BIOLOGICAL SAFETY

BSL-1LABORATORY PROCEDURES

PURPOSE OF TRAINING

The purpose of this training is to explain:

1) Good microbiological practices
2) The concept of risk groups for biohazardous materials
3) Biological safety levels
4) Practices and procedures appropriate for Biosafety Level 1

WHAT IS BIOSAFETY?

"Biosafety programs reduce or eliminate exposure of individuals and the environment to potentially hazardous biological agents."

Biosafety in Microbiological and Biomedical Laboratories, 5th edition, December 2009

ACHIEVING BIOSAFETY

Biosafety is achieved through implementation of various degrees of laboratory control and containment.

This is done through:

- Lab design and access restrictions
- Personnel expertise and training
- Use of containment equipment
- Safe methods of managing infectious materials in a lab setting

WHAT ARE BIOHAZARDS?

Biohazardous materials are biological materials which are known to be, or are suspected to be, hazardous to humans, animals, plants and other forms of life.

- Known pathogens (bacteria, their plasmids and phages, viruses, fungi, mycoplasmas, and parasites);
- Human and animal tissues, body fluids, blood, blood products and cell lines;
- Animal remains and laboratory animals, including insects, that may harbor zoonotic pathogens;
- Recombinant DNA;
- Biologically active toxins

RISK GROUPS

Biohazardous agents are classified according to risk groups. Risk Group 1 (RG1) is the lowest risk and Risk Group 4 (RG4) is the highest risk.

Risk Group 1

Agents are not associated with disease in healthy adult humans.

Risk Group 2

Agents are associated with human diseases which are rarely serious and for which treatment is often available.

Risk Group 3

Agents associated with serious or lethal human disease for which treatment may be available.

Risk Group 4

Agents are likely to cause serious or lethal human disease. Treatment is not usually available.

EXAMPLES OF RG1 AGENTS

- *Bacillus stearothermophilus*
- *Escherichia coli* K12 strains
- *Frankia spp.*
- *Lactobacillus acidophilus*
- *Micrococcus luteus*
- Mouse/rat cells
- Mouse/rat plasma, blood, tissues
- *Saccharomyces cerevisiae*
- *Vibrio fisheri*

MANAGING LABORATORY RISK

A risk assessment must be done after identifying the risk group of a biohazardous material to determine a level of containment for the material.

Risk assessment takes into account:

- How will the agent be handled?
- Who will work with the agent?
- What is the exposure route of the agent?
- At what scale will the agent be used?

BIOSAFETY CONTAINMENT LEVELS

There are four levels of biological containment. Each level increases in requirements for engineering controls, administrative controls and personal protective equipment.

BSL-1

BSL-2

BSL-3

BSL-4

This training specifically covers procedures for:

BIOSAFETY LEVEL 1 (BSL-1)

Low individual risk

Low community risk

- Work involves well-characterized agents not known to consistently cause disease in immunocompetent adult humans.
- There is minimal potential hazard to lab personnel and the environment.
- Work is typically done on the open bench using standard microbiological practices.
- Special containment equipment is generally not necessary, but would be determined through a risk assessment.
- Lab personnel must have specific training on the lab procedures they are performing.

BIOSAFETY LEVEL 2 (BSL-2)

Moderate individual risk

Limited community risk

- Work that poses moderate hazards to personnel and the environment.
- Lab personnel must have specific training in handling the pathogenic agents in their lab work.
- All procedures that can create aerosols or splashes are performed in a biological safety cabinet (BSC) or other physical containment.

BIOSAFETY LEVEL 3 (BSL-3)

High individual risk

Moderate community risk

- Work is done with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation exposure route.
- Lab personnel must receive specific training in handling pathogenic and potentially lethal agents.
- All procedures are performed in a BSC or other physical containment.
- The BSL-3 lab has specific lab design requirements.

BIOSAFETY LEVEL 4 (BSL-4)

High individual risk

High community risk

- Work is done with dangerous and exotic agents.
- Aerosol transmitted lab infections are frequently life threatening or fatal and there are no vaccines or treatments.
- Lab staff have specific training for the extremely hazardous pathogens they are working with.
- BSL-4 lab design, PPE, training, etc. are highly specific.
- Primary and secondary containment is required.

MODE OF TRANSMISSION

Biohazardous agents can be transmitted via:

- Direct contact
- Mucous membrane exposure due to splash or aerosol
- Ingestion
- Inoculation/sharps injury
- Inhalation
- Fomites

What are fomites?? Any inanimate object that can carry pathogenic organisms - examples are pens, pencils, notebooks, cell phones, iPods, or any object in the lab.

ELEMENTS OF CONTAINMENT

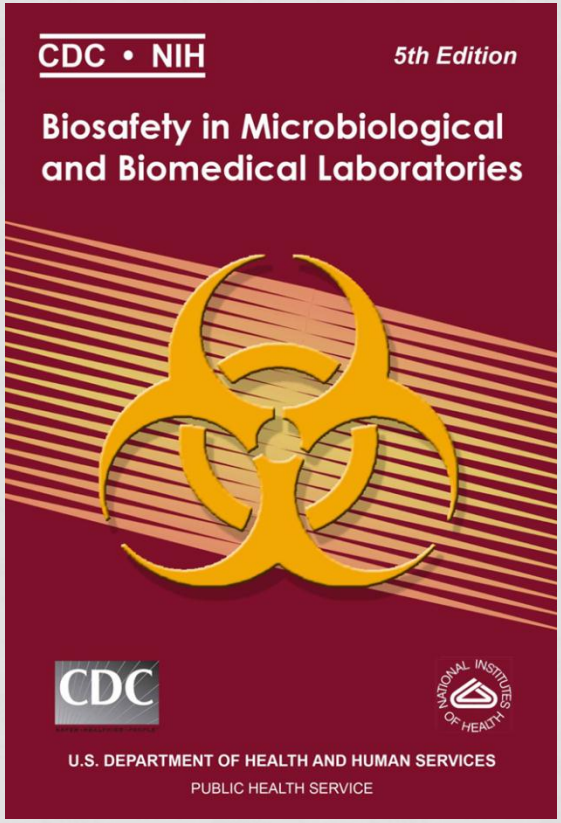
Each biosafety level is associated with certain criteria for:

1. Standard Microbiological Practices
2. Special Practices
3. Safety Equipment, including primary barriers and PPE
4. Laboratory Facilities

THE "BMBL"

The CDC-NIH publication "Biosafety in Microbiological and Biomedical Laboratories" outlines all four elements of containment for each biosafety level in detail.

http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm



STANDARD MICROBIOLOGICAL PRACTICES

WORKING IN A BSL-1 LAB

Standard Microbiological Practices:

- Restrict or limit access to the laboratory when working
- Eating, drinking, smoking, applying cosmetics and handling contact lenses are prohibited!

FOOD AND DRINK STORAGE

Food and drink can be stored and consumed in **non-lab** areas only.

Do not store any materials for human consumption:

- In the lab on benches or shelves
- In lab refrigerators or freezers
- In or on your desk if your desk is in the lab

If you need materials that could be for human consumption for lab projects, clearly mark them as "**Lab Use Only**"

WORKING IN A BSL-1 LAB

Standard Microbiological Practices continued:

- No mouth pipetting

- Minimize splashes and aerosols

AEROSOL CONTROL

How are aerosols generated?

- vortexing, centrifuging, expelling pipets
- aspirating, removing tops from tubes/flasks

Minimize exposure to aerosols:

- Use a biological safety cabinet, face shield or bench shield
- Wrap tube cap with gauze
- Keep tubes capped
- Use centrifuge safety cups

WORKING IN A BSL-1 LAB

Standard Microbiological Practices continued:

- Decontaminate work surfaces daily

Always use a disinfectant that is appropriate for the agents in your lab. Bleach is a very effective surface disinfectant.

BLEACH (SODIUM HYPOCHLORITE)

Regular household bleach used for surface disinfection must be freshly diluted each day.

A 1:10 preparation is sufficient.

1L= 100mL bleach + 900mL water

WORKING IN A BSL-1 LAB

Standard Microbiological Practices continued:

- Decontaminate wastes

Solid and liquid wastes must be decontaminated in the autoclave or with a chemical disinfectant prior to entering the municipal waste stream.

Sharps must always be collected in a puncture resistant, hard walled container.

LIQUID AND SOLID WASTES

- If using an autoclave to decontaminate liquid or solid wastes, you must complete the training "Autoclaving Biohazardous Waste" to ensure you are using it properly.

- To chemically disinfect liquid waste:
  - Use 10% final volume of bleach to waste
    - 10% = 1 part bleach to 9 parts liquid waste
  - For liquid that has a high organic load, use 20% final volume
    - 20% = 2 parts bleach to 8 parts liquid waste
  - Bleach must contact waste for 30 minutes for proper disinfection
  - Dispose into a laboratory sink if there are no other hazards present in the liquid, like chemicals or radioisotopes

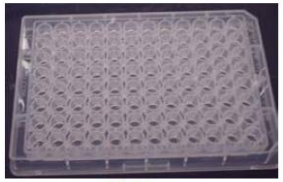
SHARPS AND RED BAG WASTE

- If you are not autoclaving solid waste, the waste is "red bag waste" and is collected in the biohazard box located in the lab area.

- Sharps waste must be contained **in a sharps container** and placed in the biohazard box once full and sealed.
  - Needles, syringes, broken glass that is contaminated, razors
  - Never manipulate sharps, such as recapping needles!

BIOLOGICAL WASTE STREAMS

SOLIDS


Plates


Tubes


Gloves

Contaminated Paper


Serological Pipettes


Pipette Tips

CONTAMINATED SHARPS


Razors


Needles/Syringes


All glass products

CLEAN GLASS


Cuvettes Tubes

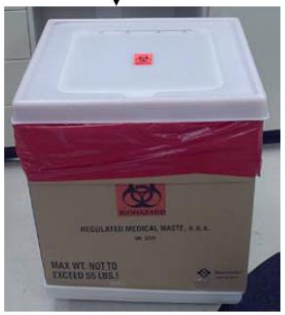

Slides

Pasteur Pipettes

Glass that has been chemically disinfected can go in the blue and white glass boxes.


Autoclave bagged waste

OR



Red bag waste for incineration

NO CHEMICALS OR RADIOLOGICALS ALLOWED IN ANY BIOLOGICAL WASTE STREAM!!!!

WORKING IN A BSL-1 LAB

Standard Microbiological Practices continued:

- The universal biohazard symbol must be posted at the entrance of the lab when biohazardous agents are present.

- Maintain an insect & rodent control program

WORKING IN A BSL-1 LAB

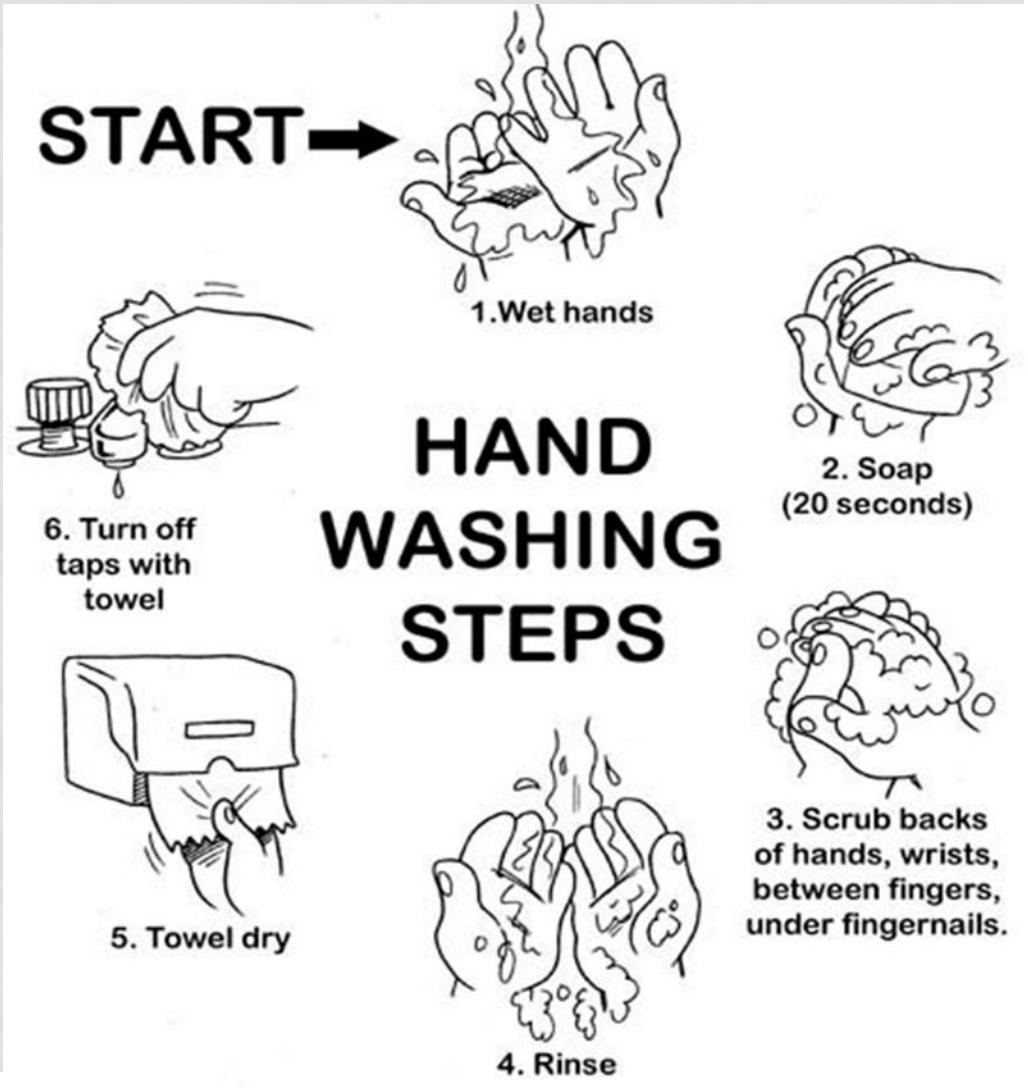
Standard Microbiological Practices continued:

- Wash Hands

Lab workers must wash their hands after they handle biological materials, after removing gloves, and before leaving the laboratory.

WASH HANDS!

Hand hygiene is considered the single most important measure for preventing transmission!



Safety Equipment

WORKING IN A BSL-1 LAB

Safety Equipment:

- Personal Protective Equipment required in a BSL1 lab includes,
  - Gloves when hazardous materials are in use;
  - Safety glasses when there is a splash potential of microorganisms, or other hazardous materials;
  - Lab coats are recommended for biological work, and required when other hazardous materials are used.

OTHER LAB PPE GENERAL RULES

- Open toed shoes are never allowed in the laboratory.
- If hazardous materials are in use, your legs should be covered (long pants, long skirt).
- **PPE is NOT allowed outside of lab areas**. Never wear your gloves or lab coat outside of the lab areas.

ONE GLOVE RULE

If you are wearing gloves make sure you do not contaminate clean areas with your gloves. Be mindful to remove your gloves before touching clean items, like the telephone, doorknob, elevator button, etc. Use the one glove rule!

Laboratory Facilities

WORKING IN A BSL-1 LAB

Laboratory Facilities:

- Labs must have doors for access control.
- There must be a sink for hand washing.
- Labs must be easily cleanable – carpets and rugs are not allowed!
- Lab furniture must be able to support anticipated loads and be easily cleaned. **Cloth chairs <u>are NOT</u> allowed!**
- Windows that open must be fitted with fly screens.

EMERGENCY PROCEDURES

EMERGENCY RESPONSE: EXPOSURE

- Alert others in the lab
- If it is a life threatening emergency, call 911
- For any mucous membrane exposures, use the eyewash, safety shower, or sink to flush the material as best you can. For skin exposures, clean the area thoroughly with soap and water.
- Notify your supervisor or Principal Investigator immediately
- Go to Health Services for evaluation when needed
- Complete an incident report and submit to supervisor and biological safety officer

UNH HEALTH SERVICES

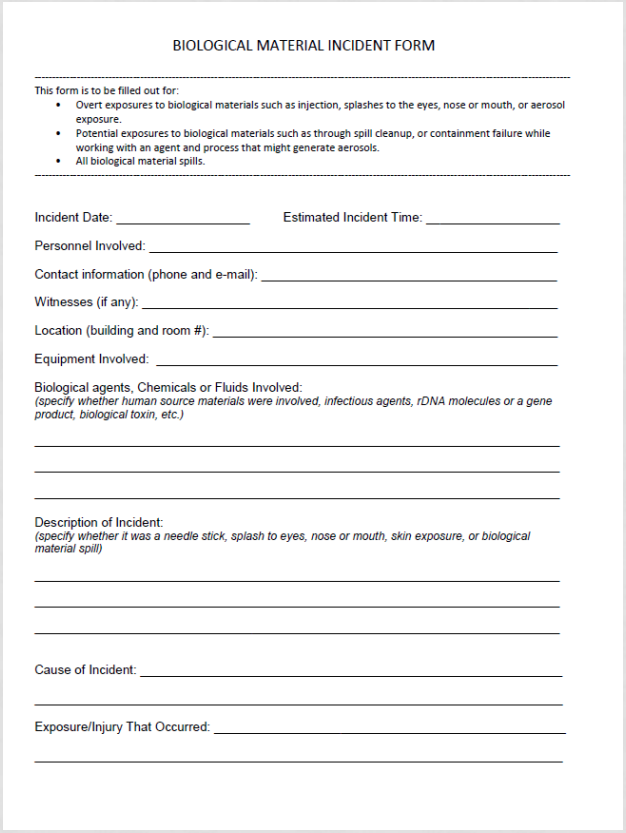
Health Services is located across Main Street from Holloway Commons and the Memorial Union Building (MUB).

EMERGENCY RESPONSE: SPILLS

- Alert others in the lab
- Isolate the area
- Don PPE, including lab coat, safety glasses and gloves
- Remove any sharps, including glass by mechanical means (forceps, scoop, dustpan/broom. **Do not use your hands to pick up sharps!**
- Place absorbents on the spill
- Apply disinfectant over the absorbents
- Allow 20-30 minutes of contact time
- Remove absorbents and properly dispose
- Re-apply disinfectant directly to surface and wipe clean
- Notify supervisor and complete an incident report

INCIDENT REPORTS

- An incident report for all biological exposures and biological spills must be completed within 48 hours of the event.



BIOLOGICAL MATERIAL INCIDENT FORM

This form is to be filled out for:

- Overt exposures to biological materials such as injection, splashes to the eyes, nose or mouth, or aerosol exposure.
- Potential exposures to biological materials such as through spill cleanup, or containment failure while working with an agent and process that might generate aerosols.
- All biological material spills.

Incident Date: _____ Estimated Incident Time: _____

Personnel Involved: _____

Contact information (phone and e-mail): _____

Witnesses (if any): _____

Location (building and room #): _____

Equipment Involved: _____

Biological agents, Chemicals or Fluids Involved:
(specify whether human source materials were involved, infectious agents, rDNA molecules or a gene product, biological toxin, etc.)

_____

_____

_____

Description of Incident:
(specify whether it was a needle stick, splash to eyes, nose or mouth, skin exposure, or biological material spill)

_____

_____

_____

Cause of Incident: _____

_____

Exposure/Injury That Occurred: _____

_____

Questions?

Contact the biological safety officer at 862-0197 for any questions about working in a BSL-1 lab.