Biosafety Manual

What constitutes a biohazard?

Biohazards are pathogenic (disease-causing) microorganisms capable of self-replication. Thus, any microbe that can cause disease in humans or animals is considered a biohazard. They may have infectious potential for humans, animals, or other living things in the environment. Included are pathogenic prokaryotic and eukaryotic microbes, viruses, and subviral agents. Potentially biohazardous agents which may produce latent (silent or non-clinical) infections must be considered biohazardous. Materials that may harbor infectious agents (such as human blood, body fluids, tissues, and cells) must also be considered biohazardous.

Recombinant organisms produced through genetic manipulation with any potential for survival in the environment or in living things and can pose a health risk for humans, animals, or other living things in the environment are considered biohazards.

What is biosafety?

Biosafety is a set of specialized practices for safe handling and disposal of infectious organisms or biological material, which may harbor infectious organisms. It includes the safe management of recombinant and synthetic DNA (rDNA) activities.

Purpose

The purpose of this manual is to specify controls and safe handling practices for micoorganisms (viruses, bacteria, fungi, rickettsia, mycoplasma, protozoans, multicellular parasites and prions), biological toxins, recombinant or synthetic nucleic acid molecules, human blood or tissues and animal cell cultures.

Scope

This written program applies to all research performed at the University of North Carolina at Charlotte (UNC Charlotte) campus as well as off campus facilities. Students are covered as well as full-time and part-time employees.

Biosafety levels

The Centers for Disease Control and Prevention (CDC) describes four biosafety levels (BSLs) in their publication, *Biosafety in Microbiological and Biomedical Laboratories 5th Edition*, commonly referred to as “BMBL”:).

The four biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. The biosafety levels are outlined in a summary table in Section IV—Laboratory Biosafety Level Criteria of the BMBL.

Table 2. Summary of Recommended Biosafety Levels for Infectious Agents

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

TABLE SOURCE: BMBL 5th Edition, *Rev 12/2009*, Section IV "Laboratory Biosafety Level Criteria", page 59

The practices and equipment prescribed for each level are specific for the organisms used, the operations performed, the routes of transmission of the infectious agents used, and the laboratory function or activity.

Biosafety Level 1 (BSL-1)
BSL-1 is appropriate for work with organisms not known to cause disease in healthy adults. Typical organisms used at BSL-1 include yeast, *E. coli* K-12, Lactobacillus spp., and asporogenic *Bacillus subtilis*. Anyone working with organisms in a BSL-1 laboratory must follow the procedures and policies of this Biosafety Manual.

Biosafety Level 2 (BSL-2)
BSL-2 is applicable to agents of moderate risk associated with human disease of varying severity. Representative organisms assigned to BSL-2 are the salmonellae, *Staphylococcus, Streptococcus, and Toxoplasma* spp. When good microbiological techniques are used, these agents can be used safely on the open bench, if the potential for producing splashes or aerosols is low. BSL-2 is the biosafety level used for work with human blood, body fluids, or tissues where the presence of an infectious agent may be unknown. Primary hazards at BSL-2 include accidental percutaneous or mucous membrane exposures, exposure of non-intact skin, or ingestion of infectious materials. Extreme care must be taken with contaminated needles or sharp instruments. Any procedure with the potential for producing aerosols or splashing should be conducted using primary containment equipment, such as biosafety cabinets, safety centrifuge cups, sealed centrifuge rotors, and covered incubator shakers. Other primary barriers should be used as appropriate, such as safety glasses, splash shields, gloves, and lab coats.

Biosafety Level 3 (BSL-3)
BSL-3 practices, safety equipment, and facilities are applicable to indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious or potentially lethal infection. Representative microorganisms assigned to this level include *Mycobacterium*

tuberculosis, St. Louis encephalitis virus, and *Coxiella burnetii*. In addition, BSL-3 practices and procedures are required for large-scale culture of bloodborne pathogens. At BSL-3, more emphasis is placed on primary and secondary barriers in order to protect personnel in nearby areas, the community, and the environment from exposure. All laboratory manipulations must be performed in a biosafety cabinet or using other enclosed equipment. In addition, access to BSL-3 laboratories must be controlled, and the ventilation system must be designed to minimize the release of infectious aerosols.

Biosafety Level 4 (BSL-4)
BSL-4 is for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the inhalation route, and for which there is no available vaccine or therapy. There is no BSL-4 research conducted at UNC Charlotte.

Biosafety levels and specific agents

Designation of the appropriate biosafety level for work with a particular pathogen is dependent on a number of factors, including virulence, biological stability, route of transmission, infectious dose, communicability of the agent, laboratory facilities to be used, procedures to be used with the agent, quantities and concentration of the agent, employee training, and availability of effective prevention or treatment.

Section VIII—Agent Summary Statements in BMBL provides guidance for selection of biosafety levels for particular agents. While the list is not all-inclusive, it contains information for many agents that are proven laboratory hazards, have high potential for laboratory associated infections, or for which the consequences of infection are serious.

Additional agent information is available on the Public Health Agency of Canada's *Pathogen Safety Data Sheets and Risk Assessment* website. While this website is an excellent source of information on disinfection, infectious dose, precautions for the laboratory, etc., the recommended biosafety level for some agents may differ from the US recommendations.

Routes of transmission of pathogens

In order to produce disease, microorganisms must have a portal of entry into the body. The portals of entry differ depending on the organism and its ability to attack or survive in certain parts of the body. Routes of transmission for agents may differ from nature when these agents are handled in the laboratory, due to the generation of aerosols and/or the volume or concentration of the agent that is present. Typical routes of laboratory-acquired infection include:

- Inhalation
- Injection through the skin via a needle or other sharp object (broken glass, sharp metal object, scalpel, etc.)
- Introduction through the mucous membranes of the eye, mouth, or respiratory tract
- Introduction through skin abrasions, cuts or otherwise broken skin, such as dermatitis or acne
- Ingestion
- Animal bites or scratches, when working with animals.

Universal precautions

The *US Occupational Safety and Health Administration* (OSHA) built upon earlier CDC publications aimed at preventing healthcare worker infections with the publication of the 1992 Bloodborne Pathogen Standard (29 CFR 1910.1030).

Per OSHA: Universal Precautions is an approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

The primary premise of universal precautions is that all human sources of blood, body fluids, and tissues should be assumed to be infectious.

Universal precautions are intended to prevent parenteral, mucous membrane, and nonintact skin exposures to bloodborne pathogens in the workplace. The assumption that everything of human origin is potentially infectious drives the use of:

- Routine use of barrier protection (gloves and other protective clothing, including masks and protective eyewear as appropriate)
- Work practices to minimize exposure (hand washing, decontamination of equipment and work surfaces, etc.)
- Engineering controls (e.g., biosafety cabinets; sharps containers, centrifuge safety cups).

Principles of biosafety

- Know and understand the biology and infectious potential of the biohazardous agents you handle.
- Handle all potential biohazards as if an infectious agent is present.
- Use the principles of good microbiological practices when handling any organism—follow prudent practices.
- Plan in advance for response to accidents, spills, and injuries.
- Use disinfectants with proven efficacy against the potential biohazards you are handling.
- Work with the appropriate physical containment level required for the biohazard you are handling.
- Accept full responsibility for your work.
- Provide documented training for new or transferred employees prior to conduct of work with biohazards.
- Remain vigilant and monitor all biosafety practices.
- Report all accidental exposures to your PI or supervisor, the UNC Charlotte medical provider and the Biosafety Program Director/Biosafety Officer (BSO).
- Dispose of biohazards and sharps properly.

Principles of good microbiological practice

- Never pipette by mouth.
- Keep your hands away from your face in the laboratory.
- Never eat, drink, take medicine, apply cosmetics, store food or handle contact lenses in the laboratory.
- Always wear protective equipment (lab coat, gloves, eye protection) as appropriate for the task.
- Change gloves frequently and discard them as biohazardous waste when they have been used to handle biohazards.
- Remove gloves and lab coat and wash hands before leaving the laboratory.
- Wash hands thoroughly after handling microorganisms, cell cultures, human blood or tissues, and before leaving the laboratory.
- Handle all pathogens or materials containing pathogens in biosafety cabinets if the potential for aerosolization exists.
- Store all biohazardous materials securely in clearly labeled, sealed containers.
- Minimize the use of sharps in the laboratory (needles, scalpels, glass Pasteur pipets, razor blades, glass, etc.).
- Never recap a used needle or otherwise manipulate it by hand.
- Dispose of needles and other sharps in puncture resistant containers ("sharps" containers).

- Label all biohazards and areas where biohazards are stored with the Universal Biohazard Symbol.
- Know the location of appropriate spill kits or other decontamination equipment.
- Clean work surfaces with an approved disinfectant after work with biohazards and at end of each work day.

Anyone working in a BSL-2 laboratory must follow the procedures and policies of this Biosafety Manual.

Handling cell cultures and human tissues

When cell cultures are known to contain an infectious agent or an oncogenic virus, the cell line can be classified at the same level as that recommended for the agent.

Unfortunately, not all cell lines have been classified, and most cell lines have not been thoroughly tested for the presence of viruses. Several vertebrate species carry complete copies of viral genomes in their DNA even though whole virus is not released from the cell. Investigators handling mammalian cells may be handling viruses unwittingly and the possibility exists that human materials may harbor HIV, HBV, or other pathogens.

Under no circumstances shall anyone work with autologous cells (cells derived from themselves) or from first degree relatives because these cells will express the tissue type of the operator and could evade the normal immune responses that recognize and destroy foreign cells. Also, it is prudent practice to avoid using one's own blood for any tissue culture experiments. No one should work with their own blood samples or those of colleagues working in the lab if the intention is to transform lymphocytes because in the advent of an accidental exposure, their immune system will not challenge the transformed cells.

It is the policy of UNC Charlotte that all human and other primate cells should be handled using Biosafety Level 2 practices and containment. Work should ideally be performed in a biosafety cabinet, and all materials should be decontaminated before discarding. Appendix H – Working with Human, NHP and Other Mammalian Cells and Tissues in the BMBL contains additional detail on the hazards, known and potential, of human cell cultures.

It is the position of OSHA that any cell line of human origin must be considered to fall under the Bloodborne Pathogens Standard (thereby necessitating BSL-2 practices and containment) even if the cell stock has been tested and found to be free of bloodborne pathogens. Even if commercial vendor testing data exists on a purchased cell line, remember that tests do not exist for every potential contaminant, some purchased cell lines are known to be infected with specific viruses, and the possibility of cross contamination after use in the laboratory is always a risk. The American Type Culture Collection (ATCC) now advises that all human cell lines be handled at BSL-2.

The following cell lines or tissues are of particular concern, whether they have been shown to harbor a pathogenic agent or not:

- Non-continuous cell lines derived directly from human clinical materials.
- Human clinical material (e.g., samples of human tissues, blood, or fluids obtained after surgical resection or autopsy).
- Non-human primate tissue.
- Cell lines producing infectious viral particles.
- Mycoplasma-containing cell lines.

In addition to the guidelines listed above for containment, the following precautions should be followed for cell lines considered to be BSL-2:

1. A biological safety cabinet is used for all cell manipulations that may create aerosols, whether or not the procedure requires sterility.

2. Reusable contaminated materials are placed into a container with a freshly prepared 1:10 dilution of bleach or other suitable disinfectant prior to being washed.

3. Disposable contaminated materials are discarded into an appropriate disinfectant or directly into biohazard waste containers and are properly disposed.

4. Disposable gloves are worn.

According to the ATCC, no cell line has been shown to harbor an infectious agent or an oncogenic virus requiring the precautions necessary for Biosafety Level 3 or 4. However, it is recommended that studies involving suspensions of HIV prepared from T cell lines be conducted using Biosafety Level 3 precautions. The ATCC provides their catalog on-line which contains biosafety information for many cell lines.

Anyone handling human or non-human primate cell lines in a BSL-2 laboratory must follow the procedures and policies of this Biosafety Manual.

Equipment and facilities

Safety equipment

Safety equipment includes containment equipment as well as personal protective equipment (PPE). The biological safety cabinet is the principal containment device used to provide protection from potentially infectious aerosols or splashes generated by procedures such as vortex mixing, uncapping stoppered tubes, sonication, homogenizing, grinding, blending, opening centrifuge tubes, heat sealing, etc. Safety centrifuge cups are another example of containment equipment, as they are designed to prevent aerosols from being released during centrifugation.

Examples of PPE that provide primary barriers to minimize exposure are gloves, lab coats, gowns, shoe covers, respirators, face shields, safety glasses, and goggles. In some situations, such as animal studies, the use of containment equipment such as biosafety cabinets may be impractical. In those cases, the choice of appropriate PPE is important in order to protect personnel from exposure to infectious agents.

Biosafety cabinets

Biosafety cabinets (BSCs) are of several designs and styles. The types of cabinets and the procedures for which each is designed are discussed in Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets of BMBL.

BSC selection should be based on a discussion between the PI and the Biosafety Program Director/BSO, dependent on information from the scientist about the materials that will be used in the cabinet. In general, a recirculating BSC (Class II Type A1/ A2/B1) may be used if no volatile toxic compounds or radioactive materials that may be volatilized are going to be handled. If a cabinet is hard-ducted to the outdoors (some Class II A2/all B1) than minute amounts of volatile toxic chemicals and radionuclides may be handled. BSCs that exhaust 100% of the air to the outdoors (Class II Type B2) are more expensive to install and maintain; therefore, they are used only when the work dictates.

The following chart from BMBL, Appendix A, summarizes BSC characteristics:

Table 2. Comparison of Biosafety Cabinet Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications: Nonvolatile Toxic Chemicals and Radionuclides	Applications: Volatile Toxic Chemicals and Radionuclides
I	75	In at front through HEPA to the outside or into the room through HEPA (Figure 2)	Yes	When exhausted outdoors[1,2]
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit (Figure 3)	Yes (minute amounts)	No
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter (Figures 5A, 5B)	Yes	Yes (minute amounts)[1,2]
I, B2	100	No recirculation; total exhaust to the outside through a HEPA filter (Figure 6)	Yes	Yes (small amounts)[1,2]
II, A2	100	Similar to II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to the outside through a canopy unit (Figure 7)	Yes	When exhausted outdoors (FORMALLY "B3") (minute amounts)[1,2]

Biosafety cabinets are certified when installed and at least annually, and whenever they are moved. This certification is performed at UNC Charlotte by an outside vendor. Contact the UNC Charlotte Biosafety Program Director/BSO if you have a BSC that needs certification.

Facilities

A properly designed facility can protect persons working inside and outside of the laboratory, as well as persons or animals in the community from exposure to infectious agents. Important elements of facility design for Biosafety Level 2 work include separation of the laboratory from public access, availability of decontamination equipment, and hand washing equipment. If the risk of airborne transmission is great, multiple secondary barriers may become necessary. Design features could include specialized ventilation systems, filtering of exhaust air, airlock entrances, and controlled access zones.

Disinfection and decontamination

The OSHA Bloodborne Pathogen Standard and its interpretation documents have specific guidance for the selection of disinfectants for use in the laboratory. BMBL offers guidance in

Appendix B--Decontamination and Disinfection for disinfection of infectious agents (but not necessarily bloodborne pathogens).

The US Environmental Protection Agency (EPS) provides guidance and approval on various disinfectants and sterilants for specific uses online in their Selected EPA-registered Disinfectants.

The Food and Drug Administration (FDA) provides their guidance online in their FDA-Cleared Sterilants and High Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices - March 2009

In most cases, either a freshly prepared 1:10 dilution of household bleach followed by a rinse of either 70% ethanol or distilled water should be adequate for most disinfection purposes. If proposed research involves use of a disinfectant not listed in one of the resources above, then consult with the UNC Charlotte Biosafety Program Director/BSO for further information.

There are differences by definition between sterilants, disinfectants, and decontaminants.

A **sterilizer or sterilant** is an agent intended to destroy all microorganisms (viruses, bacteria, fungi, and bacterial or fungal spores) on inanimate surfaces.

A **disinfectant** is an agent intended to destroy or irreversibly inactivate specific viruses, bacteria, or pathogenic fungi (but not necessarily spores) on inanimate surfaces. Most disinfectants are not effective sterilizers.

A **hospital disinfectant** is an agent shown to be effective against *Staphylococcus aureus, Salmonella choleraesuis*, and *Pseudomonas aeruginosa*. It may also be effective against such organisms as *Mycobacterium tuberculosis*, pathogenic fungi, or certain specifically named viruses. All commercially available hospital disinfectants contain a claim of effectiveness for specific agents in their labeling. All claims of effectiveness must be substantiated by data submitted to and accepted by the EPA prior to registration.

An **antiseptic** is a chemical germicide which is formulated to be used on skin or tissue.

Decontamination refers to a procedure that eliminates or reduces microbial contamination to a safe level with respect to the transmission of infection. Sterilization and disinfection procedures are often used for decontamination.

UNC Charlotte-specific practices

Training requirements

All personnel working with biohazardous materials must receive specified training and maintain documentation of current up-to-date training regardless of their previous experience or education.

Biosafety training courses

All UNC Charlotte researchers, students, employees and visitors who have a potential for occupational exposure to bloodborne pathogens (human blood, body fluids, unfixed tissues or organs; human cell cultures, medium or other solutions; and blood, organs, or other tissues from experimental animals infected with a bloodborne pathogen) must receive OSHA Bloodborne Pathogen training. It is an OSHA mandate that this training be conducted **upon initial assignment and annually thereafter**.

Employees who have the potential to be exposed to infectious materials other than human blood or body fluids should receive Biosafety Training. For BSL-2 level work and higher, CDC requires that personnel demonstrate proficiency in standard and special microbiological practices and must receive annual updates or additional training when procedural or policy changes occur. Demonstration of proficiency can be obtained by shadowing/mentoring of new employees and this training should be documented in writing. Updates on practices and procedures for laboratory work can occur during departmental meetings or weekly lab meetings and these training sessions should also be documented in writing.

Animal caretakers who work with animals that have been exposed to potentially biohazardous agents should receive Biosafety Orientation Training and they may also need to perform, OSHA Bloodborne Pathogen training on an annual basis.

Individual responsibilities

The responsibility for assuring safe handling of biohazardous materials is shared between the employee, the laboratory supervisor (senior scientist or principal investigator), management, area safety personnel, the Biosafety Program Director/BSO, and the Institutional Biosafety Committee (IBC).

The **employees, students and visiting scientists** must become familiar with the hazards of the particular infectious agents used. They must adhere to appropriate safety precautions and use the required protective equipment and/or engineering devices. It is the responsibility of employees, students, visitors, researchers to report all accidental exposures or incidents involving biohazardous materials to his/her PI or supervisor, the UNC Charlotte medical service provider and the Biosafety Program Director/BSO.

The **laboratory supervisor**, designated as the senior scientist or principal investigator (PI), is responsible for assuring the safe handling of biohazards in the laboratory and must:

- Know the safety requirements and potential health hazards of the materials in the laboratory.
- Register biohazardous work with the IBC by completing the Biosafety Protocol form available on the Biosafety website.
- Ensure that laboratory workers reporting to him/her receive the necessary biosafety training.
- Ensure that laboratory workers adhere to the standards of "good microbiological practice" and "universal precautions" when handling human blood, potentially infectious materials, or other pathogens.
- Ensure that all accidental exposures are reported properly and promptly.

In addition, the PI is responsible for enforcement of policies and procedures that assure the safe operation of the work area. Specific responsibilities include:

- Identify personnel having potential exposure to biohazardous materials.
- Ensure that use of biohazardous materials is registered with the IBC.
- Ensure that personnel have completed all required training.
- Ensure that adequate personal protective equipment, engineering safeguards, and physical facilities are provided to protect against exposure to biohazards.
- Enforce adherence to good work and housekeeping practices in all areas where biohazardous materials are used.

Area safety coordinators and **building safety committees** are responsible for:

- Assisting and providing support to management in implementing biosafety measures
- Serving as resources regarding biosafety practices, equipment, and regulatory requirements.

- Support and assist the Biosafety Program Director/BSO in their efforts to conduct regular safety inspections in the laboratory areas.

The Biosafety Program Director/BSO responsibilities include:

- Serve as a resource to personnel in all laboratory areas as well as other areas of the institution where workers may have contact with biohazardous materials.
- Provide training materials and deliver or assist with biosafety training.
- Review and advise on applications for the use of biological materials in laboratories.
- Together with members of the IBC and/or management, conduct periodic assessments of laboratory areas for compliance with biosafety regulations, policies, and procedures.
- Identify and consult on all regulations affecting the receipt, handling, shipping, treatment, and disposal of biohazardous materials.
- Update the Biosafety Manual.
- Coordinate with the UNC Charlotte medical provider and Student Health Services for vaccinations and follow-up/emergency care for biohazardous incidents.
- Investigate laboratory incidents involving spills, near-misses and exposures, coordinate with PIs for corrective action, if appropriate.
- Conduct regular safety inspections and audits in the laboratory areas.
- Maintain a biohazard inventory for the campus.

The **Institutional Biosafety Committee** is comprised of scientists who have expertise in the area of biohazards, an occupational physician, the head of laboratory animal care, and the BSO. Responsibilities of the committee include:

- Establish policies and procedures for the proper handling of biohazardous materials.
- Review the use of recombinant and synthetic nucleic acid molecules per the NIH Guidelines.
- Review of Biosafety Protocols for the use of biological materials in laboratories.
- Serve as consultants to the biosafety officer, laboratory personnel and local safety committees as issues arise.

Registration of use of biohazards

The principal investigator of each laboratory using biohazardous materials is required to register their use of biohazards by completing a registration form. This form documents where biohazards are used, for what purpose(s), and by whom. The Office of Research Compliance manages the review process, which is carried out by the IBC. The purpose of this process is to assure regulatory compliance and safe usage of biohazards, as well as to maintain a central repository of information on use of biohazardous materials.

Registration forms and further information can be obtained online on the Biosafety website.

What do I need to register in a protocol?

- BL2 and BL3 organisms. (If you're not sure, contact the Biosafety Program Director/BSO for further information.)
- Human blood, body fluids, tissues, and human cell cultures.
- CDC/USDA Select Agents and High Consequence Livestock Pathogens.
- Recombinant or Synthetic Nucleic Acid materials covered by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.
- Work with Risk Group 1 organisms need to be registered with the UNC Charlotte Biosafety Program but do not require IBC approval prior to initiation.

Use of signage

Biohazard signs must be posted on or adjacent to, the entrance to BSL-2 and BSL-3 laboratories.

- BSL-2 laboratories include those in which certain microbial agents designated as BSL-2 as well as certain human materials (blood, body fluids, tissues, and human cell lines) are manipulated.
- BSL-3 laboratories are those which require additional engineering controls and safety precautions due to the more hazardous nature of the organisms used.
- The signs must include the name and phone number (not the phone number of the laboratory) of a contact person responsible for the laboratory, and where they can be reached after hours and the name and phone number of the Biosafety Program Director. The name(s) of the infectious agent(s) should also be listed on the sign, as well as the use of human materials. Emergency responders will need the guidance of personnel in the laboratory to clean up spills or otherwise deal with an emergency.
- If the agents in use in the laboratory necessitate any special requirements for entry (e.g., vaccinations, PPE) the requirement must be conspicuously indicated on the sign.
- If work involving biohazards is conducted on an intermittent basis, the warning sign may be removed or covered when biohazardous work is not in progress.

Biohazard signs are not routinely posted at the entrance to BSL-1 laboratories.

- By definition, agents assigned to BSL-1 are not known to cause disease in healthy human adults.
- Laboratories working with cell cultures **not** of human or nonhuman primate origin are considered to be BSL-1. Note: It is possible that biohazard waste containers may be present in such labs, as biological waste may be disposed of in a conservative manner based on risk assessments regarding the environment and public perception.
- If a BSL-1 laboratory does work of a unique nature, involves complex activities, handles extremely high volume of a BSL-1 agent, risk-based decisions for signage, waste handling, and work practices will be made by the laboratory supervisor in concert with the IBC and the Biosafety Program Director/BSO

Use of biohazard labels and tags

All samples classified as biohazards or potentially infectious materials must be labeled with the universal biohazard symbol and/or the word "biohazard". If samples are too small to label individually, or if the nature of the work makes individual labeling impractical, the container holding them (e.g., test tube rack, plastic bag) shall have a biohazard label.

Equipment which may is used to store biohazardous materials must be labeled with biohazard warning tags or labels. These labels or tags should be affixed by a means (such as string, wire, or adhesive) that prevents their loss or unintentional removal.

Places and types of equipment to which biohazard signs or warning labels should be affixed include:

- Entrances to rooms where biohazards are stored
- Refrigerators, liquid nitrogen freezers or freezers where biohazards are stored
- Containers of infectious waste
- Containers of biohazardous materials
- The outside of packages in which biohazards are transported
- Equipment which may be potentially contaminated with biohazardous materials
- Any item which may be potentially contaminated or infectious (e.g., animal cages, used containers which may have contained biohazards).

Biohazardous waste

Disposal

Examples of items that are considered to be biohazardous waste include cultures of infectious microorganisms; human blood, body fluids, or tissues; carcasses, body parts, or fluids from infected laboratory animals; or materials that contain or may have been contaminated with any of the above.

All potentially infectious waste generated in laboratories must be segregated from other refuse and placed into waste containers that are impervious to moisture, of sufficient strength and thickness to prevent expulsion, secured to prevent leakage or expulsion, and labeled with the biohazard symbol. All potentially contaminated sharps must be placed in containers that are leak proof, rigid, and puncture resistant (e.g., plastic "sharps containers"), in addition to meeting the above criteria for infectious waste containers.

Infectious waste is regulated by state and local regulations. The key requirements with regard to infectious waste are proper labeling with subsequent disposal in a safe manner. Solutions containing biohazards may also be chemically decontaminated (e.g. with a freshly prepared 1:10 dilution of household bleach) and then provided they contain no other hazardous chemicals or materials may be discharged into the sanitary sewer system.

UNC CharlotteUNC CharlotteWhen waste contains a combination of a biohazard and another hazard, such as radiation or chemical waste, the usual practice is to decontaminate the biohazard and then treat the waste as only a chemical or radioactive waste. Contact EHS or the BSO for guidance with mixed chemical or radioactive wastes.

Animal waste

Any waste associated with animal use (e.g., surgery) must be bagged and placed in containers for the disposal with animal carcasses.

Disposal of sharps

The use of sharps in the laboratory is discouraged. Alternatives to sharps, such as plastic instead of glass and the use of safety engineered devices (safety needles, safe syringes, blunt scissors, etc.) should be evaluated on an annual basis.

It is the policy of UNC Charlotte that all sharps used or generated in the laboratory, regardless of whether they were used for biological hazards or nonhazardous materials, must be disposed of in rigid, puncture-proof red sharps containers. In North Carolina, sharps include, but are not limited to, needles, scalpel blades, razor blades, capillary tubes, slides and cover slips.

Sharps as defined above must be placed in red plastic sharps containers. The container must be appropriately marked with the biohazard symbol.
If broken glass contained potentially infectious or biohazardous material, it must be disposed of in a biohazardous sharps container, or it can be chemically decontaminated and placed in the broken glass box (if this does not present an additional risk). Extreme care should be taken with contaminated broken glass so as to avoid an injury. Broken glass that is contaminated must be cleaned up by first decontaminating the spill with disinfectant, and then by using a scoop, tongs,

dustpan, etc. Never pick up broken contaminated glass with your hands, even if you are wearing gloves.

If broken glass or Pasteur pipettes were not used to manipulate biohazardous material, they may be disposed of in the broken glass boxes available in laboratory areas.

To prevent injury to employees, sharp objects must never be placed loose into a plastic trash bag.

Biohazardous sharps containers must never be disposed of in the regular trash. Due to the labeling on the containers, they must be treated as if they were potentially biohazardous, and placed in the appropriate bins. UNC Charlotte.

Following is a summary table of various sharp or pointy objects that can puncture biohazard bags, the nature of materials that may be used with them, and proper disposal containers.

Sharp Object(s)	Nature of Material Used with Sharps	Disposal Container
Needles, blades, slides, cover slips, etc.	Biohazardous or non-biohazardous	Red sharps container
Broken glass, Pasteur pipettes	Biohazardous	Red sharps container or chemically decontaminate and place in broken glass bin
	Nonhazardous	Broken glass bin
Pointy objects (pipet tips, pipets)	Biohazardous	Plastic bottle*, sharps container or pipet box => Stericycle box
		Chemically decontaminated, => regular trash or broken glass bin (glass pipets)
	Nonhazardous	Broken glass bin (glass pipets) or regular trash (plastic)

***labeled with a biohazard sticker**

Protection of maintenance personnel, contractors and other service workers

It is important that laboratory work areas are kept clean and decontaminated so that service, maintenance, and contractor personnel are not inadvertently exposed to potential health hazards. As discussed in the section "Use of biohazard labels and tags" biohazard warning signs and labels must be in place on laboratory doors and on equipment so that service and maintenance personnel are aware of the existence of potential biohazards. Service or maintenance work should be done using universal precautions if there is a potential for exposure to human blood or body fluids.

Laboratory personnel must decontaminate work surfaces and equipment before service or maintenance personnel can begin their work. While it is difficult to decontaminate all parts of some laboratory instruments, all accessible surfaces and parts should be decontaminated before any service is performed on the instrument. Follow the manufacturer's directions for decontaminating equipment, if available. At the very least, accessible parts of equipment should be wiped down with a detergent followed by an appropriate disinfectant. If unsure in regards to the appropriate disinfectant or how to decontaminate a specific piece of equipment, contact the Biosafety Program Director/BSO.

A tag or label indicating decontamination (with date and name of responsible party) should be affixed to an instrument prior to maintenance. Laboratory personnel should be available to answer any questions from maintenance personnel regarding the existence or nature of potential hazards.

Emergency procedures

Reporting exposure incidents

Employees, students, visitors and contract workers shall immediately report any incident that results in an accidental exposure from a potentially infectious agent to their supervision and to the designated healthcare provider or Student Health Center (students) and to the Biosafety Program Director/BSO. Following an exposure to a bloodborne pathogen or other potentially infectious material, EHS and the Biosafety Program Director/BSO will complete a confidential investigation and follow-up. See http://safety.UNC Charlotte.edu/forms. For exposures to human-sourced materials, the procedure is summarized in the UNC Charlotte Bloodborne Pathogens Exposure Control Plan, available at (https://safety.UNC Charlotte.edu/sites/safety.UNC Charlotte.edu/files/media/manuals/Bloodborne_Pathogens_Exposure_Control_Plan.pdf).

Treating exposures

When an exposure incident occurs, first aid should be given at the accident site –this will consist of washing or rinsing the site with copious amounts of water, or using soap and water for skin exposures. Eyes or mucous membranes should be flushed with water for 10-15 minutes. After washing the site thoroughly, the employee should proceed to the designated medical provider or Student Health (students) as quickly as possible for evaluation and treatment.

Vaccinations

All employees, visitors, students, and contract workers with the potential for occupational exposure to human blood or other potentially infectious materials (OPIM's) are offered the hepatitis B vaccine at no cost to the employee. The vaccine is administered by the designated healthcare provider or Student Health (students) under the direction of a licensed physician and in accordance with U.S. Public Health Service standards. The vaccination is given at three intervals over a 6-month period and is ninety-five percent effective in preventing the disease. If the employee, visitor, student or contract worker chooses not to receive the vaccine, he/she must sign a declination form; anyone who declines the vaccine initially may receive the vaccine at any later time should they change their mind in regards to obtaining the vaccination.

Handling of biological materials

Any infectious material that is being transported on campus must be packaged to prevent leakage and spills. Typically, this would involve packaging the material in a sealed Ziploc bag with enough absorbent inside to contain the contents of the sample. After wiping the outside of the bag with a disinfectant, this Ziploc bag should then be placed in leak-proof tight-fitting lsecondary container, labeled with a biohazard sticker. This sealed container can be hand-carried to the destination, without wearing gloves, since the outside will also be decontaminated. If an organism or specimen is being transported, it must be taken directly to the destination, without stops at restrooms, cafeteria, etc. Personal vehicles and campus shuttles are not to be used to transport biohazardous materials

Shipment of potentially biohazardous materials

All materials known or reasonably expected to contain pathogens must be packaged, labeled, and shipped in accordance U.S. Department of Transportation (DOT) and International Air Transport Association (IATA) regulations regarding the transportation of infectious or biological substances. "Reasonably expected" means that the material has been tested and found to, or is

known to contain a pathogen, or that it has been taken from a patient known to be infected with a pathogen. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi and other agents such as prions), which can cause disease in humans or animals.
All potentially infectious materials that are to be shipped or transported over public highways must also be packaged in accordance with the applicable regulations.

> **NOTE:** Anyone involved in the shipment (packaging, labeling, manifesting, etc.) of biohazardous materials **must** be properly trained in hazardous material shipping regulations procedures (including identification, classification, and packaging of infectious or biological materials). This training must be completed every 2 years. Contact the Biosafety Program Director/BSO to arrange a training session or arrangements can be made for online training through the Mayo Clinic website: http://www.mayomedicallaboratories.com/education/online/dangerousgoods/index.html

A brief description of the various regulations affecting interstate shipment, import, and export of infectious or other biological materials are found below. Contact the Biosafety Program Director/BSO for further guidance on packaging and permit requirements.

Imports: If you are bringing into the U.S. non-infectious biological material of animal origin, a USDA permit may be required. If the material is infectious and of animal origin, a CDC permit and a USDA permit are required. If the material is non-infectious of human origin, there are no requirements. If the material is infectious and of human origin, a CDC permit is required.

Exports: For exporting biological materials, it is recommended that to use a transporter specializing in importing and exporting biological materials, such as World Courier, Federal Express, or DHL. World Courier obtains any customs documents and regulatory certificates necessary for export. They also provide packaging and instructions.

PHS - CDC

The importation of etiologic agents and vectors of human diseases is subject to the requirements of the Public Health Service Foreign Quarantine regulation (USPHS 42 CFR Part 71). Under this regulation, the form "Application for Permit to Import or Transport Agents or Vectors of Human Disease" must be filed with the Biosafety Branch, Office of Health and Safety, Centers for Disease Control and Prevention. That office then issues an importation permit along with shipping labels to the importer. The importer is responsible for assuring that the foreign shipper packs and labels the infectious material according to USPHS regulations. The importer must supply a copy of the permit and the appropriate shipping labels to the shipper. For current information, refer to the CDC site: http://www.cdc.gov/od/eaipp/

Part 72 of 42 CFR addresses interstate shipment of etiologic agents. It lists bacterial, fungal, viral, and rickettsia agents to which the regulation applies. It also specifies packaging, labeling, and shipping requirements.

Regulatory Requirements

Several regulations affect the primary nature of research work as well as peripheral activities associated with that work. This section summarizes the primary regulations dealing with use, transportation, shipping, and disposal of biohazardous materials.

OSHA

The Occupational Safety and Health Administration introduced a regulation dealing with occupational exposure to bloodborne pathogens (29CFR1910.1030) in December 1991. The text of the regulation is available on the OSHA web site (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10051). This regulation, known as the Bloodborne Pathogens standard, affects all employees who have potential occupational exposure to human blood, body fluids, or tissues. The standard outlines methods to minimize exposure, including engineering and work practice controls, personal protective clothing and equipment, training, medical surveillance, hepatitis B vaccination, signs and labels.

The Bloodborne Pathogens standard mandates training for employees, students, contract workers prior to initiation of work with any human blood, body fluids, or tissues. Another requirement of the Bloodborne Pathogens standard is that each institution shall have a written Exposure Control Plan, designed to eliminate or minimize exposure to infectious agents. At UNC Charlotte, this plan can be found at: https://safety.UNC Charlotte.edu/sites/safety.UNC Charlotte.edu/files/media/manuals/Bloodborne_Pathogens_Exposure_Control_Plan.pdf
Hard copies of the standard and the plan can be obtained from the EHS Office.

Department of Health and Human Services and United States Department of Agriculture

The Federal Select Agent Program (http://www.selectagents.gov/index.html) is jointly comprised of the Centers for Disease Control and Prevention/Division of Select Agents and Toxins and the Animal and Plant Health Inspection Services/Agricultural Select Agent Program. The Federal Select Agent Program oversees the possession, use and transfer of biological select agents and toxins, which have the potential to pose a severe threat to public, animal or plant health or to animal or plant products. The Program greatly enhances the nation's oversight of the safety and security of select agents by:

- Developing, implementing, and enforcing the Select Agent Regulations
- Maintaining a national database
- Inspecting entities that possess, use, or transfer select agents
- Ensuring that all individuals who work with these agents undergo a security risk assessment performed by the Federal Bureau of Investigation/Criminal Justice Information Service
- Providing guidance to regulated entities on achieving compliance to the regulations through the development of guidance documents, conducting workshops and webinars
- Investigation of any incidents in which non-compliance may have occurred

Select Agents Regulations

Pursuant to 42 USC 262a and 7 USC 8401, select agents and toxins are a subset of biological agents and toxins that the Departments of Health and Human Services (HHS) and Agriculture (USDA) have determined to have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products. The current list of select agents and toxins can be found at http://www.selectagents.gov/Select%20Agents%20and%20Toxins.html

Anyone considering working with a select agent or toxin from the above list must consult with the Biosafety Program Director/BSO in advance and complete an IBC Protocol form, since approval for work can take several months. Approval involves an audit of facilities, procedures and security by the applicable agency (CDC or USDA) as well as FBI approval of anyone desiring access to the agents in question.

CDC-NIH

While the CDC is not a regulatory body, it has set guidelines for the safe handling of biological hazards. The CDC guidelines are recognized as the most comprehensive description of microbiological practices, laboratory facilities, safety equipment, and classification of biohazards. CDC classified etiologic agents on the basis of hazard in the early 1970's and published a booklet, *Classification of Etiologic Agents on the Basis of Hazard* (4th edition, 1974). This booklet and the concept of categorizing infectious agents and laboratory activities into four classes or levels led to the publication of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), first published in 1984 (http://www.cdc.gov/biosafety/publications/bmbl5/). The descriptions of Biosafety Levels 1-4 parallel those in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, originally published in the Federal Register in 1976 and updated most recently in 2013 (http://oba.od.nih.gov/rdna/nih_guidelines_oba.html).

DOT

The United States Department of Transportation regulates the transportation of infectious substances under 49 CFR, Parts 171,172 and 173, and 175 (Hazardous Materials Regulations: Infectious Substances). These regulations specify transportation requirements and restrictions for any material known or reasonably expected to contain a pathogen. A pathogen is a microorganism that can cause disease in humans or animals.

References

[1] Biosafety in Microbiological and Biomedical Laboratories. 2009. CDC-NIH, U.S. Department of Health and Human Services. HHS Publication No. (CDC) 21-1112 (http://www.cdc.gov/biosafety/publications/bmbl5/).

[2] CDC. 1987. Recommendations for Prevention of HIV Transmission in Health-Care Settings. MMWR 36:2S-18S (http://aepo-xdv-http://www.cdc.gov/mmwr/preview/mmwrhtml/00023587.htm).

OSHA Bloodborne Pathogen Standard. 29CFR 1910.1030.
https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10051

Public Health Agency of Canada's *Pathogen Safety Data Sheets and Risk Assessment* website:
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES (NIH GUIDELINES):
http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm

National Select Agent Registry site: http://www.selectagents.gov