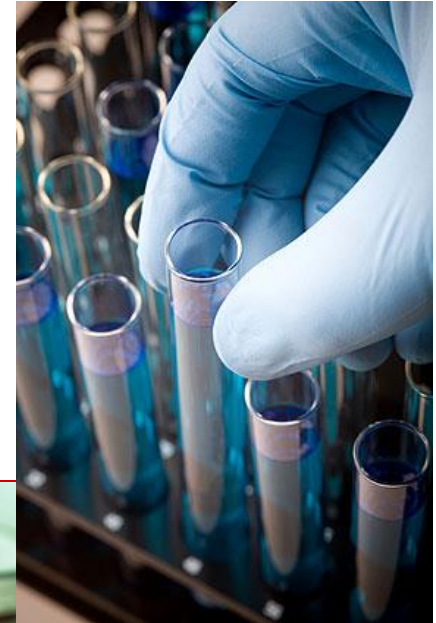


Standard Microbiological Practices: Basic Biosafety Principles & Lab Hygiene

Presented By:
Biological Safety

<http://biosafety.tennessee.edu>



Training Overview:

- This training is designed to:
 - Orient new personnel to standard microbiological practices (SMPs)
 - Inform users of risk assessments and determination of Biosafety Levels
 - Provide lab and facility expectations when working at BSL-1* (low-risk biological materials)

*This online module does not satisfy training requirements for working at BSL-2 or higher. Visit <http://biosafety.utk.edu> for additional information

Standard Microbiological Practices

What are they?

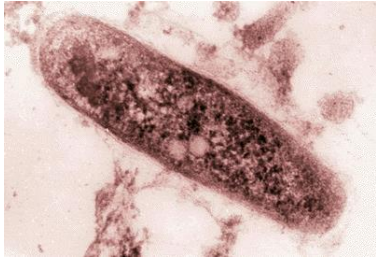
- Basic lab hygiene practices that apply to all labs where microorganisms or biological materials that contain microorganisms are manipulated.

What's their purpose?

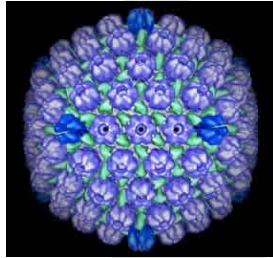
- To minimize the spread of contamination generated through the lab processes to protect both personnel, research facilities and the environment.

Types of Biohazards:

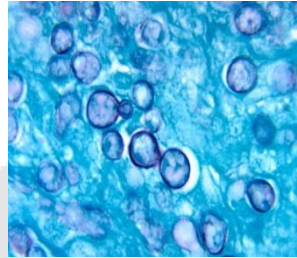
What they are....



Bacteria



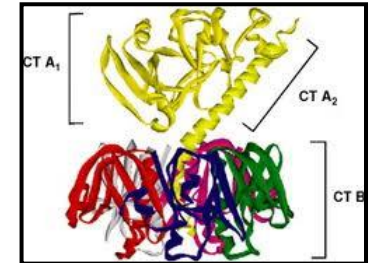
Viruses



Fungi

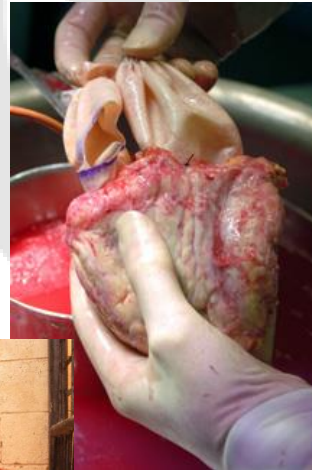


Parasites



Toxins

Biological materials that will contain them....



Biological Hazard Determination

- **Risk Assessments:**
 - **Process used to identify the potential risk posed to human health and the environment as a result of exposure to hazardous materials.**
 - **Helps to determine the type of containment, procedures and safety equipment required.**

Risk Assessment

Organism-Related Factors

- Host range
- Pathogenicity
- Availability of prophylaxis
- Route of transmission
- Viability in the environment
- Origin of the source
- Additional for recombinant DNA:
 - Nature of insert
 - Environmental impact

Procedure-Related Factors

- Concentration of organisms
- Large-scale
- Animal use
- Aerosol generation
- Experience level of personnel



Biosafety Levels

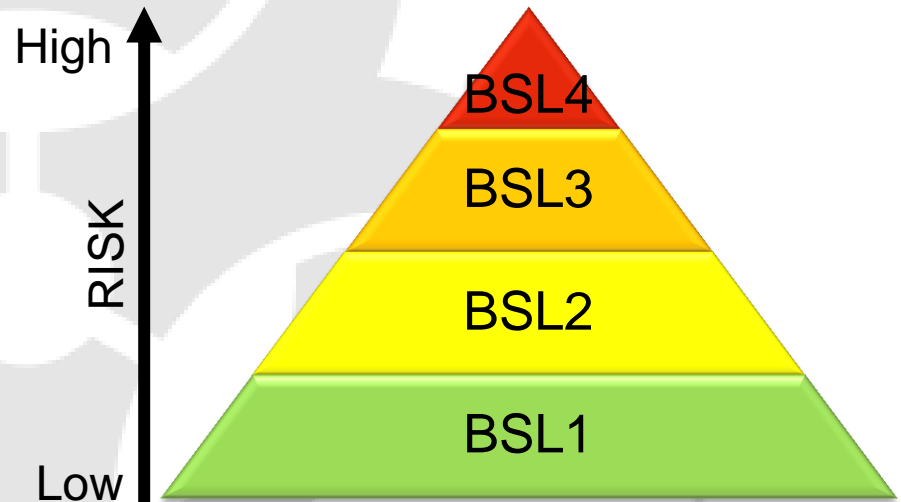
Projects are assigned to biosafety levels (BSL) based on risk assessment

A BSL is a combination of:

Lab practices and techniques,
Safety equipment and Lab facilities

To be used to:

Minimize potential exposure to a biohazard for lab personnel and others



Biosafety Levels

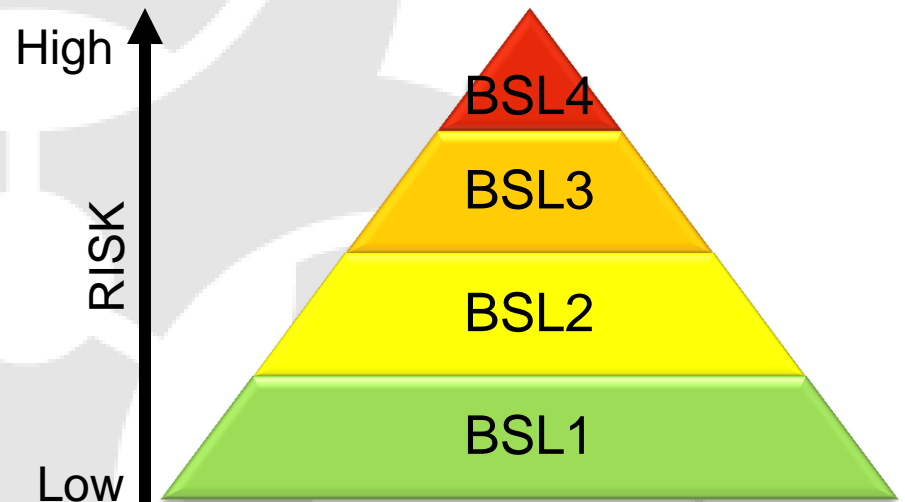
4 levels

BSL-1 least restrictive,
BSL-4 most restrictive

As the BSL increases, so does:

- The risk of the organism/materials to humans*
- Stringency of procedural and facility requirements for working with organism

* When applied for human health protection.



Biosafety Level 1

Used for work with biological agents and materials that pose minimal risk to people or the environment.

Features:

- Work on open bench
- Lab coat & gloves recommended
- Waste and work surfaces decontaminated



Standard Microbiological Practices:

Practices

- Lab Hygiene
- Hand washing
- PPE

Containment

- Aerosol control
- Sharps Management
- Biohazardous Waste management
- Disinfection and Decontamination
- Spill Remediation
- Exposure Response



Safe Work Practices: Lab Hygiene



- Do not store or consume food or drink in lab.
- Do not apply facial cosmetics in lab.
- Keep personal items out of areas where biological or other hazardous materials are manipulated or stored. **THIS INCLUDES CELL PHONES!!!**

Hand Washing

- Hand washing is extremely important in preventing lab-acquired infection and minimizing the spread of infectious materials.
- Hands must be washed:
 - After glove removal;
 - Before leaving the work area
 - Anytime the gloves may have become contaminated with infection-risk material.

How is it properly done?

Use running water and soap AND
Lather 20-30 seconds!



Waterless Hand Sanitizers

Notes on waterless hand sanitizers:

- Waterless hand sanitizers may be used as a temporary means of reducing contamination until a source of running water and soap can be reached.
- Waterless hand sanitizers are NOT an adequate replacement for hand washing with soap and water!



Personal Protective Equipment (PPE)

Lab coats

Recommended for BSL-1 labs;
required for BSL-2 labs

Lab coats serve as a “protective shell” for your street clothes.

Lab coat should be long-sleeved and should have a gathered cuff style for forearm protection.

Don't wear lab coats or other PPE for non-lab activities or into public areas (i.e., restrooms, break rooms, offices, outside, etc.).



Personal Protective Equipment (PPE)

Fluid-resistant disposable gloves

- Fluid resistant disposable gloves are recommended for all manipulations of microorganisms and related samples. These are required at BSL-2.
- Fluid resistant disposable gloves are SINGLE use!
- Wear the right size for your hand.
- Be sure to inspect gloves for defects prior to use.
- Gloves are FLAMMABLE! Do NOT “wash” gloves with alcohol and then work around ignition sources.
- A non-latex alternative should be available to prevent latex allergies (e.g. nitrile, neoprene, polyvinyl chloride)
- Clean areas such as doorknobs, keyboards, telephones, etc., must NOT be touched with gloved hands.



Personal Protective Equipment (PPE)

Use personal protective equipment (PPE) when handling or manipulating biological materials (including wastes).

Eye protection

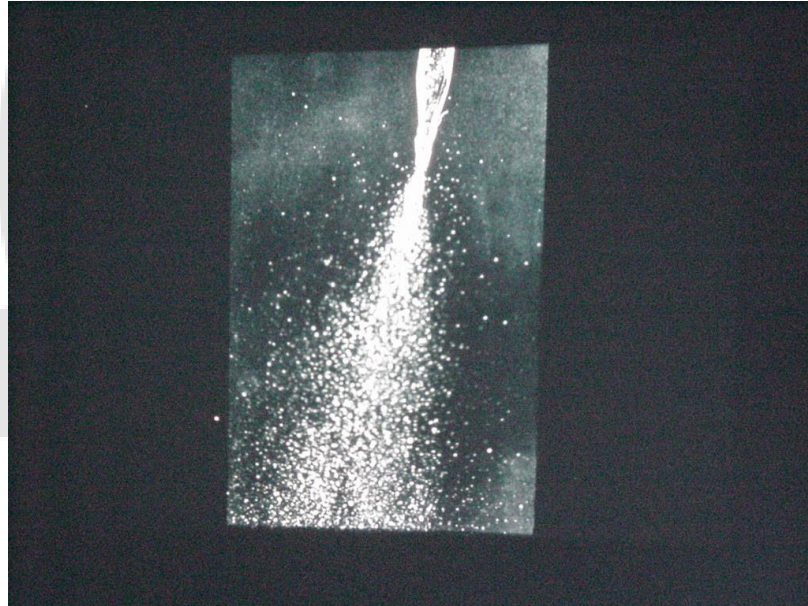
Safety glasses are recommended as a minimum level of protection for all active lab operations.



Splash goggles are required for tasks involving quantities of hazardous materials that can generate a splash.



Minimize & Contain Aerosols:



Aerosols are generated through virtually all lab processes, especially those that disrupt the surface tension of a liquid or apply mechanical force to the material...

Minimize & Contain Aerosols:

- Minimize quantities and unnecessary handling of materials when feasible.
- Confine activities with viable biological materials to designated work spaces.
- Use a Biosafety Cabinet if one is available. (Required at BSL-2 if procedures have high potential for aerosol generation.)
- Don't "dump" solid biowastes from one container to another.



Class II Biosafety Cabinet

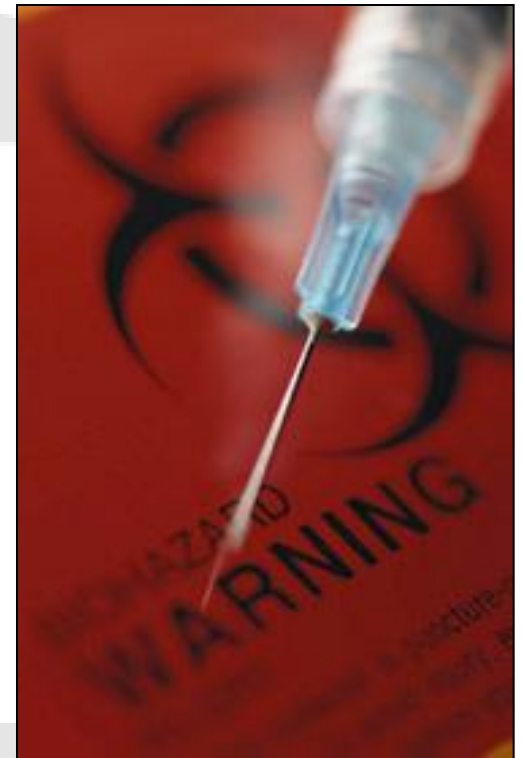
SHARPS: Safe Handling and Disposal Practices

What is considered a Biohazardous “Sharp”?

Any item that is:

- Sharp enough to puncture the skin and
- Biologically-contaminated

These items require careful handling & specific disposal



SHARPS: Safe Handling and Disposal Practices

- Eliminate use of sharps if other technologies are feasible
- Organize tasks to limit sharps exposure
- Keep sharps container within arm's reach
- Don't bend or break sharps
- Use mechanical devices for removing broken glass; do not use your hands
- Use disposables and safer sharps devices where feasible



SHARPS: Safe Handling and Disposal Practices

- Do not handle sharps with 2 hands
- Don't pass a sharp device from one hand to another
- **NO NEEDLE RECAPPING!**
 - May be exceptions based on legitimate safety considerations or collection requirements
 - Must be documented/approved by the Biosafety Office



If allowed, needles are to recapped by:

1. Placing the cap of the syringe on the counter and place the needle into the cap with one hand.
2. Picking up the syringe with the cap on the needle with one hand.
3. Clicking the cap into place with your thumb.

NO TWO-HANDED RECAPPING!



SHARPS: Safe Handling and Disposal Practices

Disposal of sharps:

- Use proper containers for disposal
- Containers must:
 - Be the right size for the job
 - Be manufactured as a sharps container
 - Have the lid installed
 - Have the lid closed when not in use or for transport



SHARPS: Safe Handling and Disposal Practices

Sharps Container Disposal:

- Permanently close container when it is $\frac{3}{4}$ full and wipe down exterior with disinfectant.
- Package for removal by medical waste contractor (Stericycle) or submit containers to EH&S during regularly scheduled hazardous waste collections.
- Contact the Biosafety Office if you need assistance.

Remember:

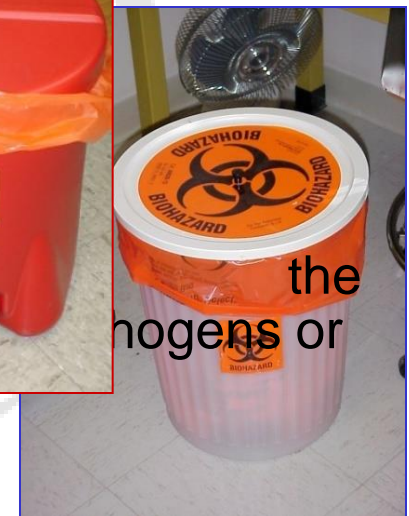
NO chemical hazards in the sharps containers! If you have mixed waste, contact the appropriate safety office for assistance!

Solid Biohazardous Waste: Proper Collection and Disposal

Solid **non-sharps** biowaste includes disposable microbiological and cell culture tubes, flasks, plasticware, contaminated PPE, paper goods, etc. contaminated with this potentially infectious or recombinant materials.

Materials should be:

- Stored in proper containers that prevent environmental release
 - durable,
 - leak proof,
 - lined with a bag
 - means of closure
 - labeled with biohazard symbol
- Liners (bags) must be red/orange or have biohazard symbol if waste contains human-derived materials.
- Containers must be closed when not in use.



Treatment of Bagged Biowaste

Autoclave treatment

- Use an autoclave that has been verified for effective waste treatment and follow the established treatment parameters (time/temperature).
- Leave bags open and add a small amount of water to bags to enhance steam exposure.
- Use secondary containment that is suitable for autoclave treatment.



Treatment of Bagged Biowaste

Autoclave treatment (cont.)

- Use care when opening autoclave after cycle.
 - Steam hazard
 - Chemical exposure hazard (possibly)
- Allow bags to fully cool.
- Bags are then accumulated in large white trash drums for disposal



REMEMBER: Red/orange bags or those labeled with a biohazard symbol are not permitted in UT dumpsters.

Plastic Serological Pipette* Disposal

- Should be segregated from other solid biowaste to prevent biohazard bag punctures, tears, leaks, etc.
- Collection container should be lined with a plastic bag (autoclavable biohazard bag if pipettes are biologically contaminated).
- Autoclave as described if biologically contaminated.



*Glass pipettes (serological and Pasteur) are best managed as sharps and should be disposed to a sharps container if biologically contaminated

Liquid Biohazardous Waste: Proper Collection and Disposal

Autoclave Treatment:

- Treat for 20-30 minutes at 121C (250F). Allow to cool, and dispose in sink.

OR

Bleach Treatment:

- Collect in a flask labeled for identification of hazards. Secondary containment should be used if vessel stored on floor or open bench.
- At 2/3 full, flasks should be neutralized with 10% bleach for 10-15 minutes followed by disposal in sink.
- Use care not to create splashes; rinse sink thoroughly after discharge.



Do not autoclave bleach-treated liquid biowaste!

Decontamination & Disinfection:

Items requiring disinfection:

- Bench top where procedures were performed (regardless of the presence of bench paper)
- Outer surfaces of storage and sample containers;
- Exposed surfaces of equipment (e.g., BSC, vortex, centrifuges)
- Any surface where spills/leaks have occurred
- Surfaces touched with gloved hands



Decontamination & Disinfection:

- **Clean surfaces of bulk contamination/spills prior to disinfection.**
- Make sure your disinfectant is adequate for your materials:
 - Is the product effective for organisms of concern?
 - Is the product designed for your application?
 - What is the dilution ratio?
 - Contact time?
 - Shelf-life?
 - What are the hazards to the user and what PPE is required?
- Refer to the manufacturer's instructions for proper dilution and use of the disinfectant.



Commonly Used Disinfectants:

Bleach Solutions

- 1:10 to 1:100 (0.5%-0.05% hypochlorite) solution of household bleach is an intermediate-level disinfectant:
 - Effective for vegetative bacteria, mycobacteria, fungi, and most viruses
- Should be prepared at time of use to assure efficacy as diluted bleach breaks down when exposed to light.
 - 1:10 dilutions are stable ~5-7 days; 1:100 dilutions are stable ~24 hours
- Rapidly “breaks down”, especially when exposed to light.
- Concentrated bleach is caustic/corrosive and can cause skin and eye burns. Splash goggles, long sleeve lab coat and gloves are needed for handling undiluted bleach. May also corrode/rust stainless steel surfaces.

Commonly Used Disinfectants:

Ethanol

- 70% is the preferred concentration to ensure adequate contact time (~10 minutes)
- Intermediate-level disinfectant
 - Effective for vegetative bacteria, mycobacteria, fungi, and most enveloped and hydrophilic viruses (it is not effective against nonenveloped enteroviruses)
- Surface-friendly but will dry and crack some plastics

Flame + Ethanol + BSC = FIRE!

Avoid using ethanol in conjunction with Bunsen burners in a BSC!



Spill Prevention & Response:

- Close primary containers if at all possible.
- If necessary, secure primary containers in a rack or other device that will keep them from shifting or tipping.
- Use a leak-resistant secondary container with a lid (if possible) to contain any leaks or spills.
- Move the materials on a cart if you are:
 - transporting a large quantity of material
 - moving from one floor to the next.



Spill Prevention & Response:

For small spills (<500 ml):

- Alert others and gather spill clean-up supplies.
- Put on the appropriate PPE for spill clean-up.
- Cover the spill with paper towels.
- Spray/flood spill with disinfectant working from outside edge of spill inward.
- After contact time (~10 minutes), wipe up the spilled material.
- Repeat disinfection and allow proper contact time.
- Wipe up disinfectant, repeat if necessary.
- Dispose contaminated clean-up materials as biohazardous waste.

For large spills (>500 ml), contact the Biosafety Office for assistance!



Exposure Response

If potentially infectious research materials enter your body through:

- Cut or puncture with a contaminated sharp
- Broken skin (cuts, scrapes, eczema, rashes, etc.)
- Splash to the eyes, nose or mouth

You must:

- Flush the exposed area (15 minutes)
- Immediately report to your supervisor
- Seek medical attention as soon as possible



Be sure to have pathogen/strain information readily available

Lab Visitors

Contractors, service personnel, and visitors:

- Should be informed about the hazards present and the lab's "code of conduct" before they spend time in the lab

Children under the age of 16:

- Should be restricted from the lab

Stranger Danger:

- Don't let strangers wander through the lab. Ask questions or refer them to your lab supervisor.

The PI and/or lab supervisor have final say on access to the lab

Lab Requirements: Restricted Access and Biosecurity Measures

Maintain control of research materials and keep them out of reach of the general public by:

- Storing biohazard materials (including wastes) in the lab.
- Store stock cultures, tubes, or plates of infectious agents in lockable devices whenever possible.
- **Lock all** storage devices that are maintained outside of the lab
 - **Biological materials stored in shared equipment must be secondarily contained to prevent leaks/spills; labeled with the contents, investigator/lab name, and date; and labeled as biohazardous if pathogens are known or likely to be present.**
- Keep doors closed and locked when the lab is not in use and restrict access to authorized personnel

These procedures keep your biological materials safe and secure while preventing lab-associated thefts (analytical balances and computer equipment are often targeted)

If you have any questions, contact the Biosafety Office

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