrance through change room where personal clothing is removed and laboratory clothing is put on;</li> <li>shower on exiting</li> <li>All wastes are decontaminated</li> </ul>	ABSL-3 equipment plus: <ul style="list-style-type: none"> <li>Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-</li> </ul>	ABSL-3 facility plus: <ul style="list-style-type: none"> <li>Separate building or isolated zone</li> <li>Dedicated supply and exhaust, vacuum and decontamination systems</li> <li>Other requirements</li> </ul>

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

ABSL	AGENTS	PRACTICES	SAFETY EQUIPMENT (PRIMARY BARRIERS)	FACILITIES (SECONDARY BARRIERS)
		before removal from the facility	supplied positive-pressure personnel suit) used for all procedures and activities	outlined in the text

## 4.2 Preventing Transmission of Zoonotic Diseases

### ***Risks for Those Who Handle Animals and Their Tissues***

Hazards associated with handling animals fall into three categories:

- Physical injuries can occur from bites or scratches (rodents, rabbits, dogs, cats, swine, non-human primates and others), including kicks or other direct injuries. The key to preventing these injuries is proper training of personnel by the animal care staff or other qualified individuals.
- 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 coat) when handling animals. Additional precautions may be posted on the animal room door.
- 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 secretion/excretion as well as the living animals can frequently transmit zoonotic diseases.

### ***Overview of Zoonotic Diseases***

Humans may 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, sign 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 occupational health physician know that he/she works with animals.

### ***Special Considerations for Pregnant Employees***

- Employees who become pregnant should contact Occupational Injury Management as soon as possible for a consultation. Refer to Emory University Radiation Safety Policy Manual at [www.ehso.emory.edu](http://www.ehso.emory.edu) on Emory University Policy on Radiation and Pregnancy.
- *Toxoplasma* sp. is a parasite 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

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

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 preexisting illness sets the stage for infection. Infection can cause acute febrile illness in pregnant women, followed by abortion, stillbirth or seriously ill premature infants. It can be acquired by direct 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.
- Zika virus can be passed from a pregnant woman to her fetus. Infection during pregnancy can cause a birth defect called microcephaly and other severe fetal brain defects. Individuals handling Zika Virus or samples potentially infected with Zika Virus, or individuals potentially exposed due to job activities, are required to complete an in-person agent-specific training and a consultation with the Occupational Health physician before initiating research activities. Zika virus primarily spreads when a mosquito infected with Zika bites the individual. Zika also can spread through sex, blood transfusion, contact with other potentially infectious materials or through a needle-stick.

### 4.3 Herpes B Virus

Although there are several nonhuman primate viruses that can cause disease in humans, Herpes B virus, is the virus of most concern to people working with macaques or macaque tissue. Herpes B virus is a neurotropic herpes virus indigenous to macaque monkeys (rhesus, cynomolgus, pig tail and stump tail). B virus infection in macaques is a mild or sub-clinical, infection; the animal may have signs of shedding with oral or genital lesions. B virus is associated with high morbidity and mortality rates in humans. The disease in humans is an acute, potentially fatal ascending myelitis and encephalitis. The greatest risk of B virus infection is associated with animal bites and scratches. However, B virus infections have occurred from contamination of broken skin or mucous membranes with oral, ocular or genital secretions from animals shedding the virus. B virus may also be present in the saliva, conjunctival and vesicular fluids; thoracic and abdominal viscera; and neurological tissues of infected macaques. Therefore, these substances, as well as tissues or cell cultures prepared from them, are potential hazards.

#### ***Working with Non-human Primates, Their Tissues and Blood***

Working with nonhuman primates, their tissues and body fluids presents unique biohazards. Nonhuman primates have their own species-specific viruses. Some of these agents have successfully transmitted to humans and have produced significant negative human consequences. The human consequences from other nonhuman primate viruses are still unknown.

#### ***First Aid Following Potential Exposure***

An exposure may be 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, cell cultures and body fluids.

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

Following a potential exposure, the most important first step is to immediately wash the site with soap and water for 15 minutes. If you experience an eye exposure, flush your eye for 15 minutes. Report the incident to your supervisor and contact the Yerkes Safety Office (404-727-8012) Cell (404-275-0963).

***Herpes B Virus Training***

Researchers handling non-human primate source materials such as tissues, fluids, cell lines, etc., should review the BARS for Herpes B Virus and contact the Yerkes Safety Office to schedule the specific in-person training.

It is extremely important that all employees working with NHP materials be informed of the post exposure requirements in the event of an exposure.

**4.4 General Laboratory Requirements for Working with Animals**

Universal precautions as well as strict adherence to ABSL-2 and BSL-2 practices and use of the appropriate Personal Protective Equipment (PPE) are necessary when handling nonhuman primates and nonhuman primate samples.

***Working 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 based on proposed research activities upon recommendation from the IBC/RHSC.
- 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.

***Work with Farm Animals***

- Cattle
  - 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.
- Swine
  - Erysipelas in pigs can be transmitted to humans causing a severe local skin infection. Therefore, pigs showing diagnostic "diamond back" lesions should be handled with care.
  - Commercial swine may carry salmonella and campylobacter.

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- Raccoons
  - 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 incinerate it. Heat is the only way to kill the eggs.
- Sheep
  - 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 respirator and protective clothing are required for individuals working with pregnant sheep and goats.
  - Infected persons can be effectively treated.
  - Skin lesions are seen on the hands after contact with sheep and goats infected with contagious ecthyma and vesicular stomatitis.
  - Rabies can be a threat from any unvaccinated cat or dog, or food animal, especially those on pasture or exposed to feral animals.
  - Aborted fetuses from swine and cattle, sheep and goats may be associated with 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).

***Work with Birds, Rabbits, Fish and Snails***

- Birds can be infected by organisms that cause diseases such as psittacosis and avian tuberculosis. Only birds with defined health status should be used in research studies.
- Rabbit skin mites such as Cheyletiella parasitovorax can cause transient rashes in humans and those working with rabbits should be conscious of possible allergic reactions.
- Salmonella is frequently harbored in turtles and other reptiles and amphibians. Transmission can be avoided by using protective clothing and good hygiene. When working with turtles and other reptiles use universal precautions and assume that they are infected.

***Work with Rodents (e.g. Gerbils, Guinea Pigs, Hamsters, Mice or Rats)***

- Contact with rodents requires precautions against such diseases as tapeworm



## TITLE:

**EHS-402, BIOSAFETY MANUAL**

infection, lymphocytic choriomeningitis (LCMV), salmonellosis and ringworm fungal skin infections. Additional concerns for investigators using certain rodents are leptospirosis and bubonic plague. Attention should also be paid to the possibility of allergic reactions.

- To protect against these agents, care should be taken to limit exposure to soiled bedding containing feces (salmonellosis, tapeworms) and urine (LCMV and leptospirosis). Gloves, safety glasses and respirators not only limit exposure to soiled bedding, but also help prevent transmission of diseases such as ringworm and fur mites when rodents are handled.
- There are infectious agents that can be transmitted to humans through rodent bites, but the incidence of these agents in modern rodent colonies is rare.

#### **4.5 Work with Arthropods**

- All research work conducted at Emory University involving arthropods (i.e. insects, flies, mites, butterflies, Drosophila) must be registered in the electronic platform.
- Contact the Biosafety Office to initiate the registration.

### **5.0 Exposure Control Methods**

Lab supervisors and primary supervisors are responsible for ensuring that control measures are in place to reduce employee exposure to biohazards. When practical, engineering controls, administrative controls, and personal protective equipment (in that order) should be used to reduce the potential for exposures.

#### **5.1 Engineering Controls**

- Engineering controls are methods of controlling employee exposures by modifying the source or reducing and controlling the quantity of contaminants released into the work environment. Examples include biological safety cabinets, fume hoods, glove boxes, and local exhaust.
- Engineering controls are the preferred primary control measure.

##### ***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:
- 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.
- Local Exhaust or Filtration: a ventilated, enclosed work space 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 pathogenic microbes, biosafety cabinets should be used.

##### ***Biological Safety Cabinets (BSCs)***

- BSCs are designed to provide personnel, environmental and product protection

**TITLE:****EHS-402, BIOSAFETY MANUAL**

when appropriate practices and procedures are followed. Three kinds of BSCs, designated as Class I, II and III, have been developed to meet various research and clinical needs. BSCs 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, BSCs are effective in containing and controlling particulates and aerosols. BSCs also complement good laboratory practices and procedures. The correct location, installation, and certification of the biological safety cabinet is 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 annually, if the cabinet is relocated, 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 from BMBL 5th edition (CDC BMBL 5th Ed Appendix A Biosafety Cabinets).
- A brief description of the different types of BSCs is as follows:
  - 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 is 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.
  - 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.
    - The Class II cabinet has four designs that differ in the amount of air that is recirculated and/or exhausted, and whether the BSC is hard-ducted to the ventilation system.

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation. Type B BSCs may also be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs. Cabinets that are type II A/B3 that are ducted require an exhaust alarm due to NSF changes in 2016. Care must be exercised when selecting the correct Class II cabinet design for these purposes. The BSO should be consulted to aid in the selection.
- Class III BSC
  - The Class III BSC is designed for work with biosafety level 4 microbiological agents 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).

***Bunsen Burners and Loop Sterilizers inside the BSC***

- Bunsen burners are not allowed inside BSCs or on the bench. 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 and the excess gas will continuously recirculate in the cabinet.
- 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 or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available or recommended.

***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.

***Safety Equipment***

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- SAFETY SHOWERS
  - Safety showers provide an immediate water drench for an affected person. Standards for location, design and maintenance of safety showers are outlined in the EHSO SAF-351 Chemical Hygiene Plan.
- EYEWASH STATIONS
  - Eyewash stations are required in all laboratories where hazardous 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 EHSO SAF-351 Chemical Hygiene Plan.
  - Eyewash stations must be tested monthly. A template to document the monthly testing can be found at [www.ehso.emory.edu](http://www.ehso.emory.edu).

## 5.2 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. Examples of unique programs include Waste Disposal, Bloodborne Pathogens Exposure Control Plan, Agent Registration, and CDC Select Agents.

### ***Medical Surveillance***

The following policies and medical surveillance guidelines have been developed to outline the responsibilities of facility management, safety, industrial hygiene and health professionals. When in doubt regarding the medical care/testing for a specific potential exposure, these professionals should be consulted. Throughout this manual, medical surveillance requirements for a specific exposure and/or job assignment have been documented (e.g., bloodborne pathogens, animal handlers, etc.). Additionally, the Biological Agent Reference Sheets (BARS) may contain medical surveillance recommendations or requirements.

- Periodic Medical Surveillance
  - Employees who are actively engaged in work with potentially biohazardous material may be provided the opportunity to update occupational and medical histories on an annual basis, based upon the agents in use or more frequently, on a case-by-case basis (i.e. post exposure to biohazardous materials). Specific testing may be offered.
- Guidelines for Handling of Biological Agents by Immunodeficient-Immunosuppressed Employees
  - When an employee's immune system is impaired by a condition such as those specified below, the risk of infection by biological agents increases. Therefore, many human or animal pathogens that previously represented little or no threat to the health of an employee must be considered a significant safety hazard. A formal evaluation of the employee's work and his/her relationship to it must be conducted to determine if continuation will jeopardize his/her health.

**TITLE:****EHS-402, BIOSAFETY MANUAL**

- Among the conditions that can adversely affect the immune system are:
  - Treatment with cytotoxic chemotherapeutic agents;
  - Treatment with adreno corticosteroids;
  - Treatment with immunosuppressive agents/drugs or certain antibiotics (contact EHM for guidance);
  - Disease processes that suppress the immune system;
  - Pregnancy;
  - Splenectomy;
  - Gastrointestinal disorders such as: colitis, ileitis, and active chronic diarrhea.
- Any employee who handles biological agents and is aware of being immunodeficient/ immunosuppressed must report this condition to the OIM office so that an appropriate evaluation of the employee's health and safety can be initiated. This communication is considered confidential. These employees should be counseled as to the advisability of working in areas where the potential for exposure to potentially hazardous organisms is present. Any limitations or restrictions shall be reported to the affected employee's supervisor by OIM.
- Vaccination Guidelines
  - For some etiologic agents, vaccines are available to provide additional protection for employees both before and sometimes following accidental exposure. The decision to offer a specific vaccine will be made by the Biosafety Committee (IBC/RHSC) and OIM taking into consideration regulations, vaccine status, personal history and potential job activities or accidental exposure.
- Pre-placement/Pre-assignment Examinations
  - When working with certain biological agents and/or biohazardous materials, a medical examination or occupational health consultation may be provided prior to assigning the individual to the area. This examination may include but is not limited to:
    - Medical/Occupational History
    - Physical Examination (by a physician or under the supervision of a licensed physician, where allowed by law)
    - Skin Test for Tuberculosis
    - Biochemical Tests (e.g., SMAC26)
    - Complete Blood Count
    - Urinalysis
    - Immunizations (if applicable)
    - Any other test deemed appropriate for the potential exposure
    - In-person consultation with the Occupational Health physician
- Termination of Project or Employment
  - Upon discontinuation of work with biological agents and/or biohazardous materials, employees may be offered the opportunity to receive a health evaluation as deemed necessary on a case-by-case basis (i.e. occupational illness, past exposure to biological agents).

***Hazard Communication***

- Biohazard Warning Sign
  - A biohazard label is required for all areas or equipment in which RG-2 or

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

3 agents are handled or stored, BSL-2 or 3 procedures are required, or where procedures involving human source materials are handled (i.e. phlebotomy rooms). The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, on equipment like refrigerators, incubators and transport containers.

- General Labeling and Signage Requirements for 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.
    - All laboratories where biohazards are stored or used are clearly labeled using the EHSO laboratory signage.
    - All other rooms and storage areas are clearly labeled with the lab signage.
    - All BSCs where biohazards are used are clearly labeled.
    - All refrigerators, freezers, centrifuges, and incubators where biohazards are used are labeled.
    - 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.
    - Equipment that is being moved to another location must be surface decontaminated and the Equipment Hazard tag needs to be affixed to the apparatus. The Equipment Hazard Tag and guidance are located at [www.ehso.emory.edu](http://www.ehso.emory.edu).
    - Equipment should not be moved from the work area without adequate disinfection or decontamination.
    - Any equipment items that leave the laboratory for service (University shop or third-party vendor) or disposal must be handled following the EHSO guidance for biohazard/decontamination tagging.

***Animal Rooms***

- PPE and work conditions to access animal rooms are defined and approved by the IACUC and the IBC/RHSC. All users are required to follow those conditions.

***Labeling and Storage of Biological Materials***

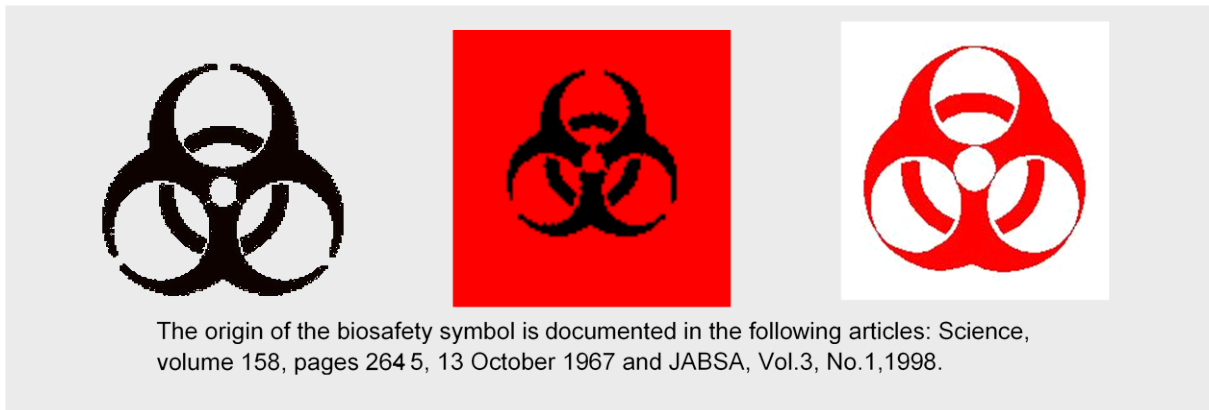
The contents of all laboratory containers shall be properly identified. One of the overriding goals of prudent practice in the labeling and identification of materials is to avoid orphaned containers of unknown materials. The labels should be written in English, and understandable to laboratory workers,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

- Sample Container Labeling and Storage Requirements:
  - 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.
  - If flammable materials are used, they must be stored in equipment that is designed for this purpose (e.g. lab safe refrigerators).
  - Personal items, e.g., food and beverages, are ABSOLUTELY prohibited in lab refrigerators, cold rooms, freezers or incubators (See Guidelines

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

- for the Consumption and Storage of Food and Beverages in Laboratory Areas)
- A variety of different biohazard labels are available through the laboratory supplies vendor (Figure 1.0).

**Figure 1.0 Examples of Biohazard Signs**



**Safety Standard Operating Procedures (SOP)**

- The PI prepares the safety SOP to inform laboratory personnel of the biological materials approved for use in research and the safety procedures and practices, including PPE, engineering controls, administrative controls, decontamination procedures, waste management, and emergency procedures to be followed in the lab.
- After the PI prepares the safety SOP, the BSO reviews it and provides feedback to the PI. The finalized version of the safety SOP is uploaded to the electronic management system.
- A template and example for the safety SOP are provided at [www.ehso.emory.edu](http://www.ehso.emory.edu).

**5.3 Recommended Work Practices**

**Pipettes and Pipetting Aids**

- When pipetting, use the following precautions:
  - Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used.
  - 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

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

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.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a BSC, a sharps containers, if needed, and a biohazard bag on holder should be available. Cover biohazard waste container when not in use.

***Syringes and Needles and Other Sharps***

- Syringes and hypodermic needles are dangerous objects that need to be handled with extreme caution to avoid accidental injection and aerosol generation.
- Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. The use of “safe” needles is highly recommended.
- Do not use a syringe and needle as a substitute for a pipette.
- When using syringes and needles, or other sharps with biohazardous or potentially infectious agents follow the Guidelines for the Safe Use of Sharps found at [www.ehso.emory.edu](http://www.ehso.emory.edu))
- Dispose of ALL needles and syringes (used or unused) in appropriate sharps containers. Do not discard syringes and needles into laboratory waste receptacles or pans containing pipettes or glassware.
- Sharps, such as razor blades or scalpels, should be kept in a container when not in use.
- Do not overfill sharps containers (2/3 filled = full). Most containers have an indicated fill line on the container.
- Keep sharps containers upright and adjacent to work area.
- DO NOT RECAP NEEDLES. If this is not a feasible alternative and you find that you MUST recap a needle the use of a mechanical device or the one-handed scoop method must be used. Contact EHSO to conduct a risk assessment.
- DO NOT BEND, CUT, REMOVE OR BREAK NEEDLES. If you find that a needle MUST be removed it must be done by a one-handed method. Throw intact needle/syringes into the sharps container for disposal.
- Use “safe” needles/syringes or sharps when appropriate.
- Use needle-locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe or syringes which involve safe needle technology.
- Plan your work to avoid quick and unnecessary movements while working with syringes.
- Where appropriate, fill or immerse syringes in disinfectant prior to disposing into sharps container. Syringes and needles that are autoclaved prior to disposal or preparation for washing can be autoclaved in a pan of disinfectant solution. Do not use bleach to disinfect this equipment.



## TITLE:

## EHS-402, BIOSAFETY MANUAL

- Use separate containers for disposable and non-disposable syringes and needles to eliminate the need to sort later.
- Wear gloves during all manipulations with needles and syringes for general safety.
- Examine glass syringes for chips and cracks, needles for barbs and plugs prior to sterilization and before use. Only use glass syringes as a last resort. Disposable syringes and needles and/or safe needle/syringe technology is preferred.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Work in a biosafety cabinet whenever possible.
- Expel excess air, liquid and bubbles from the syringe vertically into a cotton pad moistened with proper disinfectant, or into a small bottle of sterile cotton.
- If you are filling from a test tube, avoid contaminating the hub of the needle, this may result in transfer of infectious material to the hands.
- When inoculating animals be sure that your hands are BEHIND the needle to avoid punctures.
- Ensure that the animal is properly secured and restrained prior to inoculating. Be alert for any unexpected movements of the animal.
- What Goes in A Sharps Container?
  - Razor blades
  - Scalpel blades
  - Syringes (with or without needles)
  - Glass Pasteur pipettes
  - Broken contaminated glass
  - When full: seal container per instructions and discard in biohazard waste box
- Pipet Boxes can be used to discard the following materials
  - Glass or plastic pipets
  - Pipet tips
  - Wooden swabs
  - Blood tubes
  - When full: seal container per instructions and discard in biohazard waste box

**Cryostats**

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because freezing tissue usually does not inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. For guidance on safe use of Cryostats or microtomes refer to Appendix B.

**Centrifuge Equipment**

- Hazards associated with centrifugation include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions including safety precautions should be prominently posted on the unit.
- Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

is created if a tube breaks during centrifugation. Use of glass tubes should be avoided wherever possible. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters (see vacuum lines below).
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

***Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers***

- The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.
- Safety Laboratory Blenders – manufactured for laboratory use
  - Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid inactivation of biological material, and to withstand sterilization by autoclaving.
  - If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. Stainless steel jars are also available. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.
- Lyophilizers
  - Lyophilizers allow dehydration of products.
  - For guidance on safe use of Lyophilizers refer to Appendix C.

**TITLE:****EHS-402, BIOSAFETY MANUAL*****Ampules***

- Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the content. Discard the towel and ampoule top and bottom as biohazardous waste.
- Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

***UV Lights***

The following guidance on UV lights is taken from an American Biological Safety Association (ABSA) position paper.

- The CDC, NIH and NSF agree that UV lamps are neither recommended nor required in BSCs. Criteria are not available from NSF to evaluate the performance of the UV lights within a biological safety cabinet. Numerous factors affect the activity of the germicidal effect of UV light, which require regular cleaning, maintenance and monitoring to ensure germicidal activity.
- Retrofitting any equipment (e.g. UV lights) into a biological safety cabinet may alter the air flow characteristics of the cabinet and invalidate any manufacturer warranty and is not recommended.
- It is the current opinion of the American Biological Safety Association that UV lights are not recommended for use in Biological Safety Cabinet.

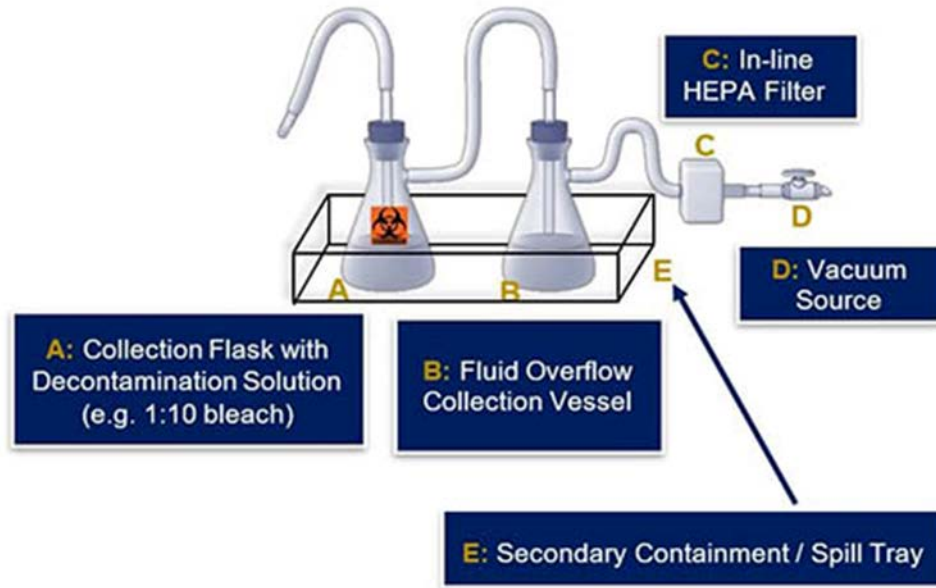
***Vacuum Lines***

- Vacuum lines shall be protected with liquid disinfectant traps. All lines exposed to bloodborne pathogens also require a High Efficiency Particulate Air (HEPA) filter or filters of equivalent or superior efficiency. Filters must be checked routinely and maintained or replaced as necessary.
- Traps should be placed in a secondary container (tray) to catch any spill if the trap tips over.
- Traps are labeled as biohazard waste (with either the text or a biohazard label)
- Figure 2 shows the different components to be included in a vacuum system to collect biohazard waste.

***Figure 2.0 Vacuum Set-Up for Biohazard Waste***

TITLE:

EHS-402, BIOSAFETY MANUAL



### ***Housekeeping***

Good housekeeping in laboratories and work areas is essential to reduce potential personnel exposures and protect the integrity of biological experiments. Routine housekeeping shall be relied upon to ensure work areas are free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

- Laboratory personnel are responsible for maintaining the cleanliness of laboratory benches, equipment and areas that require specialized technical knowledge.
- Additional laboratory housekeeping concerns include:
  - Keep the laboratory neat and free of clutter.
  - Surfaces should be disinfected regularly and free of infrequently used chemicals, glassware and equipment.
  - Access to sinks, eyewash stations, emergency showers, exits, and fire extinguishers shall not be blocked.
  - Proper disposal of all waste chemicals, biological and non-hazardous waste is essential.
  - The workplace must be free of physical hazards. Aisles and corridors should be free of trip hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, and avoidance of electrical hazards in wet areas.
  - All laboratory equipment must be cleaned, and a hazard tag completed and attached before being released for repair, maintenance, or surplus.

### ***Packaging and Transportation of Biological Materials On and Off Site***

- All biological materials shall be packaged and transported in a way that maintains the integrity of the material during normal transport conditions and

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

thus helps to ensure the safety of employees, the public and the environment.

- When transporting diagnostic and clinical specimens (both human and animal samples), biohazardous materials and recombinant DNA molecules BETWEEN buildings and floors on buildings, the following guidelines should be followed:
  - Samples need to be packaged in a sealed, leak proof primary container (e.g., plastic screw-top conical tube), which is securely positioned in a secondary leak proof and closable container (e.g., cooler, ice chest). The secondary container shall have a clearly visible biohazard symbol on the outside.
  - A list of contents as well as emergency information (e.g., PI phone number) needs to be accompanying the material (e.g., attached to the cooler in a plastic pouch).
- Transportation and shipment OFF Site:
  - The transportation and shipment over public roadways of diagnostic and clinical specimens, biological products, infectious agents and recombinant DNA molecules is regulated by national and international transportation rules. This includes specific procedures for the correct packing and packaging of these materials, necessary documentation and labeling and permits. For more information about specific shipment requirements, contact the BSO.
  - The use of personal cars for transport of biological materials is not recommended.
  - Those who transport veterinary samples (e.g. animal samples) between buildings over public roadways shall follow the guidelines established by the Division of Animal Resources (DAR).

#### **5.4 Work-Specific Guidelines**

The use of biological materials in research spaces must be registered with the Biosafety Office using the electronic platform.

##### ***Guidelines for Working with Recombinant and Synthetic Nucleic Acid Molecules***

- Refer to the EHSO Guide for recombinant DNA experiments covered by the NIH Guidelines and the institutional review required.
- All work involving recombinant or synthetic nucleic acids at Emory University must be registered with the Biosafety Office.
- Guidance for registration of biological materials can be found at [ehso.emory.edu](http://ehso.emory.edu)

##### ***Guidelines for Working with Tissue Culture/Cell Lines***

- When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG level as that recommended for the agent.
- Follow the Emory EHSO Guidelines for working with human cells and tissues in animals.

##### ***Working with Human or Non-Human Primate Cells and Tissues***

- The potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HCV and HIV, as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissues.

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- Non-human primate cells and tissues also present risks to laboratory workers such as Herpes B virus. Cells transformed with viral agents, such as SV-40, EBV, or HBV, as well as cells carrying viral genomic material present potential hazards to laboratory workers.
- Tumorigenic human cells also are potential hazards because of self-inoculation.
- Human and other primate cells should be handled using Biosafety Level 2 practices and containment;
- All work should be performed in a biosafety cabinet;
- All material should be decontaminated by autoclaving or disinfection before discarding;
- All employees working with human cells and tissues shall be included in the Bloodborne Pathogens Program (as outlined by the Emory University Bloodborne Pathogens Exposure Control Plan), and work under the policies and guidelines established by the BBP Exposure Control Plan. This includes being offered the Hepatitis B vaccine.
- Additional information can be found in the Emory University Bloodborne Pathogens Exposure Control Plan at [www.ehso.emory.edu](http://www.ehso.emory.edu).
- When using human source materials in animals, follow the EHSO Guidelines for Working with Human Cells and Tissues in Animals document located at [www.ehso.emory.edu](http://www.ehso.emory.edu).

***Guidelines for Preventing the Transmission of Tuberculosis***

- Propagation and/or manipulation of *Mycobacterium tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3 and requires Biosafety Committee approval. Contact the BSO for guidance.

***Guidelines for Clinical Laboratories***

- Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BSL-2.
- A primary barrier, such as a biological safety cabinet, should be used;
- When it is anticipated that splashing, spraying or splattering of clinical materials may occur,
- For initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*), to protect the integrity of the specimen.
- All laboratory personnel who handle human source materials shall be included in the Bloodborne Pathogens Program as outlined in the Exposure Control Plan. "Universal Precautions" need to be followed when handling human blood, blood products, body fluids or tissues.
- The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the laboratory supervisor. It is also the supervisor's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented and emergency plan procedures. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards (NCCLS).

TITLE:

EHS-402, BIOSAFETY MANUAL

***Biological Agent Reference Sheet (BARS)***

- Biological Agent Reference Sheets (BARS) review and document containment issues, facility needs, emergency response, training, vaccine recommendations and availability, serum banking and testing issues and follow up needs in the event of an exposure for a particular agent. Not all agents on site will have a BARS. BARS should be completed by the BSO with the assistance of the Biosafety Committee and the Emory University scientific community. BARS should then be communicated to those that work with the agent and made readily available (i.e. EHSO web page and posted in applicable work areas).
- PIs are responsible for communicating information contained in the BARS to those individuals who will work with or around the agent.
- BARS would include items such as specific waste disposal consideration, available prophylaxis, containment considerations and PPE considerations.

***Biological Materials Registration***

- All biological materials (for example: microbes, biological toxins, nanomaterials, recombinant and synthetic nucleic acids and derivatives) used in research shall be included in the biological registration in the electronic platform and shall be reviewed by the IBC/RHSC.
- PIs shall be prompted to update their biological registration annually, or when biological materials need to be added to the registration using an amendment.

***Bloodborne Pathogens Program and Exposure Control Plan***

- Emory University is committed to protecting its employees from risks associated with exposure to bloodborne pathogens through implementation of its Exposure Control Plan (ECP). This plan follows the requirements established by the U.S. Occupational Safety and Health Administration (OSHA) in December 1991 (29 CFR 1910.1030) and guidance provided by the Centers for Disease Control and Prevention and the World Health Organization.
- Employees at Emory University that have a reasonable anticipated risk for exposure to bloodborne pathogens need to be included in the Bloodborne Pathogens Program. As outlined in the University's ECP, these employees need to be identified and provided with the appropriate means to safely conduct their individual jobs. The following principles must be followed when employees are potentially exposed to bloodborne pathogens:
  - Minimize all exposure to bloodborne pathogens;
  - Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to bloodborne pathogens;
  - Routinely employ "Universal Precautions" when exposure to blood or potentially infectious materials is anticipated.
- All employees covered under the ECP need to complete initial training on bloodborne pathogens as well as an annual refresher course. In addition, employees shall be provided with Hepatitis B vaccination free of charge. The vaccination consists of the three-dose vaccine and antibody titer check. The specific requirements and responsibilities of PIs, laboratory supervisors, health care managers, employees and others are outlined in the ECP. Please consult this plan for further information.

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

### ***CDC and USDA Permits***

- The CDC and USDA issue permits for importing and exporting certain organisms or biological materials such as soil. The biosafety office should be made aware of all issued permits. Upload the most recent permits issued to the electronic platform.
- For additional guidance or for assistance preparing for an inspection contact the Biosafety Office.

## **6.0 Biohazardous Waste Management**

- Biohazardous waste is collected for decontamination prior to disposal. Examples of biohazard waste include waste generated from experiments involving recombinant or synthetic nucleic acids, cultures, plates, transgenic animals/plants/arthropods, sharps, etc.
- Biohazardous waste container should be labeled with the biohazard symbol and it should be lined with a red/orange bag displaying the biohazard symbol.
- Biohazardous waste container should be covered when not in use.
- Biohazardous waste is disposed of in appropriate containers accepted by the Emory University approved vendor.
  - Infectious Waste Manifests from approved vendor should be maintained for documentation and tracking.
- Sharps:
  - All sharps are disposed of in designated puncture-resistant sharps containers.
  - When the sharps container is  $\frac{3}{4}$  full, carefully close the flap and dispose of in the container accepted by the Emory University approved vendor.
  - Sharps include items that are capable of puncturing, cutting or abrading the skin such as glass and plastic pipettes, broken glass, test tubes, razor blades, syringes, and needles.
  - Sharps shall be segregated from other wastes and aggregated immediately after use in red, fluorescent orange or orange-red leak proof, rigid, puncture-resistant, shatterproof containers that resist breaking under normal conditions of use and handling, and that are marked prominently with the universal biohazard warning symbol and the word "Biohazard" in a contrasting color.
- Pipette: Plastic pipette tips and serological pipette tips used to process human body fluids or cultures of infectious agents, should be placed in a puncture-resistant "pipette" box (cardboard) that has the biohazard symbol on it. When full, these boxes are closed and disposed of in a biohazard box as shown below:





## TITLE:

**EHS-402, BIOSAFETY MANUAL**

- All biological materials containing recombinant or synthetic nucleic acids shall be disposed of according to the NIH Guidelines.
- Liquid biohazardous waste: Untreated biohazard waste should not be poured down the drain, discarded in the regular trash, or mixed with chemical waste. Bulk liquid must be treated with a final concentration of 10% bleach allowing contact time of 30 min, or overnight. Discard down the drain pouring copious amount of water.

## 7.0 Non-Contaminated Broken Glass

- Items should be discarded in a bag-lined heavy-duty cardboard box and taped shut before disposal.
- Broken glass containers with non-biohazard plastic liners should not be filled greater than  $\frac{3}{4}$  full.
- Do NOT use cardboard boxes with “biohazard” symbols printed on them.
- Usually, these are labeled “broken glass” by the manufacturer.
- Keep in mind that these boxes are very heavy if filled to the rim. Consider smaller glass disposal boxes or disposing of the boxes when the box is a manageable weight and not full.
  - Sealed broken glass boxes should be placed in the hallway for pick up.

## 8.0 Personal Protective Equipment

- PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The selection of PPE should be made according to the activities to be performed by an individual.
- PIs or lab managers should complete the PPE Hazard Assessment for Research Laboratories template to determine the PPE recommended for the activities to be conducted in the laboratory.
- The Personal Protective Equipment (PPE) Assessment Form (for Research Laboratories) is located at [www.ehso.emory.edu](http://www.ehso.emory.edu).

### 8.1 Face Protection

Splash goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face.

### 8.2 Laboratory Clothing

This category includes laboratory coats, smocks, scrub suits and disposable gowns. Long-sleeved garments or sleeve covers should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization in the event it becomes contaminated. At a minimum, a laboratory coat should be worn in all laboratories working at a BSL-2.

- Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas.

**TITLE:****EHS-402, BIOSAFETY MANUAL**

- Disposables should be available for visitors, maintenance and service workers in the event it is required.
- All protective clothing should be either discarded in the laboratory or laundered by a university approved vendor.
- Personnel shall not remove potentially contaminated items or clothing from the site.

**8.3 Gloves**

- Gloves must be selected based on the hazards involved and the activity to be conducted.
- Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents.
- Temperature resistant gloves must be worn when handling hot material or dry ice.
- Delicate work requiring a high degree of precision dictates the use of thin walled gloves.
- Protection from contact with toxic or corrosive chemicals may also be required.
- Gloves must be used when handling frozen vials.
- Wash hands after use.
- Alternatives to powdered latex gloves should be used. Powdered latex gloves should not be used in research laboratories.
- For assistance in glove selection, refer to the EHSO web site.

**8.4 Respirators**

For certain protocols and projects, respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who need respiratory protection must contact the research safety group in EHSO for assistance in selection of proper equipment and training in its usage. Personnel wearing respirators must participate in the Respiratory Protection Program (refer to EHSO web site).

**9.0 Methods of Decontamination**

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

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

To select the proper method and tools, it is important to consider, for example, the following aspects:

- Type of biohazardous agents, concentration and potential for exposure;
- Physical and chemical hazards to products, materials, environment and personnel.

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

Physical and chemical means of decontamination fall into four main categories: Heat, Liquid Chemicals, Vapors and Gases, and Radiation.

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors, gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and contact time.

### 9.1 Heat

To kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in overall shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121°C-132°C (250°F - 270° F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. To accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160°C -170°C (320°F- 338°F) for periods of 2 to 4 hours.

### 9.2 Liquid Chemicals Used as Disinfectants

- The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:
  - Nature of surface being disinfected - Porous or smooth, the more porous and rougher the surface, the longer a disinfectant will need to be effective.
  - Number of microorganism present - Higher concentrations requires a longer application time and/or higher concentration of disinfectant.
  - Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat (Table 4.0).
  - Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
  - Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.
- See Appendix D for selected disinfectants.

**Table 4.0 Increasing Resistance to Chemical Disinfectants**

DEGREE OF RESISTANCE	MICROBE	EXAMPLES
Least Resistant	Lipid or Medium-Size Viruses	Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Human Immunodeficiency virus
	Vegetative Bacteria	Pseudomonas aeruginosa Staphylococcus aureus Salmonella choleraesuis
	Fungi	Trichophyton sp.

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

		Cryptococcus sp.
		Candida sp.
	Nonlipid or Small	Poliovirus
	Viruses	Coxsackievirus
		Rhinovirus
More Resistant	Mycobacteria	Mycobacterium tuberculosis;
		M. bovis
	Bacterial Spores	Bacillus subtilis
		Clostridium sporogenes

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often very aggressive (corrosive) and toxic. Some of the more common ones are below:

#### ***Alcohols***

Ethyl or isopropyl alcohol in concentration of 70% to 90% is good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time.

#### ***Formalin***

Formaldehyde is a human carcinogen and creates respiratory problems at low levels of concentration.

#### ***Glutaraldehyde***

This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses.

#### ***Phenol and Phenol Derivatives***

The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops).

#### ***Quaternary Ammonium Compounds (Quats)***

Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses.

#### ***Halogens (Chlorine and Iodine)***

Chlorine-containing solutions have broad-spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Diluted solutions may be kept for extended periods if kept in a closed container and protected from light.

**See Appendix D for characteristics of selected disinfectants.**

### **9.3 Vapors and Gases**

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

**TITLE:**
**EHS-402, BIOSAFETY MANUAL**

**Table 5.0 Comparison of the Most Common Vapors and Gases Used for Decontamination**

VAPORIZED HYDROGEN PEROXIDE	ETHYLENE OXIDE	CHLORINE DIOXIDE GAS
~150-750 ppm	500-1000mg/L49-50°C	Concentration 10 mg/L 350-1,500 ppm
Contact time:	Contact time: 1-6 h	Contact time 1-2 h
Biological indicator: <i>Bacillus stearothermophilus</i>	Biological indicator: <i>Bacillus subtilis var niger</i>	Biological indicator: <i>Bacillus atropheus</i>
Advantage: No harmful breakdown products	Advantage: used for equipment that is heat and radiation sensitive	Advantages: no residue
Disadvantage: toxic but not carcinogenic PEL 0.1 ppm	Disadvantage: carcinogen and eye irritant. Absorbs to materials and needs to be degassed after exposure. Pure ETO is flammable	Disadvantage: potential incompatibility with electronics PEL 0.1 ppm

#### 9.4 Radiation

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices.

Ultraviolet (UV) radiation is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms. UV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.

#### 10.0 Use of Laser-Containing Sources

Personnel engaged in the use of Class 3B or Class 4 lasers are required to comply with the EHSO Laser Safety Program. Please refer to the Laser Safety Program document located at [www.ehso.emory.edu](http://www.ehso.emory.edu).

#### 11.0 References

For a list of Biosafety Resources, refer to [www.ehso.emory.edu](http://www.ehso.emory.edu).

The following is a list of rules, regulations and guidelines. Some of which are available from the Biosafety Resource Intranet Page.

- National Institute of Health (NIH)
- Guidelines for Research Involving Recombinant and Synthetic Nucleic Acids Molecules. These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA (rDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. Because of the committee's activity, the initial version of the NIH Guidelines was published in 1976. It has been amended and revised many times since then. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed rDNA research using the

**TITLE:****EHS-402, BIOSAFETY MANUAL**

NIH Guidelines as a minimum standard. For more information, please refer to the Guidelines for Working with Recombinant NA section in this manual and the NIH Guidelines for Research Involving Recombinant DNA Molecules.

- Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH). The CDC and NIH publish the CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Edition manual, 2009 (BMBL). In 1984, the CDC/NIH published the first edition of the BMBL. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with a variety of infectious agents in various laboratory settings. The BMBL has been revised several times and is commonly seen as the standard for biosafety. The BMBL was used as the basis for this biosafety manual.
- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard. In 1992, the Occupational Safety and Health Administration (OSHA) promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions. Consequently, an Exposure Control Plan was established to protect employees from exposure to HIV, Hepatitis B and other bloodborne pathogens. For more information, please refer to Bloodborne Pathogens Exposure Control Plan paragraph on page 44 of this manual and the Exposure Control Plan at [www.ehso.emory.edu](http://www.ehso.emory.edu).
- Department of Health and Human Services (HHS): Additional Requirements for Facilities Transferring or Receiving Select Agents. In 1996, HHS published a set of rules that require facilities and institutions to be registered and approved to transfer or receive certain biological agents and toxins. HHS requires companies to comply with the BMBL (see above) and OSHA's Laboratory Safety Standard 29 CFR 1910.1450.
- State of Georgia regulations on medical waste management:  
<http://www.envcap.org/statetools/rmw/ga-rmw.html>
- Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:
  - United Nations
  - Recommendations of the Committee of Experts on the Transportation of Dangerous Goods
  - International Civil Aviation Organization (ICAO)
  - Technical Instructions for the Safe Transport of Dangerous Goods by Air
  - International Air Transport Association (IATA)
  - Dangerous Goods Regulations
  - U.S. Department of Transportation
- U.S. Public Health Service
- U.S. Postal Service
- U.S. Department of Labor, OSHA
- 49 CFR Parts 171-178
- 42 CFR Part 72

**TITLE:****EHS-402, BIOSAFETY MANUAL**

- 39 CFR Part 111
- 29 CFR 1910.1030
- Importation permits are required for certain infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine.
- The Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR PER 340. The importation of etiologic agents is also governed by the following federal regulation: USPHS 42 CFR - Part 71 Foreign Quarantine. Part 71.54 Etiologic agents, hosts, and vectors.

TITLE:

EHS-402, BIOSAFETY MANUAL

## Appendix A: Spill Kit Essentials and Steps to Clean-Up Spill

All personnel working in research laboratories should know the location of the biological spill cleanup kit, the basic steps to clean up small spills, who to call in case of an emergency and the reporting procedures.

Before you start your work with biological agents:  
Identify the location of the nearest spill kit  
Check the contents of the Biological Spill Kit  
Written spill procedure including emergency phone numbers



Contents:

- Bottle with nozzle to prepare the decontamination solution, for example 10% Bleach
- Paper towels and other absorbent materials such as kitty litter or sand
- Multiple pairs of nitrile gloves
- Gown/lab coat
- Shoe covers
- Eye protection
- Forceps to pick up sharps, including broken glass
- Brush and Dustpan to clean up broken glass in contaminated liquid, should be resistant to decontaminant solution after use
- Biohazard bags

### Emergency Numbers:

- EHSO Spill Response Team: 7-2888  
Emory Police: 404-727-6111

### If a spill occurs outside the biosafety cabinet:

- Notify others that a spill has occurred, control traffic to the area.
- Remove any contaminated clothing or PPE by folding the contamination inward and dispose of as biohazard waste.
- Wash potentially contaminated body parts with germicidal soap. Shower if necessary.
- Allow aerosols to settle before returning to the spill area.
- Don PPE: gown/lab coat, two pairs of nitrile gloves, eye protection (safety goggles), shoe covers, and respiratory protection as appropriate.
- Prepare disinfectant, i.e. 10% bleach.
- Cover spill area with paper towels.
- Pour disinfectant over towels from edges of spill to center, be careful not to splash or create aerosols.
- Allow 30 minutes of contact time.
- Collect contaminated paper towels using the brush and dustpan. Discard in biohazard bag.
- Pick up any sharps, including broken glass, with forceps and discard in sharps container.
- Use brush and dustpan to recover any leftover broken glass in contaminated



**TITLE:****EHS-402, BIOSAFETY MANUAL**

liquid.

- Decontaminate dust pan.
- Exchange outside pair of nitrile gloves as often as needed while cleaning the spill.
- Wipe area with disinfectant, wipe down outside of bags, containers, and equipment involved in the spill with disinfectant and dispose of wipes as biohazard waste.
- Close biohazard bags by turning as a loose goose neck.
- Doff PPE and dispose of as biohazard waste in a separate container.
- Wash hands with enough soap and water.
- Complete incident report in [PeopleSoft: https://hrprod.emory.edu](https://hrprod.emory.edu); Self Service; Workplace Health; click HOME

TITLE:

EHS-402, BIOSAFETY MANUAL

## Appendix B Safety Checklist for Operating a Small Cryostat or Microtome

- The cryostat or microtome is an instrument that uses sharp blades to cut thin sections of fixed or unfixed tissue. The use of these instruments in the laboratory presents a laceration hazard which can result in an exposure to bloodborne pathogens or other infectious materials.
- Prior to operating this instrument ensure that you have updated your Emory EHSO Research Lab Safety, BBP and Biosafety training
- If handling human source materials, ensure that you have received the three doses of Hepatitis B vaccination and checked for antibody titers.
- A designated microtome/cryostat trainer in your lab should provide an instrument orientation, explain all safety aspects, and supervise first time operation
- Proper minimum PPE must be worn: safety glasses, lab coat, disposable gloves and cut resistant (Kevlar or stainless steel mesh) gloves if changing blades.
- Use protectors/guards for knife-edges that may extend beyond microtome knife holder. **Guards should never be removed.**
- No blades or other sharp edges on countertop and surrounding area
- Lock the hand wheel and guard the blade (and foot pedal if applicable) before any tissue manipulation
- Ensure a clear distance between hands and blade
- Use brushes or appropriate tools to position and collect samples
- Use a magnetic tool to remove the blade if necessary
- Use a brush to sweep unwanted sections If leaving the instrument momentarily, lock the wheel and engage the blade guard.
- Solutions used to stain potentially infected frozen sections should be considered contaminated.
- Put a sign on the machine to warn others it is currently in use. Do not leave motorized microtomes running unattended.
- When work is complete, leave the area clean and free of any sharp objects. Properly decontaminate equipment including reusable blades after each use.
- Instrument blades should be disposed in a sharps container or properly secured
- Unwanted sections and reagents should be disposed properly.
- Do not move or transport a microtome with knife in position.
- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or any other disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination
- Document modified from [https://blink.ucsd.edu/files/safety-tab/research/Safety\\_Checklist\\_Operating\\_Cryostat\\_Microtome\\_small.pdf](https://blink.ucsd.edu/files/safety-tab/research/Safety_Checklist_Operating_Cryostat_Microtome_small.pdf)

TITLE:

EHS-402, BIOSAFETY MANUAL

## Appendix C Safety Checklist for Using Lyophilizers

The process of using a laboratory scale lyophilizer presents unique hazards. These hazards include but are not limited to extreme pressure changes, a potential for glassware to explode or implode, and the possibility of frostbite or tissue damage associated with exposure to cryogenic materials. Here is a checklist for the safe use of a laboratory lyophilizer.

- **Lyophilizer Use**
  - Inspection of glassware & seals
  - Freezing of sample in liquid nitrogen (includes hazards of liquid nitrogen)
  - Placing of sample on lyophilizer and evacuating vessel
  - Repressurizing/removing sample (includes flask failure)
  - Maintenance (oil changing and refrigerator coil cleaning)
  - Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit.
  - If possible, sample material should be loaded in a BSC.
  - The vacuum pump exhaust should be filtered to remove any hazardous agents.
  - After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected.
  - If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized, and vapor traps should be used wherever possible.
  - Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced.
- **Inspection of glassware and seals**
  - Use only glassware designed for the lyophilizer!!
  - The lyophilizer, when in good working order, should have a pressure of 5-50 millitorr (normal atmospheric pressure is 760 torr), as indicated on the LED screen. (If the pressure is outside of this range, contact one of the people listed above.) This means that all glassware attached and under vacuum on the lyophilizer represents a significant implosion hazard.
  - All glassware used on the lyophilizer must be free of any visible defect (cracks, chips, or scratches), no matter how seemingly minor. Any glassware that is defective in this way must not be used under any circumstances. If such glassware is found, it should be discarded. The seals themselves are somewhat more forgiving, in that a defective seal reduces the vacuum. However, defective seals should still be removed from service.
  - A greater risk is present in seals that connect two pieces of glass. All seals of this type place rubber between joined pieces of glass. If these seals are used improperly, the glass pieces come into contact and scratch each other during installation. These scratches compromise the integrity of the glass thus creating a potentially serious implosion hazard. Install any seals of this type carefully to avoid this; if you are

## TITLE:

**EHS-402, BIOSAFETY MANUAL**

uncertain of how to do this do not proceed until you have received further training from one of the contact people given above.

- Handling liquid nitrogen
  - Liquid nitrogen is a hazardous material. As such, it presents a severe frostbite hazard.
  - PPE: When using liquid nitrogen, wear proper eye protection and protective gloves.
    - Proper eye protection will depend on the splash hazard but should at a minimum include chemical safety glasses. If larger volumes of liquid nitrogen are being use or if the potential for a splash is greater, chemical goggles and a face shield may be necessary. **NEVER WEAR A FACE SHEILD WITHOUT PROPER GLASSES OR GOGGLES.**
    - Protective gloves should be approved for liquid nitrogen splash hazards and are typically designated CRYO gloves by manufacturers. Be aware, however, that the gloves offer only very transient protection, in that if liquid nitrogen soaks into the material they will freeze themselves, becoming the hazardous agent! Remove any gloves or articles of clothing that become saturated with liquid nitrogen. In addition, as it boils and attains the gas phase, liquid nitrogen displaces all oxygen in the vicinity. Therefore, in closed spaces liquid nitrogen is an asphyxiation hazard.
  - Never use liquid nitrogen in an area that is not well ventilated.
- Modified from: <http://www.pharmatips.in/Articles/Pharmaceutical-Equipment/Injection/Lyophilizer-Standard-Operating-Procedure.aspx>

**TITLE:**
**SAF-310, BIOSAFETY MANUAL**

## Appendix D. Characteristics of Selected Disinfectants

DISINFECTANT CATEGORY	ALCOHOLS	ALKALI	ALDEHYDES	HALOGEN CHLORINE	HALOGEN IODINE	HALOGEN PEROXYGEN COMPOUNDS	PHENOLS	QUATERNARY AMMONIUM COMPOUNDS
COMMON ACTIVE INGREDIENTS	ethanol, isopropanol	calcium hydroxide, sodium carbonate (SC), calcium oxide	Formaldehyde (FA), glutaraldehyde, ortho-phthalaldehyde,	sodium hypochlorite (bleach), calcium hypochlorite, chlorine dioxide	povidone-iodine (PVI)	hydrogen peroxide/ accelerated HP, peracetic acid, potassium peroxymonosulfate	ortho-phenylphenol, orthobenzylpara-chlorophenol	benzalkonium chloride, alkyldimethyl ammonium chloride
COMMERCIAL NAMES	Ethanol; isopropanol	Soda ash; washing soda	Synergize®	Clorox®, Wysiwash® ProKure V MB-10		Rescue®, Oxy-Sept 333®, Virkon-S®	One-Stroke Environ®, Pheno-Tek II®, Tek-Trol®, Lysol®	Roccal-D®, DiQuat®, D-256®
RECOMMENDED CONCENTRATION	70% isopropyl alcohol	4% solution of SC is effective against FMD	37-50% FA by weight- 2% formalin for most viruses	10% dilution of household bleach= 5250–6150 ppm.	PVI @ 7.5-10% solution	HP @ 5- 20% peracetic acid @ 0.2%	5 %	0.05 to 0.2% quat, and require 10 min to achieve disinfection
RECOMMENDED USE	Bacteria <b>Limited on viruses</b> Fungi Mycobacterium	Bacteria Virus Fungi Mycobacterium Spores	Bacteria <b>Limited on viruses</b> Fungi Mycobacterium Spores	Bacteria Viruses Fungi Mycobacterium Spores	Bacteria Viruses Fungi Mycobacterium <b>Limited on spores</b>	Bacteria Viruses Spores <b>Limited on Fungi and Mycobacterium</b>	Bacteria Viruses Fungi Mycobacterium	Bacteria <b>Enveloped viruses</b> Fungi Spores
PRECAUTIONS	Flammable	Very caustic May contain arsenic	Carcinogenic	Toxic gas released if mixed with strong acids or ammonia	Concentrated iodine compounds can irritate the skin.	Concentrated chemicals are irritants and may cause chemical burns of the skin and eyes	May be toxic to animals, especially cats and pigs	Irritant Caustic
FACTORS AFFECTING EFFECTIVENESS	Inactivated by organic matter	Variable	Inactivated by organic matter, hard water, soaps and detergents	Rapidly inactivated by organic matter	Rapidly inactivated by organic matter	Effective in presence of organic matter, hard water, soaps, and detergents	Effective in presence of organic matter, hard water, soaps, and detergents	Inactivated by organic matter, hard water, soaps and anionic solutions

 Modified from: <http://www.cfsph.iastate.edu/Disinfection/Assets/CharacteristicsSelectedDisinfectants.pdf>