BIO200 - Biosafety Training for Laboratory Researchers

Introduction

Your Job and Hazard Questionnaire (JHQ) indicates that you are planning to work with biological or biomedical materials within a laboratory, or supervise an employee who works with this type of material.

OR

You have been identified by the Institutional Biosafety Committee (IBC), your supervisor or Environmental Safety and Health (ESH) Coordinator as being required to take this course and obtain training in the basic principles of biological containment of pathogens and toxins.

Course Objectives

By the end of this course, you will be able to:

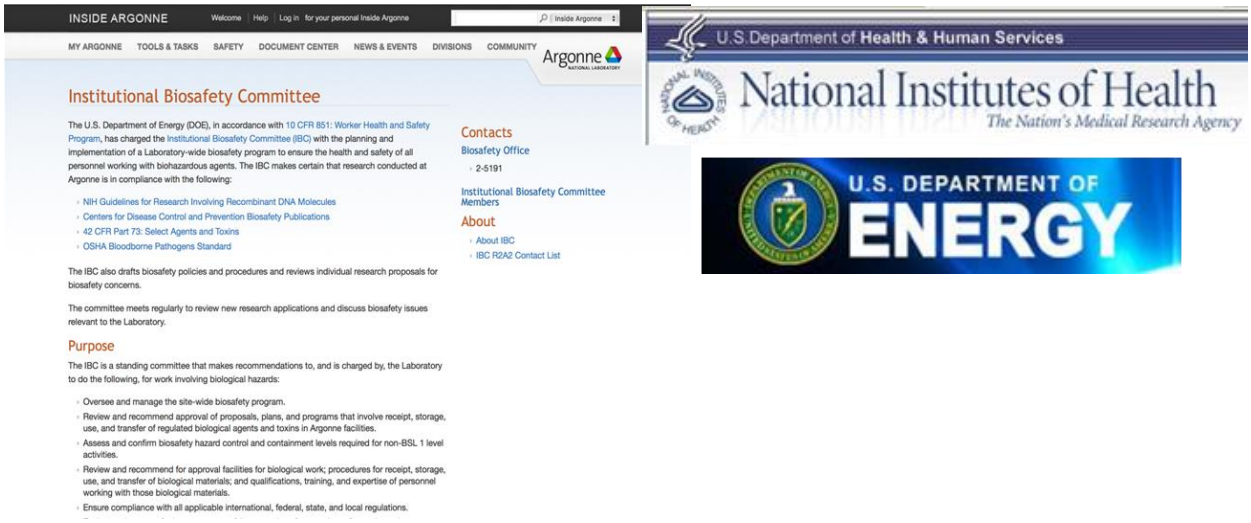
- Identify the purpose of Argonne's Institutional Biosafety Committee (IBC)
- Recognize the classes of biological hazards and toxins
- Determine how occupational exposure to biohazardous materials can occur
- Recognize the Center for Disease Control's (CDC) Infectious Agents Risk Group classifications
- Describe specific procedures for avoiding sharps injuries and aerosol exposure
- Distinguish the types of protection offered by safety cabinets
- Identify the appropriate methods for disinfection and disposal of biohazardous material
- Identify the guidelines and regulations that govern off-site/ on-site movement, storage and transport of biohazardous materials
- Determine the appropriate emergency response to infectious and biological toxic material spills

Argonne's Biohazard Policy

Argonne has a Biosafety Manual that can be accessed on the Institutional Biosafety Committee (IBC) website. For more information - click here.

The *Biosafety Manual* will be the basis for general biosafety guidelines at Argonne. Laboratory personnel will be expected to follow practices outlined in this manual and prudent practice specific to the projects in which they are involved that have been developed in collaboration with the IBC and/or their division Environment, Safety, and Health (ESH) coordinator.

Anyone not in compliance with this manual will be subject to committee review and disciplinary action, as well as any mandated reporting to oversight federal agencies.



Purpose of Argonne's Institutional Biosafety Committee (IBC)

The IBC is a standing committee that makes recommendations to, and is charged by the Laboratory to do the following, for work involving biological hazards:

- Oversee and manage the site-wide biosafety program.
- Review and recommend approval of proposals, plans, and programs that involve receipt, storage, use, and transfer of regulated biological agents and toxins in Argonne facilities.
- Assess and confirm biosafety hazard control and containment levels required for non-BSL 1 level activities.
- Review and recommend for approval facilities for biological work; procedures for receipt, storage, use, and transfer of biological materials; and qualifications, training, and expertise of personnel working with those biological materials.
- Ensure compliance with all applicable international, federal, state, and local regulations.
- Evaluate adequacy of relevant aspects of Argonne plans for security, safeguards, and emergency management.

The IBC is needed:

- Prior to initiation of the research
- At regular intervals during the activity
- When a change of protocol occurs
- When new technologies are introduced

Regulations

The regulations that govern biohazards and biosafety are at both the federal and state level and are promulgated by multiple agencies.

NEVER assume that a material is safe or legal to handle without discussing it with your ESH Coordinator and/or the Biological Safety Officer (BSO) for your area.

Contacts for questions about Biosafety Risk Assessment

There are three primary sources for biosafety risk assessment on site:

- Site-wide Biosafety Officer
- Advanced Photon Source (APS) BSO
- IBC

Classes of Biological Hazards

Biological Toxins

Any toxic substances that can be produced by microorganisms, animals, and plants. Examples include:

- Botulinum toxins, tetanus toxin, and staphylococcal enterotoxins, which are produced by animals.
- Ricin toxin, Trichothecene mycotoxins, and abrin, which are produced by plants.

Infectious Agents

Includes viruses, bacteria, fungi, rickettsii and parasites. This category may include animal-derived material which may harbor zoonotic disease agents (zoonotic are diseases that can be transmitted to man).

Prions

Protein-based agents such as Transmissible Spongiform Encephalopathy (TSE) agents and tissue from animals that may be infected with zoonotic diseases.

Potentially Infectious Material (PIM)

All human and non-human primate tissue, blood, cell lines and explants, and products derived from the previous categories.

Regulated by a Government Agency

Biological material which poses no human health risks but is regulated by a government agency Examples include:

- Animal and plant pathogens which pose a risk to the local environment.
- Material derived from the former whose movement is monitored by the USDA and/or the Dept. of Commerce.

Opportunistic agents

Risk Group 1 organisms that cause disease due to exposure circumstances or lower immunity.

Biological Toxins

Biological toxins are any toxic substances manufactured by biological organisms.

They include both large chemicals and proteins.

Biological toxins include such material as:

- Ricin
- Botulinum toxin
- Various enterotoxins
- Snake and spider venoms and their purified components
- Many other materials are listed on the Federal Select Agent list of bio-warfare agents.

Because toxins are not living organisms, work with them is not assigned an official biosafety level.

However, at Argonne, we require Biosafety Level (BSL) 2 controls for biological toxins in accord with Centers for Disease Control (CDC) recommendations.

Prions

Prions are normal proteins of animal tissues that can misfold and become infectious; they are not cellular organisms or viruses.

Prions are associated with a group of diseases called Transmissible Spongiform Encephalopathy (TSEs). No early acute clinical indications for TSEs have been described.

After an extended incubation period of years, these diseases result in irreversible neurodegeneration. There are no vaccines or cures for TSEs.

The CDC requires that all prion work be performed under BSL2 level controls or higher.

Waste disposal from prion work involves incineration of solid waste and soaking of surfaces and liquids with sodium hydroxide and detergent and/or full strength bleach.

Human and Animal Cell Culture

Employees planning to work with human/non-human primate cells or tissue (including established cell lines) must contact the Biosafety Officer to obtain Bloodborne Pathogens for Researchers training and be offered a Hepatitis B vaccine series from HEW.

Work with this material must be carried out in a BSL2 facility which has been inspected by the IBC prior to start of work.

In general, animal cell work is not considered a biohazard unless the animal cells are purposely infected with pathogens and/or are carrying rDNA constructs which code for toxic or oncogenic sequences.

Possession, Use, and Transfer of Biological Select Agents and Toxins

Argonne **DOES NOT** have a license to possess Select Agents.

- This is a long process which involves significant infrastructure construction and employee clearance by the FBI.

Select Agents

Select Agents (SAs) are highly dangerous potential bio warfare agents as defined by the federal government. www.cdc.gov/od/sap/docs/salist.pdf

SA and SA toxins are a small subset of infectious agents and toxins.

There are, however, attenuated forms of certain SAs and exempt quantities of SA toxins that can be worked with at BSL2 containment that are not covered by the SA rules.

Occupational Exposure to Biohazardous Material

- Inoculation
  - Pricking, jabbing or cutting the skin with contaminated instruments such as needles, scalpels and glassware; from animal bites or scratches, or through contact with breaks in the skin such as shaving cuts or other personal injuries.

- Ingestion
  - Mouth- pipetting, eating, drinking, smoking, applying cosmetics, lip balm and splashes

- Splashing into the face and eyes

- Spillage and direct contact

The CDC classifies infectious Agents into Risk Groups (RG) by degree of handling risk

Below are various types of risk groups.

Risk Group 1 - RG1 agents are agents that do not make healthy adult humans sick; such as cloning strains of E. coli. **(RG1) - Don't Drink)**

Risk Group 2 - RG2 agents are agents that make healthy adult humans sick but which rarely are serious and for which interventions are often available; such as Hepatitis B virus.
(RG2) - Don't Touch)

Risk Group 3 - RG3 agents are agents that cause serious or lethal disease for which interventions may be available. These agents usually have a high individual risk but a low community risk (i.e. are not communicable person to person); such as West Nile Virus. **(RG3) - Don't Breathe**)

Risk Group 4 - RG4 agents are agents that cause serious or lethal disease for which interventions are not usually available; such as Ebola and Marburg Viruses. **(RG4) – Do Not Do in Illinois**)

The CDC defines Biosafety Levels for handling to reduce risk of Lab-acquired infections

Biosafety levels involves a combination of practices and controls which include:

Standard microbiological practices

- Personal protective equipment (PPE)
- Aerosol-reduction handling protocols

Safety equipment (Primary barrier)

- Biosafety cabinet
- O-ringed rotor buckets

Laboratory facilities (Secondary barrier)

- Biohazard sign on entrance
- Authorized personnel only entry requirements

Biological material handling is usually assigned to a Biosafety Level that defines the required handling practices based upon the risk posed to the worker.

Each Biosafety Level defines a different working environment

Below are various types of Biosafety Level and risk groups.

Biosafety Level 1 (BSL1) - This is a standard Argonne wet laboratory

Biosafety Level 2 (BSL2) - These laboratories have access limited to authorized personnel and can have special equipment such as biosafety cabinets. Human/non-human primate tissue culture must be performed in a BSL2 facility.

Biosafety Level 3 (BSL3) - A BSL3 lab requires filtered air flow and other controls to limit exposure to airborne hazards.

- Argonne has one BSL3 beamline sector at the APS and the University of Chicago has a regional biocontainment laboratory on site.

Biosafety Level 4 (BSL4) - A BSL4 lab is a highly controlled environment where employees breathe filtered air coming in from outside the lab.

- There are only a handful of these facilities in the country: CDC in Atlanta, Galveston, TX and USAMRIID in MD.

Argonne does NOT have a BSL4 facility.

Sharps Safety in the Laboratory

Studies have shown that 42% of injuries occur when used needles are recapped. Therefore, when using sharps safety devices, activate the safety device as soon as possible after use.
PROMPTLY DISCARD ALL NEEDLE AND SYRINGE UNITS IN SHARPS CONTAINERS AFTER USE.

- Do not clip, recap, or separate needles from the syringe.
- Deposit needles and syringes directly into sharps containers.
- Do not disconnect the needle from the syringe unless you are using a sharps safety device and you have first activated the safety feature.
- Never bend, break, or shear needles.

See LMS-PROC-263 for additional information.
(Disposal of Sharps in Laboratories)

Sharps Safety

Plastic pipettes do not break as easily as glass and thus are not considered a sharps hazard. Use **2ml polystyrene pipettes** in place of glass Pasteur pipettes for tissue culture work and fluid aspiration.

Use **Disposable scalpels** such as the **BD Bard-Parker™ Protected Disposable Scalpel** which features a locking retractable shield.

Plastic inoculation loops/spreaders should be used in place of inoculation spreaders formed from glass rods or Pasteur pipettes. Use plastic loops to spread colonies on agar plates. Accidental breakage is virtually eliminated and thus no sharp edges are created which could lead to accidental exposure to contaminants.

Sharps Disposal Guidelines

All sharps, including those with safety devices already activated, must be disposed of in a sharps container.

- Never over-fill sharps containers. This invites compression of waste, which has led to many needle stick injuries.

Broken glassware must be picked up by mechanical means (e.g., with tongs or dust pan and brush) – **never pick up broken glass directly with the hands.**

- Decontaminate instruments used to pick up broken glass after their use and prior to storage with an appropriate fluid such as bleach.

Aerosol Exposure

In addition to avoiding obvious sources of infection such as splashes, cuts, accidental inoculation or ingestion, laboratory workers should also be aware that many pathogens, when airborne, may cause infection if inhaled.

- Inhalation of infectious aerosols is a primary cause (estimated 80%) of laboratory-acquired infections.
- Aerosolized micro-organisms are generated during most routine laboratory procedures involving manipulation of liquid suspensions, including blending, sonicating, centrifuging, and heat sterilization.

Aerosol Producing Activities

Examples of aerosol producing activities that can result in accidental aerosol exposures.

Centrifugation and its steps:

- Filling centrifuge tubes
- Removing plugs or caps from tubes after centrifugation
- Removing supernatant
- Re-suspending sedimented pellets
- Blowing out pipettes and pipette tips
- Sorting cells
- Shaking, vortexing, or stirring tubes
- Pulling needles out of out of septa while filling a syringe
- Pouring liquids

Preventing Biological Aerosol Production

All operations which may result in the production of aerosols or splashes, must be performed in a biosafety cabinet.

Once formed, aerosols can be captured by high efficiency particulate air (HEPA) filters in a biosafety cabinet (BSC) or removed from the laboratory by room ventilation methods.

In general, avoid frothing and the production of air bubbles. Follow safe work practices and employ safety precautions to avoid creation of aerosols.

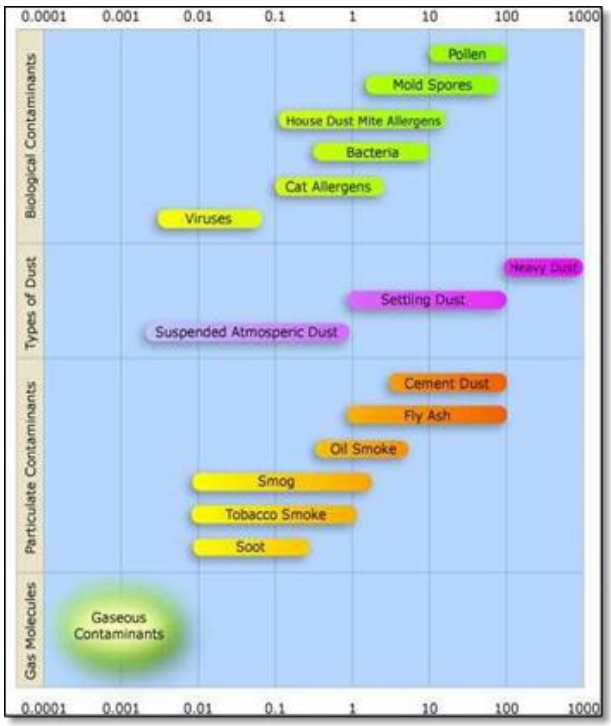
While the use of safe work practices and the application of available safety devices is recommended, their use is not a substitute for good technique.

Biosafety Cabinets in BSL2 Facilities

Any operations that may generate a splash or aerosol hazard are required to be performed in a biosafety cabinet (BSC).

The common element to all classes of BSCs is the HEPA filter. This filter removes particles of 0.3 microns with an efficiency of 99.97% (see chart).

While HEPA filters are effective for trapping particulates and infectious agents, these filters will not capture volatile chemicals or gases.



Types of Biosafety Cabinets

There are three types of biological safety cabinets. See below for more information on each class of biosafety cabinet.

Class I – A Class I biosafety cabinet will provide personnel and environmental protection, but not product protection.

This type of biosafety cabinet is an open-front negative pressure cabinet. The exhaust air from the cabinet is filtered by a HEPA filter. It is similar in air movement to a chemical fume hood, but usually has a limited fixed work access opening and the exhaust air must be HEPA filtered to protect the environment.

Class II – A Class II biosafety cabinet will provide personnel, environment, and product protection.

The Class II vertical laminar-flow biological cabinet is an open-front, ventilated cabinet. This cabinet provides a HEPA-filtered mass airflow within the work space. The exhaust air from the cabinet is also filtered by HEPA filters.

Class III – A Class III biosafety cabinet offers the highest degree of personnel and environmental protection.

The Class III biosafety cabinet is a totally enclosed ventilated cabinet of gas-tight construction. Operations within the cabinet are conducted through attached rubber gloves. When in use, it is maintained at a negative air pressure of at least 0.5 inches water gauge. Supply air is drawn into the cabinet through HEPA filters. The cabinet exhaust air is filtered by two HEPA filters, installed in series, before discharge outside of the facility. **The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility's ventilation system.**

Biosafety Cabinet Basics

This 10-minute video covers the basics of how to work with a biosafety cabinet in a way that maximize its ability to protect you from biohazards. (http://youtu.be/9FCKMAdxuMY)

Disinfection Agents

There are a number of methods for the decontamination of biohazardous material, including:

- Chemical
- Thermal (autoclave and incineration)
- UV treatment

Commonly used disinfectants include:

- Alcohols (such as ethanol)
- Aldehydes (such as formaldehyde and glutaraldehyde)
- Halogens (including household bleach)
- Iodophors (such as Betadine)
- Quaternary ammonia disinfectants (such as Roscal and BacDown)

Various hazards require different disinfection agents as well as contact times. A risk assessment based upon the type of material to be handled must include the definition of an appropriate disinfection agent for that material.

Disinfection and Waste Disposal

The Illinois EPA requires that any biological waste that poses an infection hazard be disposed of by incineration (as Potentially Infectious Medical Waste) or by EPA-defined disinfection protocols.

Steam autoclaving at 121° C/21 psi for a minimum of 60 minutes is required by Illinois law for appropriate disinfection of potentially infectious waste and disposal in the regular trash. This includes all human/primate cells and tissue as well as infectious agents.

All waste must be stored prior to disinfection in appropriately labeled, tightly covered, leak proof biohazard containers.

Autoclaves must be:

- Tested monthly with live spore-containing indicators
- Certified annually by an outside contractor

Off Site Movement

The Department of Transportation (DOT) regulates the transport of hazardous biological material such as infectious tissue, agents or toxins.

- Follow the DOT guidelines when shipping potentially infectious material such as human tissue or human tissue-derived materials.
- Infectious material affecting only animals fall under a different category from that affecting only humans.

Biological toxins are covered under 49 CFR 172.101 as toxic chemicals (hazard class 6.1 UN 3172).
Information about packing and labeling such material for transport off-site as well as on site can be obtained from Argonne's Shipping Department in Building 46 and/or the BSO.

Transport of Biohazards on Site

Transport of biohazardous material on site within a building (i.e. between the BSC and the freezer; between adjoining laboratory rooms; between sectors at the APS) or walking the material to another building **does NOT** fall under DOT regulations. **The transportation of biohazardous material to and from buildings on roads by vehicle is covered by DOT regulations**.

Transportation of infectious or toxic bio-materials within the facility must be in a secured leak-proof, unbreakable container with an appropriate label. An absorbent material such as Kim-wipes or paper towels should be added to the container in case of breakage.

At Argonne, the Biosafety Officer has a bright orange biohazard-labeled marine tackle box available for use in the movement of biohazardous material. Contact the BSO for use and availability.

Emergency Response

Infectious and Biological Toxic Material Spills. If the spill is:

- Within a contained space (i.e. biosafety cabinet or sealed rotor head):

- Follow the spill protocol laid out in the laboratory's approved BSL2 operating manual.

- Outside a contained space but within the facility AND can be readily cleaned up:
  - Proceed with the approved clean-up procedures but must inform the ESH Coordinator and the BSO.

- Either within the facility but not readily contained OR is outside the facility:
  1. Call 9-1-1.
  2. Remain at the location to ensure that no one is exposed to the material.
  3. Aid in the post-incident response.

Potentially Infectious or Toxic Spill Clean-up

This 3-minute video was developed at Argonne to demonstrate how to safely clean up a BSL2 level spill. (http://youtu.be/a4bJ6RLHd_I)

CONCLUSION

BIO 200 – Biosafety Training for Laboratory Researchers

This concludes BIO 200.
You will now be directed to the learning measurement exercise.

Please click here to proceed