

# Basic Biosafety Training (OHS\_BIO303) Course Material

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## Introduction

Welcome to the **Basic Biosafety Training (OHS\_BIO303)** Course Material. This one-time training course is intended to provide individuals working with infectious agents with information on how to conduct a Biological Risk Assessment.

### UAB

UAB adheres to the National Institutes of Health (NIH) requirements for biological research, published in the 5<sup>th</sup> edition of the [\*\*Biosafety in Microbiological and Biomedical Laboratories \(BMBL\)\*\*](#). The [\*\*UAB Biosafety Manual\*\*](#) explains the operation of the Biological Safety Program and provides guidelines for all university personnel for the safe performance of experiments involving biological agents.

### Objectives

At the conclusion, participants should be able to:

1. Perform a detailed Biological Risk Assessment, based on agent and procedure-specific properties.
2. Define the different Biosafety Levels, list the minimum controls required, and describe the type of agents appropriate for each level.
3. Implement the principles of biological containment.

## Key Terms

- **Animal Biosafety level (ABSL1-4)** – A set of graded biocontainment levels that define minimum precautions necessary to safely work with different classes of infectious agents in animal models. The levels of containment range from the lowest (ABSL1) to the highest (ABSL4).
- **Biohazard** – An agent of biological origin that produces harmful effects on humans, (i.e., microorganisms, toxins, and allergens derived from those organisms).
- **Biological Risk Assessment** - Includes identifying agent- and procedure-specific considerations to identify risks, and to then assign appropriate lab practices, engineering controls, and personal protective equipment (PPE) needed to mitigate those risks. An [\*\*Agent-Specific Data & Safety Plan Template\*\*](#) has been developed to facilitate this process.

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- **Biosafety** – The applied use of practices, procedures, specialized facilities, and safety equipment (layers of containment) to mitigate risk from infectious microorganisms.
- **Biosafety level (BSL1-4)** – A set of graded biocontainment levels that define minimum precautions necessary to work with different classes of infectious agents safely. The levels of containment range from the lowest (BSL1) to the highest (BSL4).
- **Chance** – The likelihood of something happening.
- **Containment** – Practices, and barriers used for managing infectious materials that are designed to reduce or eliminate the risk of release or accidental exposures.
- **Disinfection**: A procedure reducing the level of microbial contamination by eliminating nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects.
- **Hazard** – Any source of potential damage, harm, or adverse health effects.
- **Inactivation**: A chemical (e.g., bleach) or physical (e.g., autoclave) process of rendering a biological agent inactive.
- **Incubation period**: The period between exposure to an infection and the appearance of the first symptoms.
- **Risk** – The probability that harm, injury, or disease will occur.
- **Risk group (RG)**: Graded classification used to rank the inherent risks of an infectious organism, with lowest risk group rated at “RG1” and the highest designated as “RG4.” Not all microorganisms are assigned a Risk Group.
- **Vector**: An organism that can transmit or distribute the agent.

## Conducting a Biological Risk Assessment

When performing a risk assessment, it is important not to underestimate or overestimate the risks involved with the proposed work. Minimizing the risk may lead to unsafe work practices, such as failure to wear the appropriate PPE. Conversely, overestimating the threat can increase the precautionary burden on personnel, which may result in shortcuts and lack of attention to actual hazards. It is better to err on the side of more stringent safeguards but within reason.

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Training, technical skills, and willingness to develop good work habits are critical in determining the relative risk for a specific lab. Facility safeguards and proper care and maintenance of containment equipment are also essential components of risk mitigation.

## Vital Considerations

### Risk Groups

The microorganism's properties are used to make a risk group determination. The [BMBL](#) contains Agent Summary Statements. If an Agent Summary Statement does not exist for the specific agent listed for the project, contact the UAB Biosafety Program at (205) 934-2487 for help in determining the agent's hazardous characteristics.

| Risk Group Classification | NIH Guidelines  | Examples of Agents   |
|---------------------------|---|--|
| Risk Group 1              | <b>(No or low individual and community risk)</b><br>Agents not associated with disease in healthy adult humans  | <i>E. coli K-12, S. cerevisiae(yeast), Lactobacillus, Bacillus subtilis, Bacillus cereus, Penicillium notatum, Staphylococcus epidermidis, AAV</i>                   |
| Risk Group 2              | <b>(Moderate individual risk; low community risk)</b><br>Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.                                 | <i>Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, Cryptococcus neoformans, Adenoviruses</i>                        |
| Risk Group 3              | <b>(High individual risk but low community risk)</b><br>Agents associated with severe or lethal human disease for which preventive or therapeutic interventions may be available  | <i>Yersinia pestis (black plague), SARS virus, Mycobacterium tuberculosis, Monkeypox virus, Francisella tularensis, Burkholderia pseudomallei, Chikungunya virus</i> |
| Risk Group 4              | <b>(High individual and community risk)</b><br>Agents likely to cause severe or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk) | <i>Ebola virus, Marburg virus, Lassa virus</i>   |

### Agent-Specific Properties

- Strain:** There are often a number of strains available for a specific pathogen. It is preferable to use the least virulent or pathogenic strain available that meets your endpoints.
- Host Range:** Describes the types of organisms that could be infected with the agent. For example, a virus can infect specific species of plants, animals, or even specific cell types.
- Vector Range:** Refers to the geographical range or distribution of the vector (e.g., ticks, mosquitos)

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- **Infectious Dose:** Is the number of microorganisms required to cause an infection in the host
- **Medical Options**
  - Prophylaxis: Vaccinations or post-exposure measures to prevent an infection
  - Treatments: Therapeutic options available to dampen or reverse an illness
  - Surveillance: Monitoring individuals for adverse health effects that may be caused by the agent
- **The Severity of Disease if Contracted:** How severe is the disease once contracted? Are treatments available? If so, how do they affect the outcome?
- **Natural and Laboratory Based Modes of Transmission:** Routes of potential transmission in the lab may differ from the agent's natural way of transmission. In the laboratory setting, additional routes of infection may be possible due to the production of infectious aerosols, hand-to-mouth or hand-to-eye transfer, as well as punctures by contaminated sharps, and bites from infected animals.
- **Environmental Stability:** How long (hours, days, weeks, months) the agent remains infectious outside of a host.
- **Genetic Modifications:** Genetic modifications should be considered for deliberate or unintentional increases in resistance to antibiotics or other treatments, increased pathogenicity, or improved survival when released into the environment. Potential risks associated with genetic changes may not be evident until generated data is analyzed.
- **Regional Prevalence:** What is the current geographical prevalence of the agent (indigenous, emerging, or exotic)? Public safety and the environmental consequences of a release will factor into the relative risk of an agent.
- **Disinfectants:** The chemicals used to disinfect potentially contaminated work surfaces and the conditions in which they are adequate for your agent, including the:
  - Effective concentration, shelf-life, and contact time required.
  - Effective temperature.
  - Amount of organic matter present (e.g., soil, feces, and blood).
  - Type and condition of instruments, devices, and materials to be disinfected.
- **Inactivation Procedures:** Published methods should be used for chemical or physical inactivation of infectious agents if they are to be used for subsequent analyses and considered non-infectious. Alternatively, the inactivation methods should be validated to assure the procedure adequately protects staff from exposure.

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## Procedural Risk Factors

Proper work practices and the correct use of safety and laboratory equipment provide the primary defense against exposures. Workers must protect themselves, other staff, the public, and the environment from exposure to hazardous agents. Poor work practices by any member of the team can negate any safeguards or controls in place. PI's should evaluate staff for proficiency in appropriate techniques for handling infectious agents and provide agent-specific training before beginning any work.

- **Aerosol Producing Procedures:** A wide variety of processes and equipment used in laboratories cause aerosol generation. Aerosols of infectious agents may go undetected and are considered the probable source of many Laboratory Associated Infections (LAIs). Common lab procedures, such as centrifugation, pipetting, sonication, vortexing, flow cytometry, and blending, can potentially create aerosols. Many of the particles produced are airborne, posing a prolonged risk for respiratory exposure. Aerosol risks can be estimated using an agent's particle size, aerosol concentration, aerosol viability, and infective dose by inhalation.
  - Contamination of hands, mucous membranes, or work surfaces can also result from deposits of larger airborne particles. Containment and good laboratory practices should be used to lessen the risk from aerosols as well as careful and proficient techniques that reduce aerosol generation.



Consult the literature to learn more about the potential for [Laboratory Associated Infection \(LAIs\)](#). However, the absence of LAI reports in the literature concerning a specific agent does not reduce or eliminate the need to conduct a Risk Assessment.

- **Animal Models:** Infected animals pose a procedural risk, due to their unpredictability and potential to bite, scratch, aerosolize, or shedding of infectious agents. Shedding can occur in the feces, urine, and saliva of infected animals. If your studies involve infected animals, the literature on potential shedding should be reviewed to determine if cages, bedding, and other surfaces that were in contact with an infected animal can serve as potential sources of exposure. Animal-to-animal transmission among cage-mates is one indicator of possible shedding. However, lack of animal-to-animal transmission does not lessen the risk of exposure when handling infected animals.

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- **Method of Exposure:** How is the agent administered to animal models (e.g., aerosols or injection)? If not utilized correctly, aerosol exposures pose a risk to equipment. The use of syringes poses a threat for percutaneous exposure.
- **Amplification by Infection:** If an animal model supports replication of the agent, there will likely be an increase in infectious material. If so, biting and shedding are considered. If not, when will the animal no longer transmit infectious materials?
- **Latent or Adventitious Agents:** Animal models, as well as human- and animal-derived tissues, may harbor infectious agents that are not an intended part of the [study](#) (e.g., Herpes Viruses, Hepatitis B, Hepatitis C, Human Immunodeficiency Virus, Lymphocytic Choriomeningitis Virus, Hanta Virus). Therefore, the potential for infectious agents within animal models or tissues used in the study is a possible risk.

## Sharps Policies

Policies for the safe handling of sharps (needles, scalpels, pipettes, and broken glassware) are required. Laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Examples:

- Avoiding the use of needles that are bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
- Placing used disposable needles and syringes in puncture-resistant containers used for sharps disposal.
- Placing non-disposable sharps in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
- Using a brush and dustpan, tongs, or forceps to remove broken glassware rather than handling directly. Plasticware should be substituted for glassware whenever possible.

**Types of Biological Containment Controls:** “Containment” encompasses the methods, facilities, and equipment for the safe handling and storage of infectious materials, all aimed at preventing human exposures or release of these agents into the environment.

- **Elimination or Substitution:** The most effective means for eliminating risk is to remove the hazard. If the complete omission of the agent from the studies is not feasible, another option to consider is the use of attenuated strains or surrogates rated at a lower risk group.

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- **Administrative Controls:** Administrative controls include requirements for training, access control, SOPs, handwashing, sharps policies, and requiring the enrollment in [Occupational Medicine Programs](#).
- **Workplace Practices:** Strict adherence to standard microbiological practices and techniques is the most critical element of containment. A laboratory-specific biosafety manual must be drafted, adopted and specify the hazards in the lab. This manual also designates the appropriate practices and procedures for risk mitigation and describes incident response procedures in the event of an exposure. Adequate training on these practices and procedures should be documented for everyone at risk by these hazards.
- **Engineering Controls:** Engineering controls are devices or equipment designed as primary barriers to mitigate exposure risk.
  - **Primary barriers:** Biosafety Cabinets (BSC) and Centrifuge Safety Cups are examples, both of which are designed to protect from infectious aerosols and droplets. Use Personal Protective Equipment (PPE) in conjunction with engineering controls, but it can also serve as a primary barrier in cases where it may be impractical to work inside a BSC.
  - **Secondary barriers:** The design and proper function of the facilities where infectious agent work will be conducted serve as secondary barriers for protecting personnel, the public, and the environment. The facility requirements vary, based on the procedures and transmission routes of the specific agents handled. Directional airflow, the number of air changes per hour, HEPA-filtered exhaust, the presence of airlocks, and anterooms are all examples of secondary barriers.

## Assigning Containment Levels

One of the last steps of a Biological Risk Assessment is to determine the Biosafety Level (BSL). These are ranked 1 through 4, with BSL1 specifying the least stringent requirements, and BSL4 the most stringent.

### **Biosafety Level 1**

A Biosafety Level 1 (BSL1) laboratory is for research using well-characterized agents not known to cause disease in healthy adult humans and cause a minimal potential hazard to laboratory personnel and the environment. Research appropriate for a BSL1 lab may include work with specific animal cell lines or work with non-pathogenic bacteria or yeast (such as *E. coli* DH5a or *Saccharomyces cerevisiae*).

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Laboratory Supervisors must inform those working with biological materials of any potential hazards related to individual health status. Those with health issues are encouraged to discuss work with their personal physicians regarding potential risk.

## Standard Microbiological Practices

Standard microbiological practices are common to all Biosafety Levels. Microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment. These apply to BSL's. However, depending on what you are working with, the expected microbiological practices for you will be more stringent.

- Ensuring all lab staff have completed and are proficient in all required training. Proof of training must be available upon request.
- Limiting access when conducting experiments.
- Washing hands after removing gloves and before leaving the lab.
- Eating, drinking, smoking, applying cosmetics, and handling contact lenses are prohibited.
- Storing food for human consumption is prohibited.
- Using mouth pipetting is **not** allowed!
- Implementing safe handling of sharps.
- Minimizing splashes and aerosols.
- Decontaminating cultures, stocks, and contaminated waste.
- Removing gloves before exiting laboratory.
- Posting and updating UAB emergency notification on the entrance door of the lab.

## Personal Protective Equipment (PPE) and Facilities

The lab should have:

- Doors for access control
- A sink near the lab exit for handwashing
- Have work surfaces that are easily cleaned/disinfected
- Furniture designed for easy cleaning and disinfection

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- Minimum PPE:
  - Lab coats
  - Gloves, when handling hazardous materials
  - Face and eye protection, when splashing might occur

## ***Biosafety Level 2***

A Biosafety Level 2 (BSL2) laboratory is for research with agents of moderate potential hazard to laboratory personnel and the environment. BSL2 requirements build on those listed for BSL1. Refer to the BMBL for a full list of [\*\*BSL2 and ABSL2 requirements\*\*](#).

### **Special Practices:**

- Requiring laboratory personnel complete training on the specific pathogenic agents used in the lab. They must demonstrate proficiency and have supervision by competent scientists. This training must be documented and presented upon request.
- Posting Biohazard Signs at lab entrance doors (listing agents used and entry requirements) and the symbol posted on equipment used for processing biohazardous material.
- Limiting access to the lab during work.
- Increasing precautions for sharps handling and disposal.
- Offering personnel available and appropriate vaccinations.
- Decontaminating equipment and work surfaces regularly (including after completion of work, after any spill of potentially infectious material, before servicing and before being taken out of service).

### **Safety Equipment**

- Using Biosafety Cabinet, Centrifuge Cups, or other physical containment devices for aerosol-producing procedures. Ensuring Biosafety Cabinets (BSCs) and Emergency Eyewash Stations are readily available. BSC's should be certified annually.
- Protecting vacuum lines with liquid disinfectant traps and inline HEPA filters.
- Verifying mechanical ventilation systems provide an inward flow of air and that the community spaces outside of the lab are not exposed to recirculated air.
- Determining the proper handling and disposal of all laboratory waste.

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## Personal Protective Equipment (PPE) and Facilities

- Always wear lab coats when in the laboratory. Remove lab coats before leaving the lab, and do not take them home.
- Eye and face protection (goggles and a face shield, or other splatter guards) are used for anticipated splashes or sprays of hazardous materials when handled outside the BSC or containment device.
- Gloves are worn to protect hands from exposure to hazardous materials. Glove selection depends on your risk assessment. You should never wash/reuse disposable gloves or wear them outside the lab. Your lab should provide alternatives to latex gloves. You should be:
  - Changing gloves when contaminated or compromised
  - Removing gloves and wash hands when work with hazardous materials is completed
  - Disposing of used gloves with other contaminated laboratory waste. Implementing handwashing protocols

### ***Biosafety Level 3***

A Biosafety Level 3 (BSL3) laboratory is for research with indigenous or exotic agents that may cause severe or potentially lethal disease through the inhalation route of exposure. Completion of all procedures involving the manipulation of infectious materials conducted within BSCs or other physical containment devices must be in a BSL3 facility.



Additional training is required for agents and processes requiring work to be conducted in a BSL3 Facility. No BSL4 work is allowed at UAB. Therefore, BSL3-4 containment levels are not included in the scope of this course material. For more information, refer to the [\*\*BMBL\*\*](#).

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## Reviewing Your Assessment

The “last step” in a risk assessment is an ongoing review of the containment controls assigned, specifically regarding their efficacy in preventing exposures to, or releases of, infectious agents. Technological advances may have improved or developed engineering controls that are more practical to the laboratory applications involved, and new knowledge, gleaned from LAIs, literature, or practical/hands-on experience, may warrant refinements to the controls assigned. Regulatory and granting agencies may require your risk assessment be formally reviewed and approved by the Institutional Biosafety Committee (IBC) and OH&S. If you have any questions or problem that arise during the risk assessment process, contact UAB OH&S at (205) 934-2487.

### ***Lab-Specific Biosafety Manual***

The risk assessment process is designed to prevent loss of containment and exposures. However, accidents can and will occur. Your manual should be:

- Detailing exposure-response procedures for all hazardous agents in the lab.
- Linking detailed spill response procedures or exposure-response plans (including different types of exposures) to the agent-specific risk assessment.
- Listing appropriate reporting instructions for all response procedures (particularly if medical attention is required).
- Documenting training on the agent-specific risk assessment and exposure-response procedures for all lab members at risk.

### ***Reporting***

All spills outside of primary containment (biological safety cabinet or another device) that involve dangerous materials, recombinant or synthetic nucleic acid molecules, or organisms containing recombinant or synthetic nucleic acid molecules must be reported immediately to the Biosafety Officer (BSO) at (205) 934-2487 who will notify the IBC.



Incident reporting is not for establishing fault, but to facilitate the development of measures to prevent similar incidents from recurring.

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## Exposure Response Procedures

- Mucosal membranes:
  - Flush mucous membranes with water for 15 minutes
  - Notify your Supervisor/Manager as soon as possible
  - Seek medical attention (see below)
- Dermal:
  - Wash affected areas with soap and water for 15 minutes
  - Notify your Supervisor/Manager as quickly as possible
  - Seek medical care (see below)
- Inhalation/Ingestion:
  - Seek medical attention for post-exposure prophylaxis, if available

## Medical Response Options:

- Be aware of the symptoms associated with the agent you work with so that you can report and seek medical attention.
  - **Researchers (not hospital/medical care associated exposures)**
    - During Work Hours (Monday-Friday, 7am-4pm)
    - The Workplace Clinic, 1201 11th Avenue South, Birmingham, AL 35205 (205) 930-7007 After hours or weekends: UAB Emergency Departments
    - A completed [\*\*Initial Medical Evaluation Authorization Form\*\*](#), signed by a Manager/Supervisor, should accompany any campus employee seeking treatment.
  - **Hospital Employees (hospital/medical care associated exposures)**
    - Needlesticks and other incidents (including exposures to blood or body fluids) call the Rapid Response Team (RRT) at (205) 934-3675 or page 934-3411.

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## Conclusion

You have reached the end of the **Basic Biosafety Training (OHS\_BIO303)** Course Material. You should now take the assessment. The passing score is 90%. You have three chances to complete the assessment successfully. Failing all three attempts means that you fail the course and must start over.

### Other Training

- **Biohazardous Infectious Waste (Medical Waste):** If you generate or handle medical waste in or from research laboratories at UAB, it is required you complete [Medical Waste Management for Labs \(OHS BIO301L\)](#).
- **Radioactive Waste:** If you handle, pack, or manifest Radioactive Waste Materials, it is required you complete [Radiation Safety Waste Handling and Packing \(OHS RS105\)](#).
- **Hazardous Waste:** If you generate, handle, pack, or electronically sign a manifest requesting hazardous waste for pickup or disposal, it is required you complete [Hazardous Waste Handling and Packing \(OHS CS055\)](#).
- **Chemical Safety:** If you handle chemicals that are considered hazardous in the course of your work, it is required you complete [Chemical Safety Training \(OHS CS101\)](#).
- **Bloodborne Pathogens:** UAB Campus Employees whose job duties put them at an increased risk for exposure to bloodborne pathogens, are required to complete [Bloodborne Pathogens Training \(OHS BIO500\)](#).
- **Biosafety Cabinets and Fume Hoods:** Anyone that will be conducting work or research in a Biosafety Cabinet, Fume Hood, or Clean Air Station, is required to complete [Biosafety Cabinets and Fume Hoods \(OHS BIO304\)](#).
- **Recombinant or Synthetic Nucleic Acid Molecules:** If you are working with recombinant or synthetic nucleic acid molecules it is required you complete [NIH Guideline for Recombinant and Synthetic Nucleic Acid Molecules \(OHS BIO305\)](#).
- **Personal Protective Equipment:** If you are required to wear Personal Protective Equipment while you conduct your work or research, it is strongly recommended for you to complete [Personal Protective Equipment \(PPE\) \(OHS OHS100\)](#).

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OH&S has many training courses available to all UAB active employees and students. A [decision tree](#) is available to assist you in choosing the right training courses to supplement the knowledge/skills you may need at work. If you have any questions or comments contact OH&S at (205) 934-2487.