



University of Toledo



Laboratory Bio-Safety Level 1 and 2 Training

University of Toledo

Environmental Health and Radiation Safety

Objectives

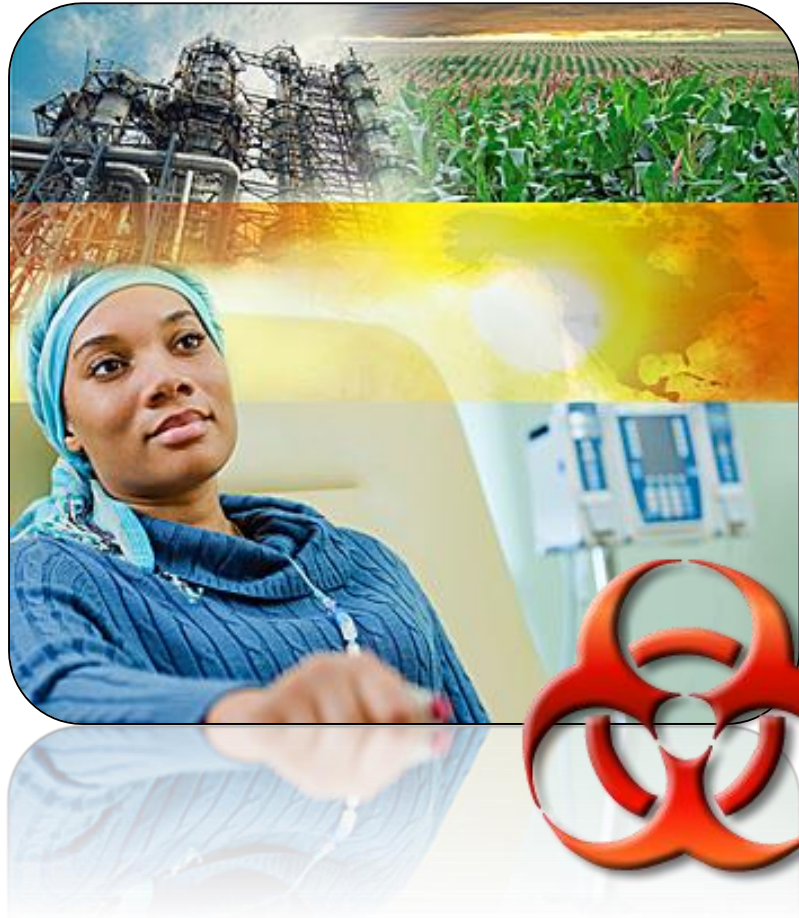
- ∞ Develop ability to apply appropriate measures to protect oneself and the environment from biological hazards
- ∞ Identify, locate, and efficiently use key biosafety resources
- ∞ Describe the ways that containment facilities and equipment establish and maintain primary and secondary barriers
- ∞ Understand how to apply knowledge of work practices that are used to prevent exposures

What is a Biohazard?

- ☞ An **agent** of biological origin that has the capacity to produce harmful effects on humans
- ☞ This includes:
 - microorganisms
 - toxins and allergens derived from those organisms
 - allergens and toxins derived from higher plants and animals



What is Biosafety



∞ Biosafety is:

- *The application of combinations of laboratory practice and procedures, laboratory facilities, and safety equipment when working with potentially infectious microorganisms.*

∞ Designed to protect human health and prevent release of pathogens into the environment.

What is Biosecurity?

- ∞ Biosecurity refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens and toxins



Biosafety Vs. Biosecurity

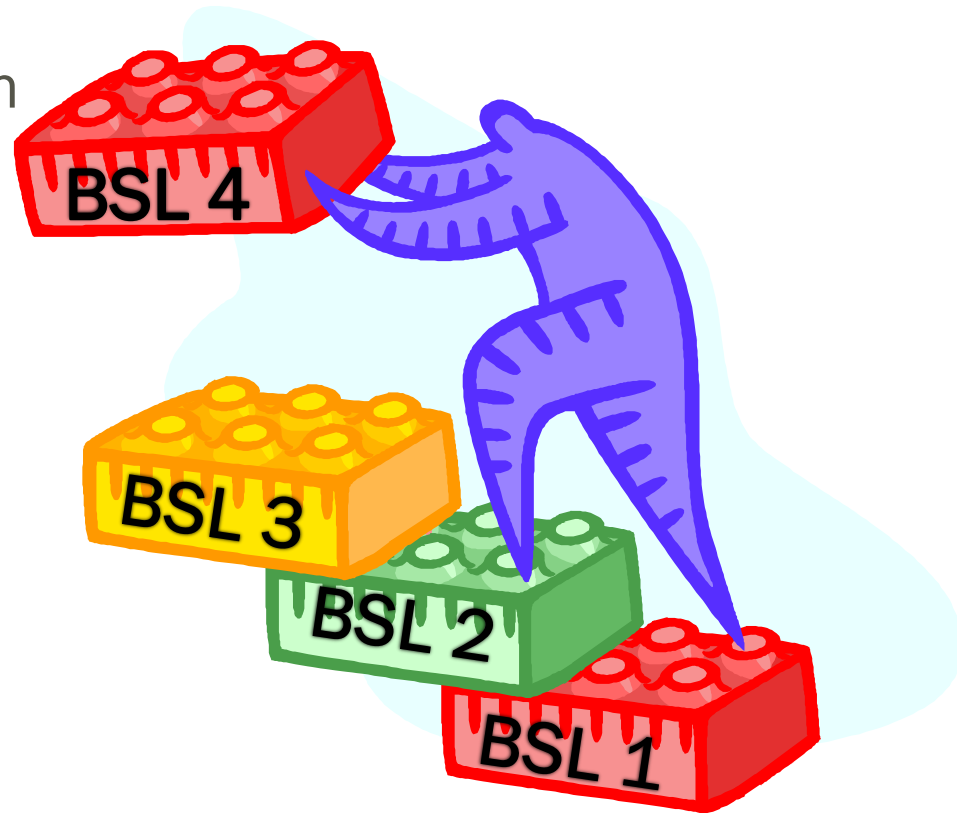
☞ **Biosafety** = measures to prevent *accidental* release and/or exposure

☞ **Biosecurity** = measures to prevent *intentional* release and/or exposure



Biosafety Levels – CDC/NIH

- Four levels of control appropriate for research with infectious agents with different levels of risk.
- Ranges from no risk for healthy people (BSL 1) to high risk of life threatening disease (BSL 4).



Biosafety levels

- ☞ **BSL1** - agents not known to cause disease.
- ☞ **BSL2** - agents associated with human, animal, or plant disease.
- ☞ **BSL3** - indigenous/exotic agents associated with human disease and with potential for aerosol transmission.
- ☞ **BSL4** - dangerous/exotic agents of life threatening nature.

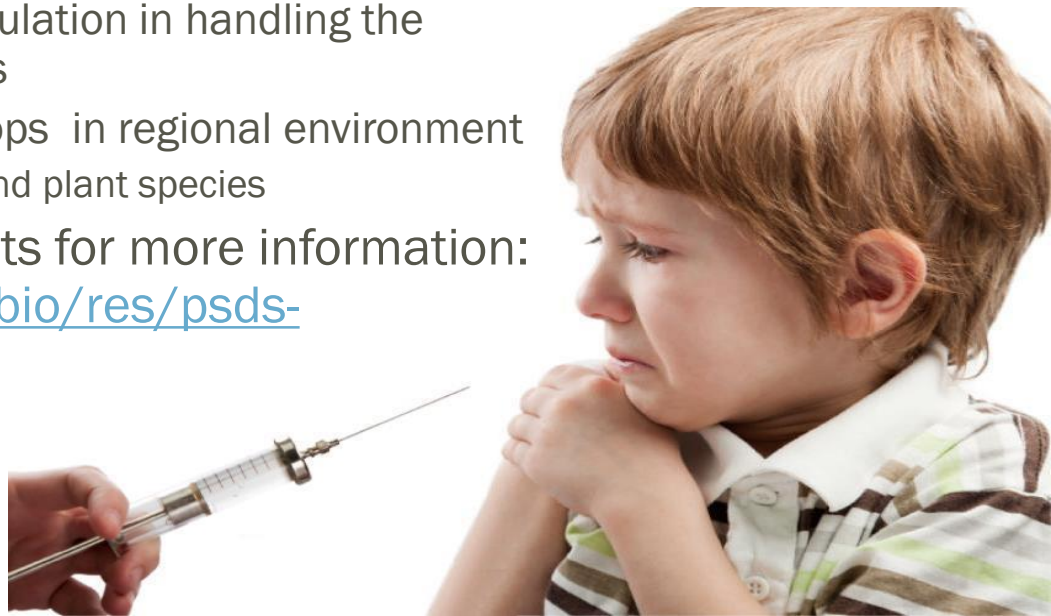
Biosafety Levels 1-4

- ∞ Increasing levels of personnel & environmental protection & appropriate guidelines for:
 - Laboratory Practices and Techniques
 - Standard Practices and Special Practices
 - Knowledge of supervisor and personnel
 - Lab specific SOPs/Biosafety manual
- ∞ Safety Equipment (Primary Barriers)
- ∞ Laboratory Facilities (Secondary Barriers)
- ∞ Buildings (Tertiary Barriers)



Biosafety Level Selection

- ∞ Selection of appropriate Biosafety level is based on certain characteristics of the infectious agent, including:
 - Pathogenicity of material - disease incidence/severity.
 - Documented route of transmission (blood borne, airborne, ingestion).
 - Availability of protective immunization (HBV Vaccine) or effective therapy.
 - Risk of exposure created by manipulation in handling the agent & caring for infected animals
 - Risk of spread to local animals/crops in regional environment
 - agriculturally important animals and plant species
- ∞ Go to Pathogen Safety Data Sheets for more information:
<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>



All Biosafety levels - Hand Washing

☞ Process:

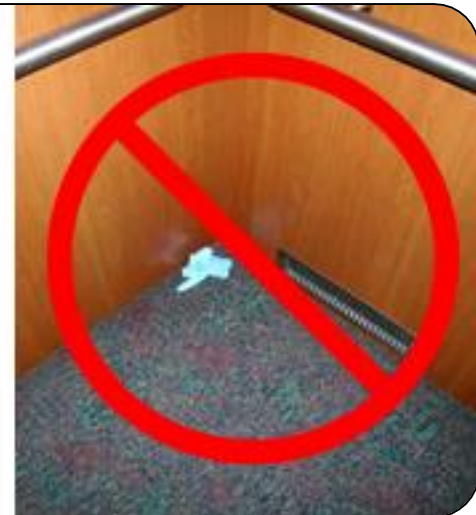
- Warm, running water w/mild, preferably liquid soap, not required to be antibacterial.
- Rub hands together vigorously for at least 15 seconds: scrub between fingers, under nails, tops & palms of hands.
- Rinse with warm, running water.
- Dry with disposable paper towel.

☞ Alcohol gels are not encouraged in lab setting



All Biosafety Levels - Personal Protective Equipment (PPE)

- ∞ PPE provides a barrier between a route of exposure and the hazard
- ∞ Protective clothing
 - Lab coat
 - Disposable latex or non-latex exam gloves:
 - When torn or contaminated, remove gloves, wash hands, and put on new pair
- ∞ PPE should NOT leave the work area!
- ∞ Create dedicated area for putting on and taking off gloves



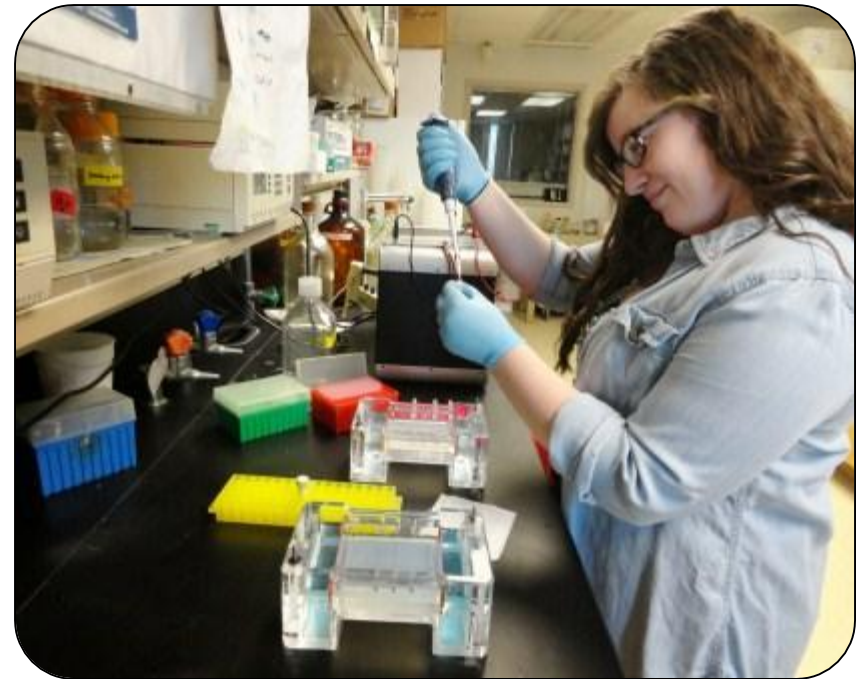
All Biosafety Levels - PPE

- ☞ Face protection worn if risk of aerosols:
 - Safety goggles
 - Face mask
 - Surgical Mask vs. Respirator
- ☞ Other appropriate PPE if necessary
 - gown, face shield, booties, etc.
 - dependent upon the circumstances.
- ☞ These devices should be used in combination with BSC and other containment devices
- ☞ **Remember:** PPE only protects yourself, while work practices protect everyone

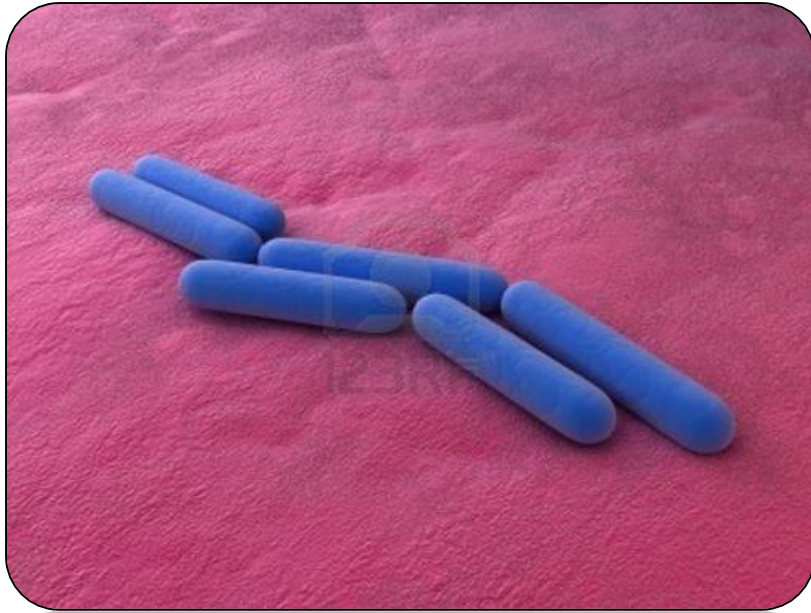


Biosafety Level 1 (BSL-1)

- ∞ Practices, safety equipment and facilities are appropriate for undergraduate and graduate work in teaching/research laboratories.
- ∞ Like 1st year biology labs and labs working with biomaterials not known to cause disease in healthy adults
- ∞ Can generally be done on open bench top using proper microbiological technique



Biosafety Level 1

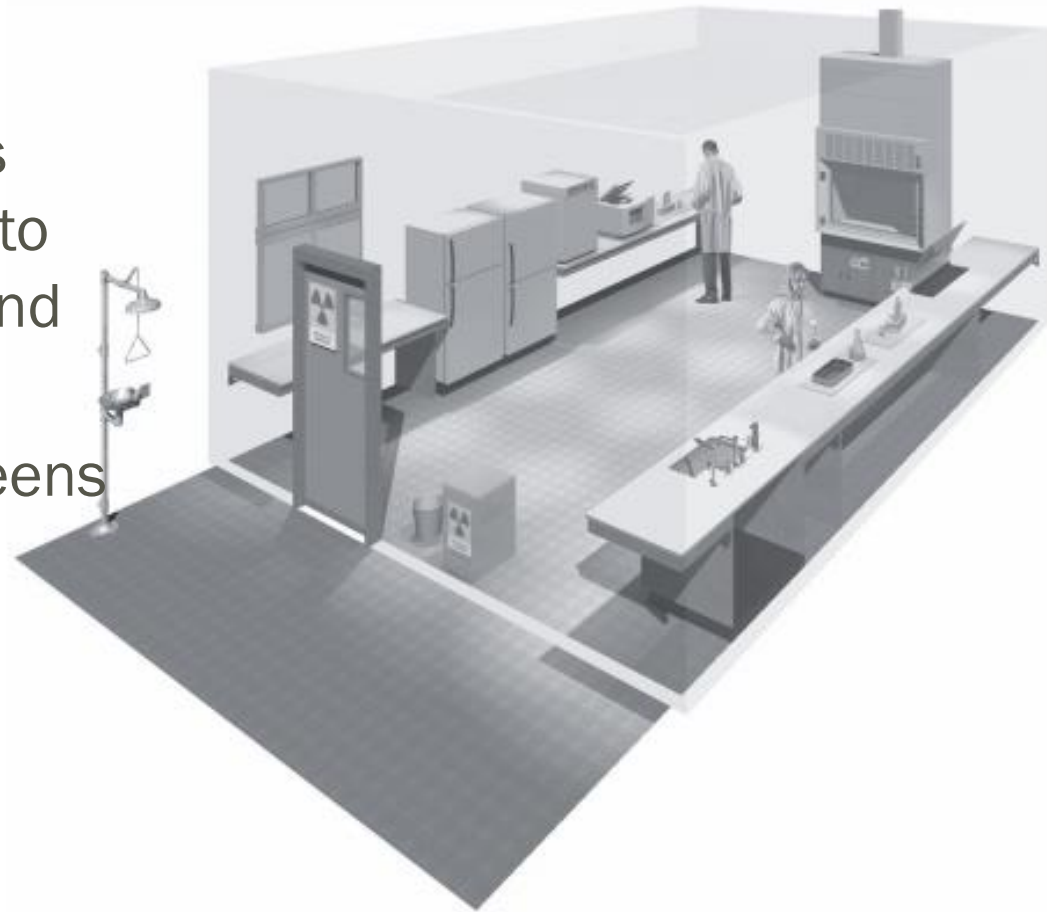


☞ Suitable for work involving well-characterized agents not known to cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment.

- Bacillus subtilis
- Infectious canine hepatitis virus
- Non-entero hemorrhagic E. coli
- Exempt recombinant DNA experiments

BSL-1 Facility Design (Secondary Barriers)

- ☞ Laboratories have doors
- ☞ Sinks for hand washing
- ☞ Walls, ceilings and floors should be smooth, easy to clean, decontaminate, and impermeable to liquids
- ☞ Windows must have screens



BSL-1 Standard Microbiological Practices

- ☞ Restrict/limit access when working
- ☞ No eating, drinking, etc.
- ☞ No mouth pipetting
- ☞ Minimize splashes and aerosols
- ☞ Decontaminate wastes
- ☞ Decontaminate work surfaces daily
- ☞ Maintain insect & rodent control program



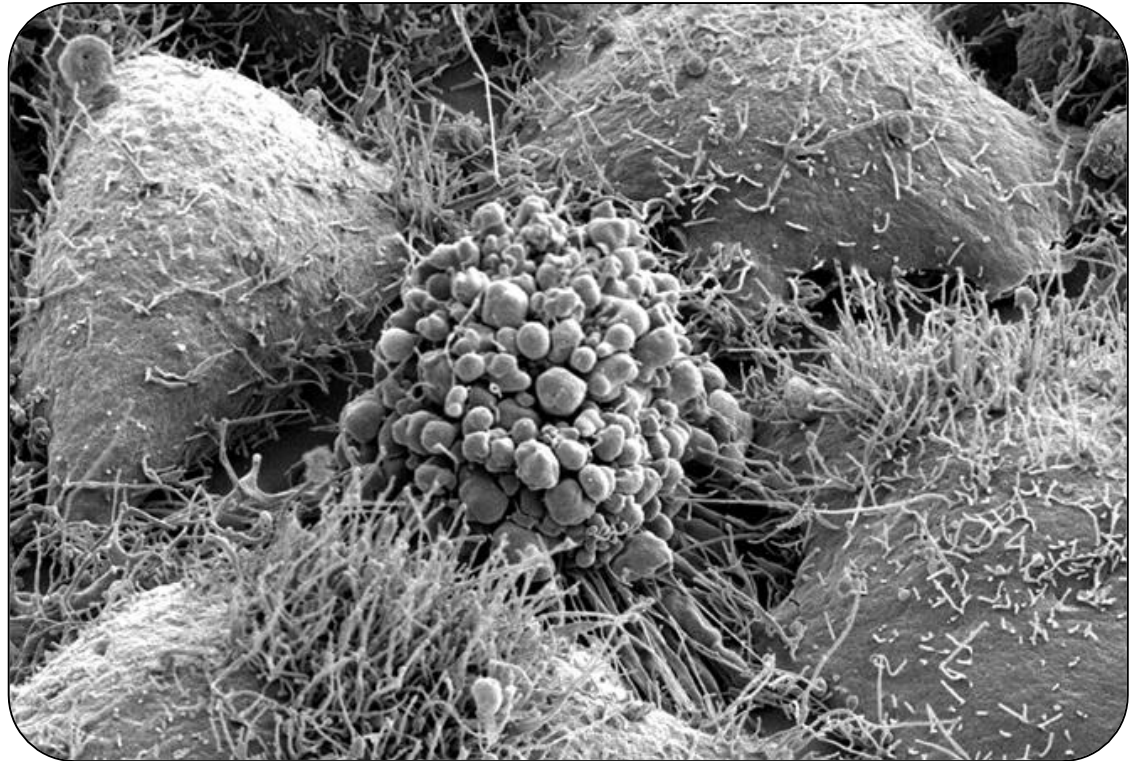
ATTOMED

Biosafety Level 2 (BSL-2)

- ☞ Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community.
- ☞ BSL-2 builds upon BSL-1
- ☞ BSL-2 agents are associated with human disease of varying severity. (TB&HIV)
- ☞ **REMEMBER!!** Bringing certain agents in from environment will call for a BSL2 designation in order to propagate and contain in lab.
- ☞ **No Children in BSL2 or BSL3 Labs. Must be 18 years of age.**

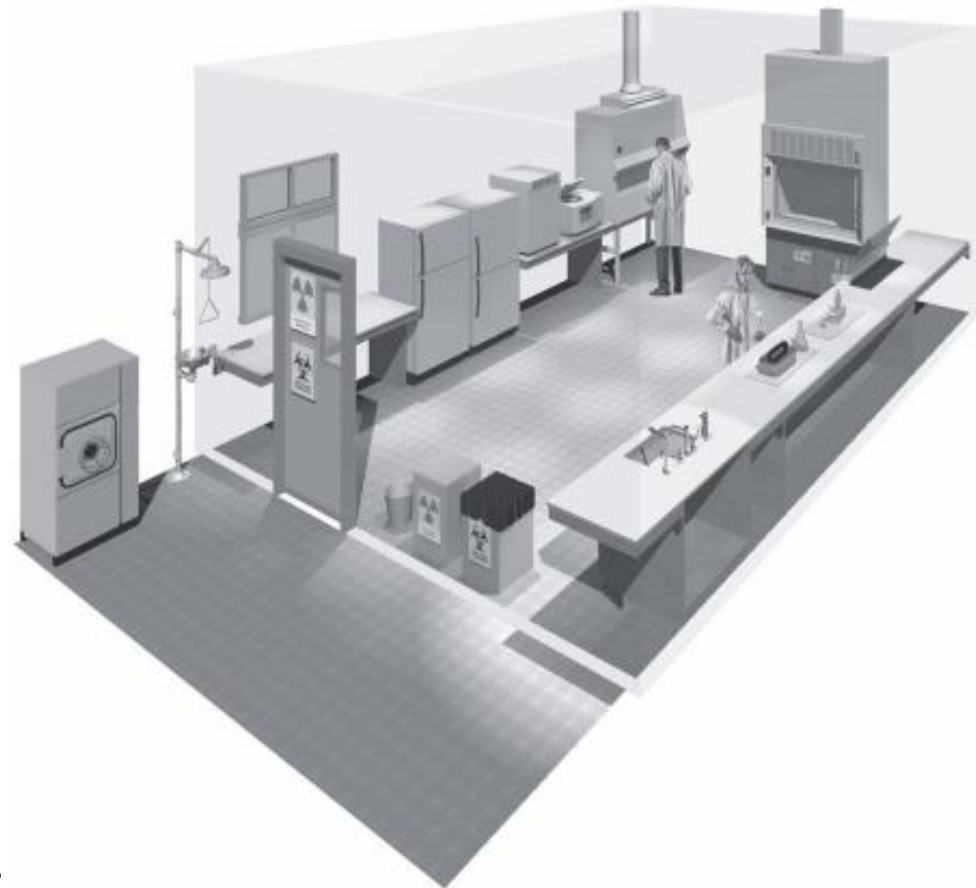
Common BSL-2 Agents

- ✎ Adenovirus
- ✎ Human Blood & Blood Products
- ✎ HeLa Cells
- ✎ Salmonella spp
- ✎ Legionella spp
- ✎ Plasmodium spp



BSL-2 Facility Design (Secondary Barrier)

- ☞ Lab doors lockable/self-closing
- ☞ Sink for hand washing.
- ☞ Work surfaces easily cleaned and impervious to water.
- ☞ BSCs for aerosol-generating procedures
- ☞ Eyewash readily available and tested weekly by lab staff
- ☞ Air flows into lab without recirculation to non-lab areas.
- ☞ Room under negative pressure.
- ☞ Anything that stores agent or manipulation occurs must be labeled with biohazard sticker



BSL-2 Special Practices

- ☞ All persons entering the lab must be advised of potential hazards and meet entry requirements
- ☞ UT Biosafety Manual Available
- ☞ Biosafety SOPs specific to lab
- ☞ Annual classroom or online test #126
- ☞ Specific training from PI with annual updates
- ☞ Animals and plants not associated with research are not permitted
- ☞ Lab personnel must demonstrate proficiency in practices prior to work
- ☞ Lab equipment should be routinely decontaminated and must be after spills and before repair or removal



**CAUTION
AUTHORIZED PERSONNEL ONLY**

BIOSAFETY LEVEL: 2

BIOLOGICAL AGENT: M. tuberculosis, M. kansasii, M. fortuitum, etc.

PRINCIPAL INVESTIGATOR: WILLIAM HELMINK

TELEPHONE NUMBER OF LAB: 6646

REQUIRED IMMUNIZATIONS: N/A

SPECIAL HAZARDS: N/A

PPE REQUIRED: Gloves, Lab coat, work in BSC

LABORATORY EMERGENCY CONTACT: Contact Through Operator X4000

**IMMUNOCOMPROMISED PERSONNEL MAY BE AT INCREASED RISK DUE TO
EXPOSURE TO PATHOGENIC ORGANISMS**

PROCEDURES FOR ENTERING/EXITING THE LABORATORY: Don PPE listed above or required by the manager of this facility prior to entering. All disposable PPE should be discarded in a red biohazard bag. Non-disposable PPE should stay in the laboratory or be managed as prescribed in your SOP. Additional information can be found on the biosafety web page at <http://www.utoledo.edu/depts/safety/biosafety.html>.

- Biohazard warning sign posted at lab entrance
 - Obtain sign from EH&RS

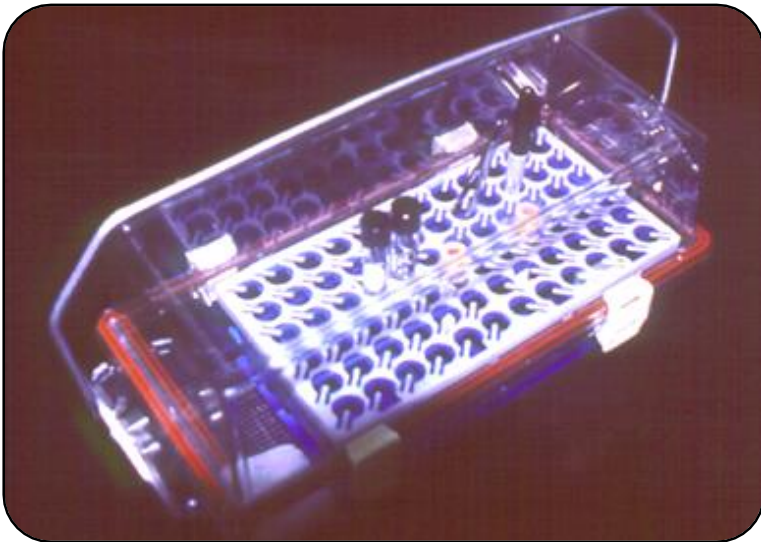
BSL-2 Safety Equipment (Primary Barriers)

- ☞ Use biosafety cabinets (class II) for work with infectious agents involving:
 - Aerosols and splashes
 - Large volumes
 - High concentrations
- ☞ Use centrifuges with sealed rotors and centrifuge safety cups.
- ☞ Do not use syringes for mixing infectious fluids



BSL-2 Safety Equipment (Primary Barriers)

- ✎ Transport of specimens within the facility
 - Cultures, tissues, specimens of body fluids, etc., are placed in a secondary container with a cover that prevents leakage during collection, handling, processing, storage, transport or shipping.



Biosafety Level 3 (BSL-3)

- ☞ Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease might have serious or lethal consequences.
- ☞ Additional Training is required for BSL3 work, consultation should be made with the Biosafety Officer before ordering, or working with any BSL3 agents



Animal Biosafety Levels (ABSL -1,2,3,4)



- ❧ Many of the same practices apply as above except special attention is paid to the fact the animals present additional exposure opportunities for lab workers.
- ❧ Shedding in urine, feces, blood, body fluids and exhaled air may pose a hazard for workers and researchers.

Recombinant DNA

- ∞ The NIH has developed specific standards that must be followed for research involving recombinant DNA as described in their publication; Guidelines for Research Involving Recombinant DNA Molecules.

[NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES](#)

[\(NIH GUIDELINES\)](#)

[November 2013](#)



Engineering Controls

- ☞ Safety equipment includes biological safety cabinets and a variety of enclosed containers.
- ☞ Biologic Safety Cabinets should be used whenever there is potential for aerosol production.
 - [CDC/NIH: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets 5th edition, 2013 version.](#)

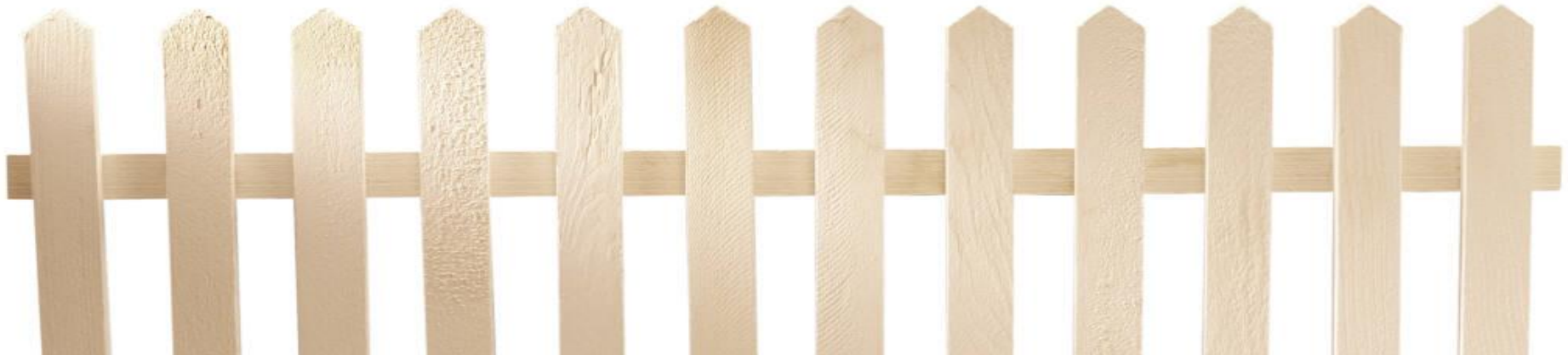


Primary and Secondary Containment

- ✎ A comprehensive bio-safety program for a research facility using biological agents can be developed by using a strategy of primary and secondary containment.
- ✎ Primary containment is the protection of personnel and the immediate laboratory or production environment.
- ✎ Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials.

Engineered Primary Barriers

- ✎ A primary barrier is imposed between the agent and the personnel.
- ✎ A primary barrier is designed to confine and isolate the agent from the individual manipulating the agent and provide protection to other persons in the laboratory room.
- ✎ Primary barriers can be designed to enclose simple manipulations (pipetting) or complex processes such as continuous-flow centrifugation.



Engineered Primary Barriers



- ✎ Primary barriers generally are represented by BSC and possibly glove boxes.
- ✎ Consist of physical barriers
 - impervious surfaces such as metal sides, glass panels, rubber gloves, and gaskets

Biological Safety Cabinets

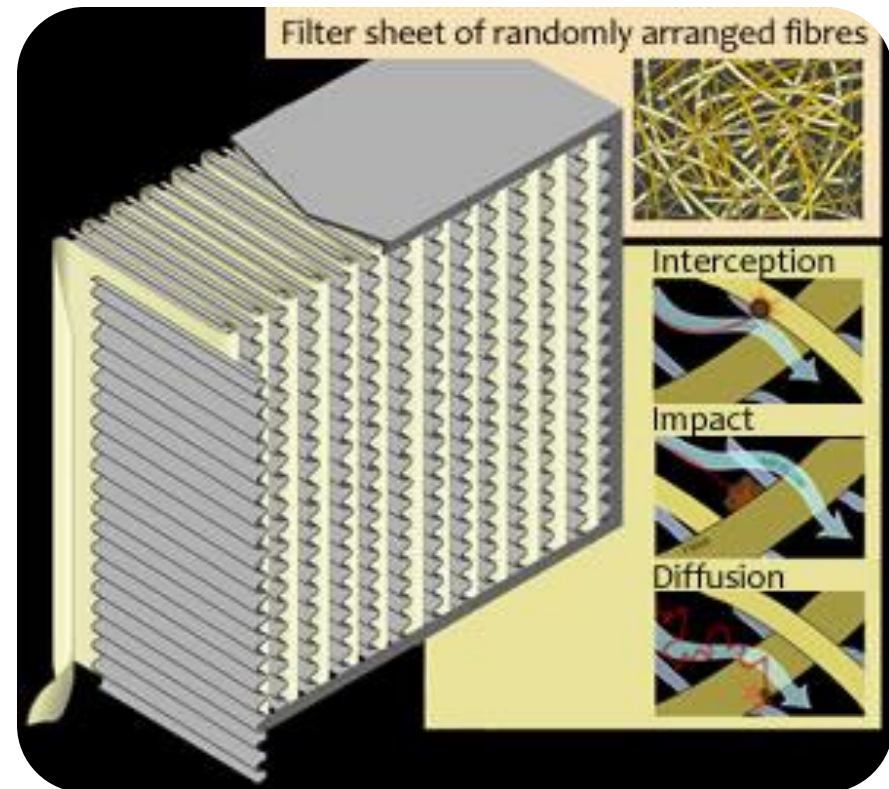
- ☞ BSC's are typically Class II Type A cabinets which means:
 - The air is cleaned as it goes into the hood to protect product
 - Then the air is then pulled through the unit away from you for your protection
 - Then finally it is HEPA filtered exhaust to protect you and the environment.
 - 70% recirculated into hood
 - 30% exhausted into room

- ☞ You should always check cabinet's annual certification prior to working in the hood.

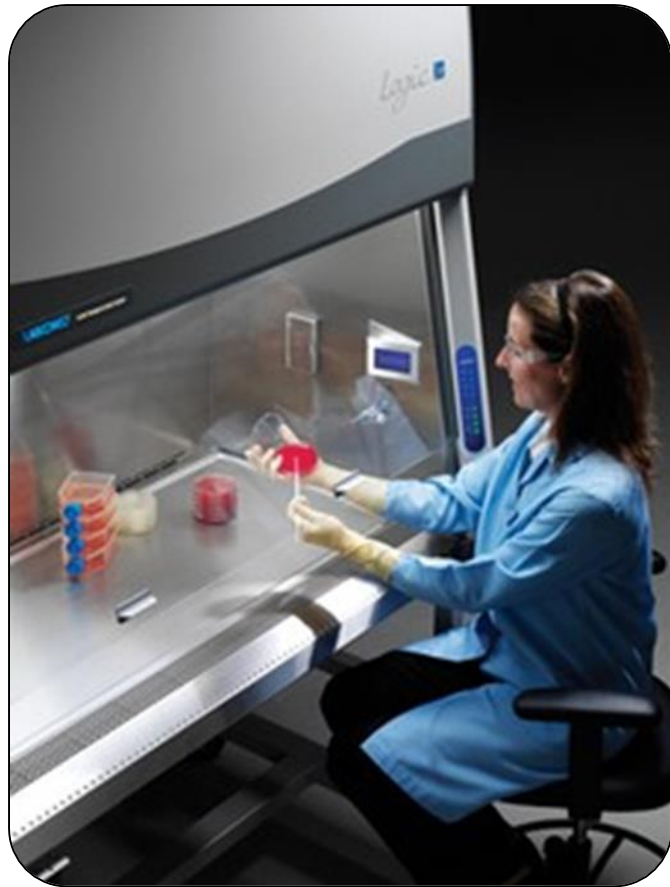


Biological Safety Cabinets (BSC's)

- Utilize HEPA Filtration: “High efficiency particulate air” filter. Remove contaminants through mechanical filtering process.
- Does not protect from chemicals: vapors pass through and may expose workers if not exhausted directly to outside.
- Chemicals and heat may damage HEPA filter.



Biological Safety Cabinets (BSC's)



Best Practices:

- Set up interior of cabinet from clean to dirty (Right or left-handed work)
- Automated pipetter's, tips and dirty tray
- Minimize movement in and out of cabinet (slow and deliberate)
- Open flames not recommended (Fire/Flow)
- Disinfection of work surfaces
- Avoid unnecessary clutter (grills and flow path)



1

6

2

7

3

8

4

9

5

Photo Property of Baker Company

Administrative Controls

Environmental Health and Radiation Safety

- We have the primary responsibility for the safety of all employees, faculty, students, patients and visitors at UT
- We develop policies and procedures regarding safe and healthy practices at UT and also enforce and monitor adherence to them.
- We respond to spills, investigate accidents, train and instruct personnel, run the medical surveillance program



Institutional Biosafety Committee (IBC)



- ∞ This group consists of individuals with expertise in a variety of biological hazards in the research setting.
- ∞ The committee functions to review research protocols and practices across the campus.
- ∞ New protocols:
 - Contact:
 - Elaine Joseph
 - 419-383-4251
 - IBC Website
 - <http://www.utoledo.edu/research/RC/biosafety.html>

Safety and Procedure Manuals

- ☞ Institutional Biosafety Manual
 - <http://www.utoledo.edu/depts/safety/docs/HM-08-009.pdf>
- ☞ Laboratory Safety & Health Manual and Institutional Chemical Hygiene Plan
 - <http://www.utoledo.edu/depts/safety/docs/HM-08-026%20Appendix%20B.pdf>
- ☞ UT Procedures and Policies
 - <http://www.utoledo.edu/depts/safety/UT%20Procedures%20and%20Plans.html>



Medical Surveillance

☞ Possible needs:

- Tetanus Shot for Live Animal Contact
- Hep B for Human Blood & Body Fluid Exposure
- PPD for TB
- Other Vaccination (Rabies, Measles)
- Respirator Clearance?



Housekeeping



- ∞ Housekeeping practices are probably the second most important biosafety procedure within the laboratory.
- ∞ Cleaning procedures and schedules are paramount in limiting exposure to biohazardous materials.
- ∞ Materials must be cleanable
 - spilling biological agents into upholstered chairs will contaminate the chairs
 - No carpeting in Biological labs

Purpose of Housekeeping

- ☞ Primary function
 - Prevent the accumulation of wastes that might harbor microorganisms that are a threat to the integrity of the biological systems under investigation;
- ☞ Might enhance the survival of microorganisms inadvertently released in the experimental procedures



Disinfectant



- ☞ 70% Isopropyl Alcohol has been shown to be only minimally effective against some agents.
- ☞ 10% Bleach Solution is the better choice
 - Bleach will harm stainless steel if not rinsed
- ☞ Every time you complete an experiment you should clean the work station, piece of equipment or the surface you have contacted.

SHARPS Waste

- ☞ Anything with a point or edge capable of piercing or cutting human skin
 - Hypodermic needles, with syringe.
 - IV tubing w/needles attached
 - Razors, scalpels, microtome blades
 - Contaminated Pasteur pipettes
 - Lancets
 - Contaminated broken glass
- ☞ Do not overfill containers
- ☞ Locate containers conveniently
- ☞ Do not place on floor
- ☞ Use dog dish if on countertop
- ☞ Do not recap, bend, or manipulate sharp by hand before disposal



Sharps Waste Problems



Left: Sharps sticking out of Sharps Waste container.
Right: Sharps Waste container past full line.

Sharps Waste Problems



Left and Right: Cardboard box is not an appropriate Sharps Waste container.

No labels. No lids.

Biological/Infectious Waste Containers

- ✂ Bags Must be in rigid, puncture-proof, leak proof secondary containment
- ✂ Labels must be affixed to at least one side of container
 - Labeled with the word “Biohazard” , With a biohazard symbol, Or a combination of both
- ✂ Closed during transport



Test Time



5

What goes into Red Bins



- ☞ Items contaminated with human or animal blood, body fluids or tissue.
- ☞ Cultures/stocks of infectious agents
 - including waste from production of biologicals, discarded vaccines, and culture dishes.
- ☞ Materials and microorganisms used in recombinant DNA research.
- ☞ **NO SHARPS!**

Waste Pick-Up Request

Submit requests for bin pickups or replacements at:



Main Campus:
EH&RS
530-3600
Wolf Hall 4216



Health Science Campus:
Environmental Services
383-5353

Additional Information

- ☞ Contact EHRS at 419-530-3600 or visit the website
 - <http://www.utoledo.edu/depts/safety/>
- ☞ The University Biosafety Manual can provide more info on:
 - Use of Recombinant DNA
 - Viral Vectors
 - Plasmids
 - Biological Safety Cabinets

