

BIOSAFETY

The safe handling and disposal of hazardous biological materials is an integral component of the Laboratory Safety Program. Hazardous biological materials include bacteria, viral agents, fungi, and protozoans, plus agents produced by means such as recombinant DNA technology. Part of the risk to laboratory workers from these agents stems from the fact that they are invisible to the naked eye and hence their presence may be undetected. Another risk is their potentially contagious nature, allowing them to spread from person to person. Thus an infection acquired in the laboratory could be disseminated far from its original source.

The routes of exposure to biological hazards include: absorption (through the skin), ingestion, inhalation, and parenteral (*injected* through the skin). Even biological agents not normally transmitted by an airborne vector can be transferred through the air by the production of aerosols. An aerosol is liquid or particulate matter dispersed in air in the form of a fine mist. Aerosol production should be eliminated or minimized whenever possible. When this cannot be done, specific controls and safety devices must be used to limit exposure.

Hazards and Precautions

The guiding principle for working with biohazards is *containment* which includes the use of safe methods for managing infectious agents with the purpose of minimizing or eliminating exposures to personnel and the outside environment. Containment begins with good laboratory practices supplemented by proper safety equipment and facility design. Together these elements address the administrative (good laboratory practices) and engineering (safety equipment and facility design) controls required to safely work with infectious agents.

Good Laboratory Practices

The Principal Investigator (PI) is responsible for the appropriate risk assessment of the biological hazards in the laboratory or research facility. All personnel must also be properly informed about these hazards as well as trained on how to deal with them. Training should include the use of good laboratory practices and standard microbiological techniques. Some of these include:

- ! Minimization of the quantities of samples and specimen.
- ! Careful maintenance of the physical integrity of containment equipment.
- ! Appropriate decontamination practices for the equipment and the working area.
- 1. Use of proper personal protective equipment

EHS offers periodic training, through the Professional Development and Training Office, on the proper management of biohazards and the use of good laboratory practices. Special sessions can be arranged for a department or research group. Contact **EHS** for more information.

Primary Barriers (Safety Equipment)

Primary barriers include engineering controls such as safety equipment designed to minimize or eliminate exposure to hazardous biological materials. Primary barriers range from safety packaging such as stoppered containers and centrifuge safety cups to biosafety cabinets. However, even with primary barriers in place, the use of good laboratory practices and standard microbiological techniques must be applied to provide maximum protection from exposure.

Secondary Barriers (Facility Design)

Facility design can provide barriers of protection for personnel and the general community from accidental releases

of hazardous biological agents. The type of research and the risk of transmission of these agents will determine the extent of the barriers needed for protection. A secondary barrier can be as simple as the identification of a separate area in a laboratory through appropriate signage ("Restricted Area," "Authorized Personnel Only," etc.) or as involved as an entire specifically-designed building providing containment and isolation from the surrounding community (e.g., CDC/NIH Biosafety Level 4 Facility in Atlanta, Georgia).

All secondary barriers should address signage, access restrictions, personal protective equipment, ventilation, waste disposal, safety equipment, and facility decontamination. Contact **EHS** for additional information regarding the necessary facility design for conducting activities using hazardous biological materials. All operations using these materials must be approved by **EHS** before use.

Biosafety Levels (BSLs)

There are four levels of biosafety recommended by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). Each BSL outlines combinations of good laboratory practices and techniques, safety equipment, and facility designs appropriate to conduct research functions or activities with various infectious agents. A summary of the specific CDC/NIH guidelines for each BSL can be found in *Table VI*.

Biosafety Level 1

Level of containment that relies on standard microbiological practices.

Biosafety Level 2

Level of containment applicable to clinical, diagnostic, teaching and other facilities working with indigenous moderate risk agents present in the community and associated with human disease of varying severity.

Table VI Summary of Recommended Biosafety Levels For Infectious Agents¹

Biosafety Level	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults.	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human Disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: ! Limited access ! Biohazard warning signs ! "Sharps" precautions ! Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: ! Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: ! Controlled access ! Decontamination of all waste ! Decontamination of laboratory clothing before laundering ! Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective laboratory clothing; gloves; respiratory protection as needed	BSL-2 plus: ! Physical separation from access corridors ! Self-closing, double door access ! Exhausted air not recirculated ! Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted laboratory infections; or related agents with unknown risk of transmission	BSL-3 practices plus: ! Clothing change before entering. ! Shower on exit ! All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs <u>in combination with</u> full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus: ! Separate building or isolated zone ! Dedicated supply, exhaust, vacuum, and decon systems ! Other requirements outlined in the text ¹

¹The information in this table was taken from CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories 4th Ed.*, HHS Publication No. (CDC) 93-8395, May 1999.

Biosafety Level 3

Level of containment applicable to facilities working with indigenous or exotic agents having potential respiratory transmission, and causing serious, potentially lethal infection. Primary hazards to personnel are auto-inoculation, ingestion and exposure to infectious aerosols.

Biosafety Level 4

Level of containment applicable to working with dangerous and exotic agents which 1) pose a high risk of life-threatening disease, 2) are transmitted via aerosol and 3) have no available vaccine or therapy.

Responsibility

The PI is primarily responsible for the determination of the necessary level of biosafety. EHS can provide information and assistance to aid the PI in defining this level. The use of agents requiring BSL 2 or higher must be approved by EHS and, when required, by the Institutional Biosafety Committee (IBC). Additional information can be found in the most recent version of the CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories.

Control of Infectious Agents

Standard Operating Procedure (SOP)

Safety precautions against biohazards start with the preparation of the research protocol or Standard Operating Procedure (SOP). The SOP must outline the methods that will be used, the storage and handling of biological agents including the appropriate biosafety level, and access restrictions to the research area. Information on the general guidelines for the creation of this document can be found in the section of this Manual on Standard Operating Procedures. The SOP must be reviewed and approved by EHS before operations begin. Contact EHS with additional questions and concerns on the development and approval of the SOP.

Potential Exposure Situations

In the research laboratory there are a number of situations in which workers may be exposed to infectious agents:

- ! *Laboratories specializing in infectious disease studies.* In this case the personnel must be fully aware of the risks and specifically trained in the proper procedures to handle the disease agent safely. Personnel should also be immunized against the disease agent when possible.
- ! *Laboratories in which studies are conducted on specimens from patients or human subjects.* Since some diseases may be transmitted by persons who do not exhibit the symptoms of the infection, all specimens from patients (blood, saliva, urine, feces, exudates, tissues, tissue cultures, etc.) should be handled with the same precautions that would be taken if they were known to be infected (i.e., Standard Precautions, formerly known as Universal Precautions). All individuals working in these laboratories are covered under the University's Bloodborne Pathogens Policy and Procedures (Exposure Control Plan) and must comply with all requirements of this policy, including appropriate training, Hepatitis B vaccination and medical follow-up of exposure incidents.
- ! *Laboratories where infectious materials are present.* Non-technical personnel who enter these laboratories as well as those who receive materials from them may have a risk of exposure. Safe transport of potentially infectious materials from "Point A" to "Point B" should use an appropriate, properly labeled secondary container (e.g., a blood or urine sample might be placed in a self-closing plastic bag labeled with the PI's name).

Infectious materials should never be stored in common-use refrigerators or cold rooms unless they are in

clearly marked, separate containers with proper identification and the name of the responsible PI.

- ! *Procedures which may produce infectious aerosols.* All activities with infectious agents should follow the guidelines of the appropriate biosafety level. Where there is a possibility of aerosol creation, work should be performed in a biological safety cabinet or provision should be made to limit potential exposure.
- ! *Accidental releases of infectious agents in the research area.* Spills of infectious agents must be promptly contained and effectively decontaminated. The PI is responsible for being familiar with the requisite conditions for inactivating the agent involved. Glassware must be constructed to withstand the centrifugal forces used without breaking. Centrifuge heads must never be run above their rated speed. Personnel should be instructed in advance on the appropriate procedures for periodic decontamination of centrifuges and other equipment used with infectious agents. Additionally, a thorough decontamination with the appropriate biocide must be performed on equipment in which an accidental release has occurred. All disinfectants used must be tuberculocidal and EPA approved.
- ! *The use of laboratory animals.* Although strict precautions are taken by the Division of Veterinary Resources in purchasing animals from certified suppliers under quarantine, this does not completely eliminate the risk of infected animals. Exposure to infectious agents can occur from intentionally infected animals and even healthy animals can carry zoonotic disease which can lead to severe and painful infections. See the section on Working with Animals for more information.
- ! *Activities using sharps (needles, blades, capillary pipettes, slides, etc.).* These items may transmit infections to people using them and also to housekeeping personnel who encounter them in trash containers. These objects should never be disposed in common waste receptacles. University of Miami policies require that these items be placed in an approved plastic sharps container, and treated as infectious waste for disposal. Contaminated hard plastics (pipettes, culture flasks, petri dishes, etc.), when broken, may become sharps and should be treated as such. See the section on the Disposal of Biomedical (Infectious) Waste for more information.
- ! *The storage and consumption of food in any laboratory area.* Eating, drinking or smoking in the area where infectious agents are present is strictly forbidden. The use of laboratory glassware for eating or drinking, and the storage of foodstuffs in laboratory refrigerators or cold rooms, is also not permitted.

Recombinant DNA Research

Recombinant DNA (rDNA) research procedures are regulated under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). rDNA molecules are defined by these guidelines as "molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell" or "molecules that can result from the replication of those described above." Each PI proposing rDNA research must complete and submit an rDNA Questionnaire to the IBC of the University for approval.

Initial Risk Assessment

An initial risk assessment must be conducted by the PI based on the agent Risk Group classification found in the "Classification of Human Etiologic Agents on the Basis of Hazard" section of the NIH Guidelines. These guidelines classify agents into four Risk Groups based on the relative pathogenicity to healthy adult humans by the following criteria:

- ! Risk Group 1. Agents that are not associated with disease in healthy adult humans.
- ! Risk Group 2. Agents associated with disease in healthy adult humans, which is rarely serious and for which preventive or therapeutic interventions are often available.

- ! Risk Group 3. Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.
- ! Risk Group 4. Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

The establishment of Risk Groups is based on the potential effect of a particular agent on a healthy adult human and does not take into account factors such as individual susceptibility, preexisting diseases, medications, immunocompromised individuals, pregnancy, breast feeding, etc.

Physical Containment

Researchers must provide the necessary physical containment to confine organisms containing rDNA molecules and to reduce the potential exposure to personnel and the general environment. Containment can be achieved through the recommendations of BSLs 1-4 where appropriate. Additional information on specific containment practices and requirements can be found in the NIH Guidelines. Contact **EHS** for more information.

Transferring or Receiving Select Agents

Certain highly infectious and toxic agents are capable of causing substantial harm to human health and welfare. These materials are called *Select Agents* and are subject to federal regulation under **42CFR Part 72**. The Select Agents regulation mandates that special requirements be fulfilled before a listed agent can be transferred to an institution. These requirements include 1) registration of any facility prior to receiving or transferring any of the select agents and 2) submission of a form (CDC EA-101) prior to actual transfer. Research using these agents requires prior written approval from **EHS**.

Researchers proposing the use of a listed agent must contact **EHS** sufficiently in advance to allow completion and approval of all required paperwork. *Table VII* lists the Select Agents covered by this regulation at the time of publication of this Manual.

Other Restrictions

The deliberate transfer of a drug resistant trait to microorganisms listed in *Table VII* is prohibited by the NIH Guidelines for Research Involving rDNA Molecules. This restriction is designed to prevent the intentional development of a drug resistant variant of the listed microorganisms, which could eliminate the effectiveness of that drug in controlling infection in humans or animals.

Exemptions

The following are criteria for exemption from the regulation:

1. Products subject to regulation under the **Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 136 et seq.)** and the **Toxic Substances Control Act (15 U.S.C. 2601 et seq.)** are exempt.
2. Additional exemptions for otherwise covered strains will be considered when CDC reviews and updates the list of Select Agents. Individuals seeking an exemption should submit a request to the CDC that specifies the agent or strain to be exempted and explain why such an exemption should be granted.

Table VII List of Select Agents¹

<p>Viruses</p> <ol style="list-style-type: none"> 1. Crimean-Congo hemorrhagic fever virus 2. Eastern Equine Encephalitis virus 3. Ebola viruses 4. Equine Morbillivirus 5. Lassa fever virus 6. Marburg virus 7. Rift Valley fever virus 8. South American Hemorrhagic fever viruses (Junin, Machupo, Sabia, Flexal, Guanarito) 9. Tick-borne encephalitis complex viruses 10. Variola major virus (Smallpox virus) 11. Venezuelan Equine Encephalitis virus 12. Viruses causing hantavirus pulmonary syndrome 13. Yellow fever virus <p>Exemptions: Vaccine strains of viral agents (Junin virus strain candid #1, Rift Valley fever virus strain MP-12, Venezuelan Equine encephalitis virus strain TC-83, Yellow fever virus strain 17-D) are exempt.</p>	<p>Fungi</p> <ol style="list-style-type: none"> 1. <i>Coccidioides immitis</i> <p>Toxins</p> <ol style="list-style-type: none"> 1. Abrin 2. Aflatoxins 3. Botulinum toxins 4. <i>Clostridium perfringens</i> epsilon toxin 5. Conotoxins 6. Diacetoxyscirpenol 7. Ricin 8. Saxitoxin 9. Shigatoxin 10. Staphylococcal enterotoxins 11. Tetrodotoxin 12. T-2 toxin <p>Exemptions: Toxins for medical use, inactivated for use as vaccines, or toxin preparations for biomedical research used at an LD₅₀ for vertebrates of more than 100 nanograms per kilogram body weight are exempt.</p>
<p>Bacteria</p> <ol style="list-style-type: none"> 1. <i>Bacillus anthracis</i> 2. <i>Brucella abortus</i> 3. <i>Burkholderia (Pseudomonas) mallei</i> 4. <i>Burkholderia (Pseudomonas) pseudomallei</i> 5. <i>Clostridium botulinum</i> 6. <i>Francisella tularensis</i> 7. <i>Yersinia pestis</i> <p>Exceptions: Vaccine strains as described in Title 9 CFR § 78.1 are exempt.</p>	<p>Recombinant organisms/molecules</p> <ol style="list-style-type: none"> 1. Genetically modified microorganisms or genetic elements from organisms on this list, shown to produce or encode for a factor associated with a disease. 2. Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins on this list or their toxic subunits.
<p>Rickettsiae</p> <ol style="list-style-type: none"> 1. <i>Coxiella burnetii</i> 2. <i>Rickettsia prowazekii</i> 3. <i>Rickettsia rickettsii</i> 	

¹The above information was taken from Title 42§CFR Part 72, *Transferring or Receiving Select Agents* and is current as of publication of this Manual. For updates and additional information, refer to the CDC/NIH Website listed in the Internet Website section of this Manual.