

# Biological Safety Training

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# About This Course

- This course is designed to introduce Carnegie Mellon University researchers to the principles and practices of biological safety.
- This course is intended for researchers who work with biological materials (bacteria, viruses, fungi, recombinant DNA, cells, human blood and blood products, naturally and experimentally infected animals, etc.)
- In addition to this course, your PI/supervisor is responsible for providing you with site-specific Biological Safety Training-handout

# Purpose of Training

- Emphasize need for adequate safety precautions in the planning, initiation, and termination of activity involving biological material that represent a real or potential hazard to the worker.
  - Number of non-microbiologists engaged in biotechnological activities is increasing.

# Course Topics

- Introduction
- Epidemiology of Laboratory Acquired Infections
- Hazard Classification-Risk Groups
- Biological Safety Levels
- Principles and Practices of Biosafety
- Other Control Methods
- Waste Management
- Decontamination
- Spill Response
- Exposure Incident Procedures

# Introduction

- **Biological Safety**-measures employed when handling biohazardous materials to avoid infecting oneself, others or the environment.
- Measures consist of:
  - Engineering controls
  - Administrative controls
  - Practices and procedures
  - Personal protective equipment (PPE)

# Introduction (cont.)

- **Biohazard** -any agent of biological origin that has the capacity to produce harmful effects in humans
- Examples of biohazards:
  - Microorganisms such as viruses, bacteria, fungi, and parasites and their toxins
  - Blood and body fluids as well as tissues from humans and animals
  - Transformed cell lines and certain types of nucleic acids

# Why practice Biological Safety?

- One principle reason:
  - Potential threat of infection in the work environment has long been recognized and appreciated by microbiologists
- Our goal in the Department of Environmental Health and Safety is to see that you leave this University as healthy, if not healthier, as when you arrived.
- Other reasons include, protecting you, your neighbor in the lab, the support staff and instructors in the lab, as well as the environment

# Why practice Biological Safety?- Regulations and Guidelines

## ■ Regulations

- Occupational Safety and Health Administration's (OSHA) General Duty Clause
- OSHA Bloodborne Pathogens Standard
- Centers for Disease Control and Prevention Select Agent Act

## ■ Guidelines

- National Institute of Health's (NIH) Guidelines for Research Involving Recombinant DNA Molecules
- Centers for Disease Control and Prevention/NIH's Biosafety in Microbiological and Biomedical Laboratories (BMBL)

# Why practice Biological Safety?- Regulations and Guidelines (cont.)

- University Specific Regulations
  - Carnegie Mellon University's Safety Plan for the Use of Biological Materials Associated Devices
- Carnegie Mellon University's Institutional Biological Safety Committee

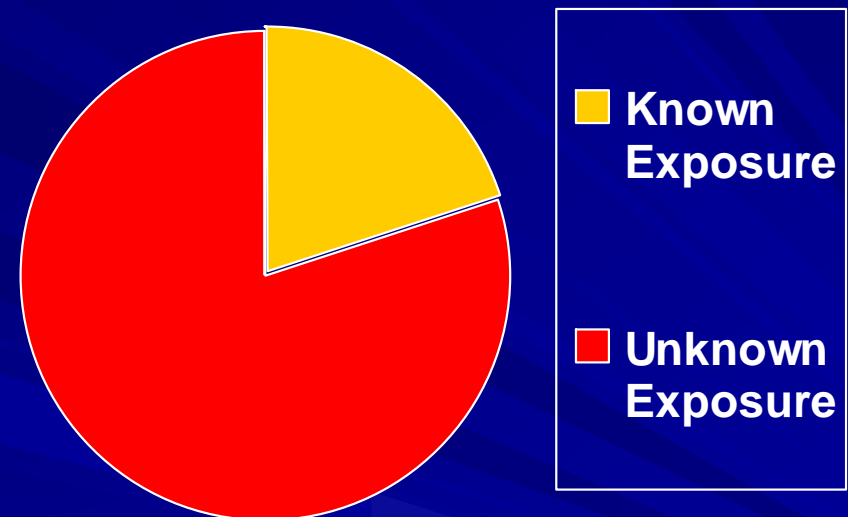
# Epidemiology of Laboratory Acquired Infections

- May be symptomatic or asymptomatic
- **Every infectious microbial agent which has been studied in the laboratory has caused infection in lab personnel**

# Epidemiology of Laboratory Acquired Infections-Means of Exposure

- Fewer than 20% of all LAIs were associated with a known accident (80% unknown or unrecognized)
- Most common modes of exposure:
  - Inhalation,
  - Ingestion,
  - Inoculation,
  - Contamination of eyes and mucous membranes

Means of Exposure to LAIs



# Epidemiology of Laboratory Acquired Infections-Means of Exposure (cont.)

<u>Route</u>	<u>Laboratory Practices an/or Accidents</u>
Inhalation	Procedures that produce aerosols
Ingestion	Mouth pipetting Splashes into mouth Eating, drinking, smoking, placing fingers into mouth Leaking contaminated items (ink pens)
Inoculation	Needlesticks Cuts from sharp objects (e.g. blades or broken glass) Animal and insect bites and scratches
Contamination of Eyes and Mucous Membranes	Spills and splashes Contact with contaminated items Transfer by hand-to-face actions

# Epidemiology of Laboratory Acquired Infections-Aerosol Production

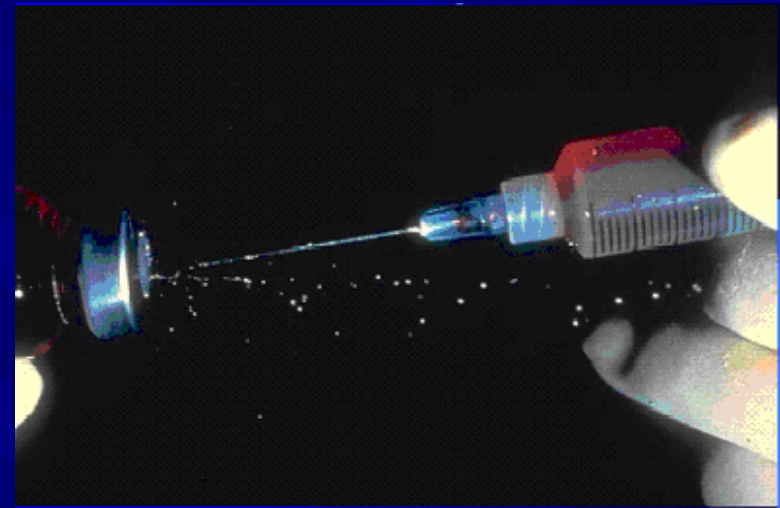
- Aerosols are solid or liquid particles suspended in a gas (air)
- All laboratory techniques/procedures are capable of generating infectious aerosols
- Such techniques/procedures include:
  - Pipetting
  - Sonicating
  - Using a blender
  - Centrifuging
  - Vortexing
  - Pouring
  - Opening culture vials
  - Dropping and breaking culture vials and flasks
  - Lypholizing

# Epidemiology of Laboratory Acquired Infections-Aerosol Production (cont.)

- Aerosols present two means of potential worker exposure:
  - Inhalation
  - Deposition onto surfaces
    - PPE, and disinfection
- Mere presence of microorganisms in the air is insufficient to cause disease!

# Epidemiology of Laboratory Acquired Infections-Aerosol Production (cont.)

- Figure 1-Withdrawing a needle from a bottle

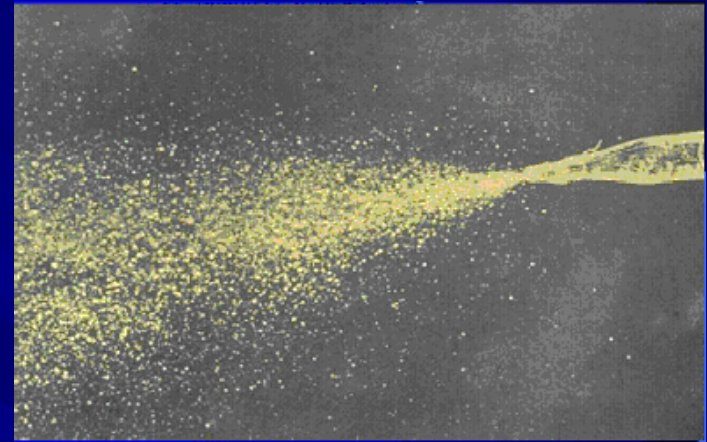


- Figure 2-Vortex mixing

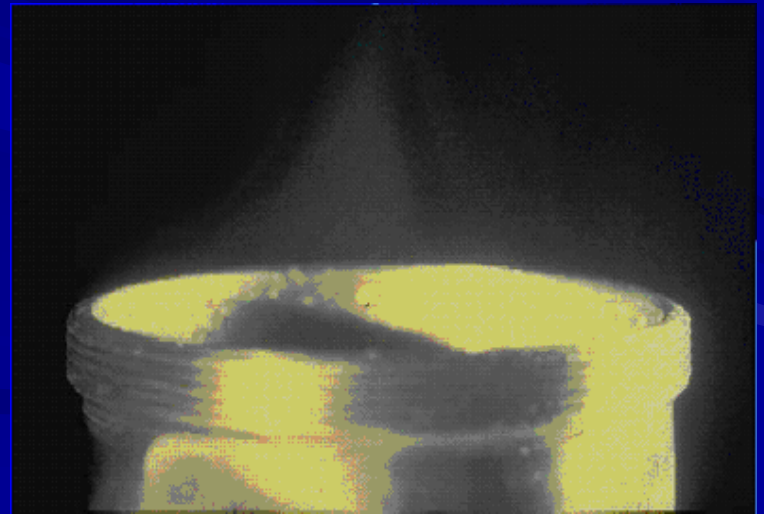


# Epidemiology of Laboratory Acquired Infections-Aerosol Production (cont.)

- Figure 3-Pipette pushing out the last drop



- Figure 4-Opening a centrifuge cup

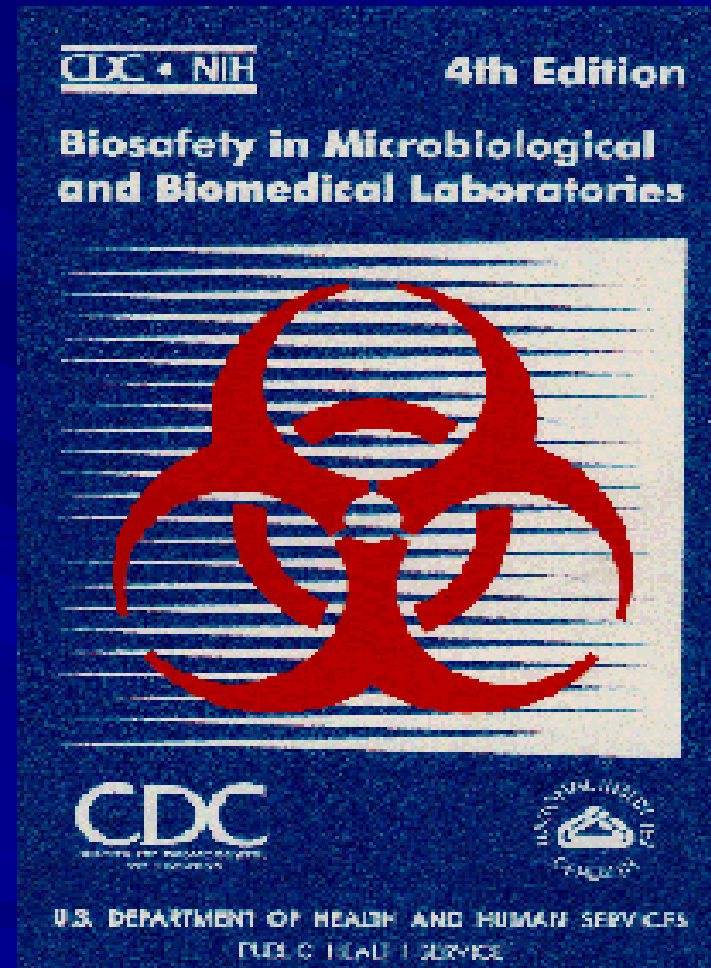


# Epidemiology of Laboratory Acquired Infections-Infectious Dose

- Infectious Dose-How many organisms does one need to be exposed to in order to become ill
- Infectious dose for humans varies and is dependent on:
  - Type of agent
  - Route of exposure
  - Health status of the host
    - Vaccination status of the host
- Viruses have a lower infectious dose!

# Hazard Classification-Risk Groups

- Biological agents are classified by the CDC and NIH according to **Risk groups** (RGs):
  - RGs range from 1-4
    - RG 1 is least hazardous
    - RG 4 is most hazardous
- Risk group(s) of the agent(s) being used will determine Biological Safety Levels (BSLs)



# Risk Groups (cont.)

- Risk Groups classification takes the following into consideration:
  - Pathogenicity of the organism
  - Mode of transmission and host range
  - Availability of effective preventive measures (e.g., vaccines)
  - Availability of effective treatment (e.g., antibiotics)
  - Other factors

# Hazard Classification-Risk Groups

## ■ Risk Group 1

- Agents are not associated with disease in healthy adult humans

- E. coli, Bacillus subtilis

## ■ Risk Group 2

- Agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

- Adenovirus

# Hazard Classification-Risk Groups (cont.)

## ■ Risk Group 3

- Agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.
  - Bacillus anthracis, Mycobacterium tuberculosis, HIV

## ■ Risk Group 4

- Agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.
  - Ebola virus

# Hazard Classification-Risk Groups (cont.)

- Recombinant DNA:
  - Risk group is determined based on source of DNA, vector used, and host
- Cell/Tissue Culture:
  - Human and primate cell line- RG 2
  - Cell lines transfected with viruses (i.e. Adenovirus)- RG 2
  - Others-RG 1
- In the event that the agent is not described in the BMBL, individuals should consult the Biosafety Officer for additional information

# Hazard Classification- Safety Sheets for Infectious Materials

- MSDS for Infectious Substances
  - <http://www.phac-aspc.gc.ca/msds-ftss/>
  - Handout-Staphylococcus aureus MSDS
- American Type Culture Collection (ATCC)
  - Cell Culture Product Descriptions
  - Handout-ATCC Product Description for NIH 3T3 Cells

# Biological Safety Levels (BSLs)

- Biological Safety Levels range from 1-4
- BSLs consist of laboratory practices and techniques, safety equipment, and laboratory facilities.
- The BSL for each lab is determined by:
  - the risk group(s) of the agent(s) being worked with
  - the documented or suspected route of transmission of the infectious agent(s)
  - the types of procedures or activities being done with the agent(s)

# Biological Safety Levels (BSLs)- BSL-1

- Work is generally carried out on open bench tops using Good Microbiological Practices
- Special safety equipment or facility design is not required
- Lab personnel have specific training in the procedures conducted in the laboratory

# Biological Safety Levels (BSLs)- BSL-2

- It differs from BSL-1 in that:
  - Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists
  - Access to the laboratory is limited
  - Extreme precautions are taken with contaminated sharp items
  - Procedures in which infectious aerosols or splashes may be created are carried out in a biological safety cabinet or other primary containment equipment
  - Standard Operating Procedures **must** be developed for the agents in use
  - Spill response procedures must be posted in lab.

# Principles and Practices of Biosafety-Containment

- The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the environment to potentially hazardous agents
- Two types:
  - Primary Containment
  - Secondary Containment

# Principles and Practices of Biosafety-Primary Containment

- Consists of techniques and specialized equipment
  - Techniques-Good Microbiological Practices
  - Equipment-primary barriers (biological safety cabinets, safety centrifuge cups, etc..)



# Principles and Practices of Biosafety- Good Microbiological Practices

- Basic code of practice
- The objectives of good microbiological practice are to;
  - prevent contamination of laboratory workers and the environment, thereby decreasing the likelihood of infection
  - prevent contamination of the experiment/samples.
    - Particularly important for cell/tissue culture work

# Principles and Practices of Biosafety- Biological Safety Cabinets (BSC)

- The BSC is the principal device used to contain infectious splashes or aerosols
- Uses directional airflow to minimize contact
- Equipped with High Efficiency Particulate Air (HEPA) filter
  - Only filters out particulates, not gases or vapors
- There are 3 classes, I, II, and III



# How a BSC Works

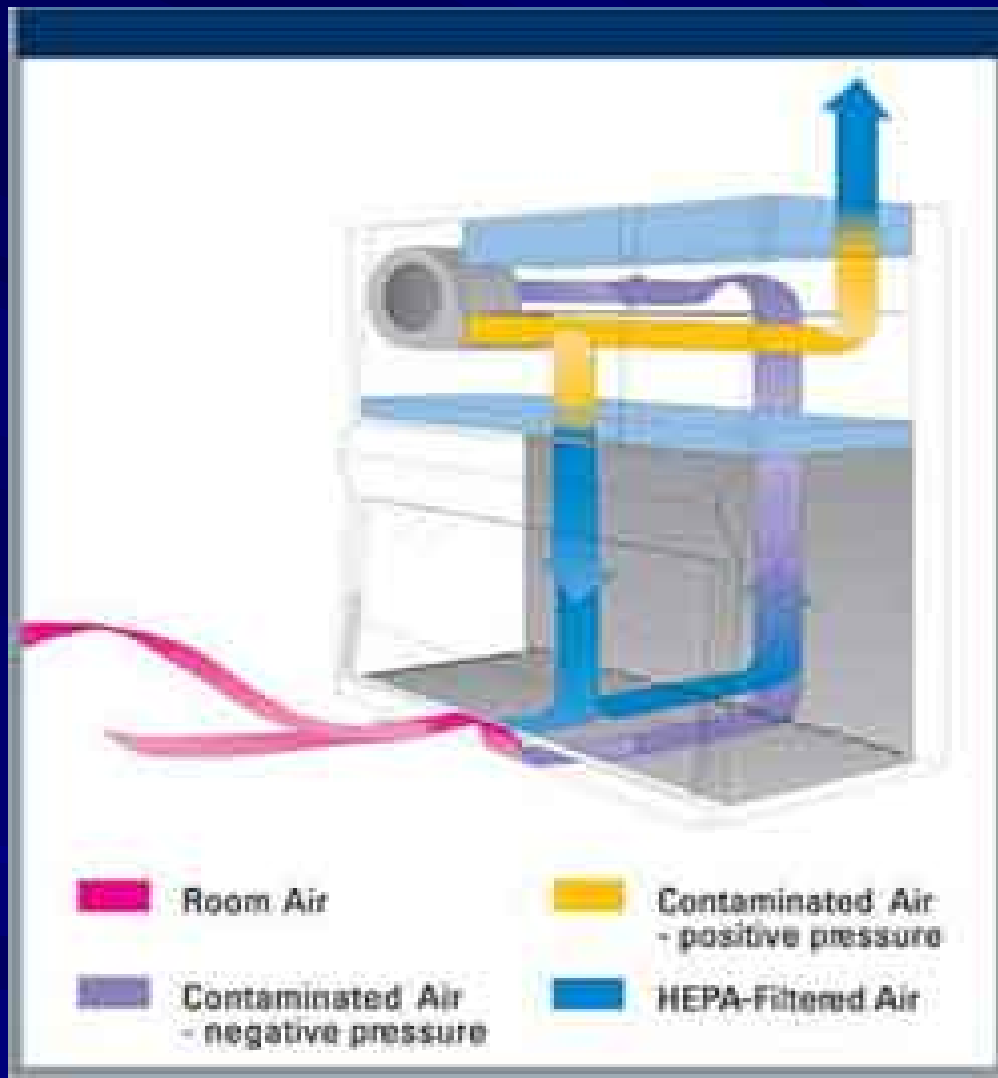
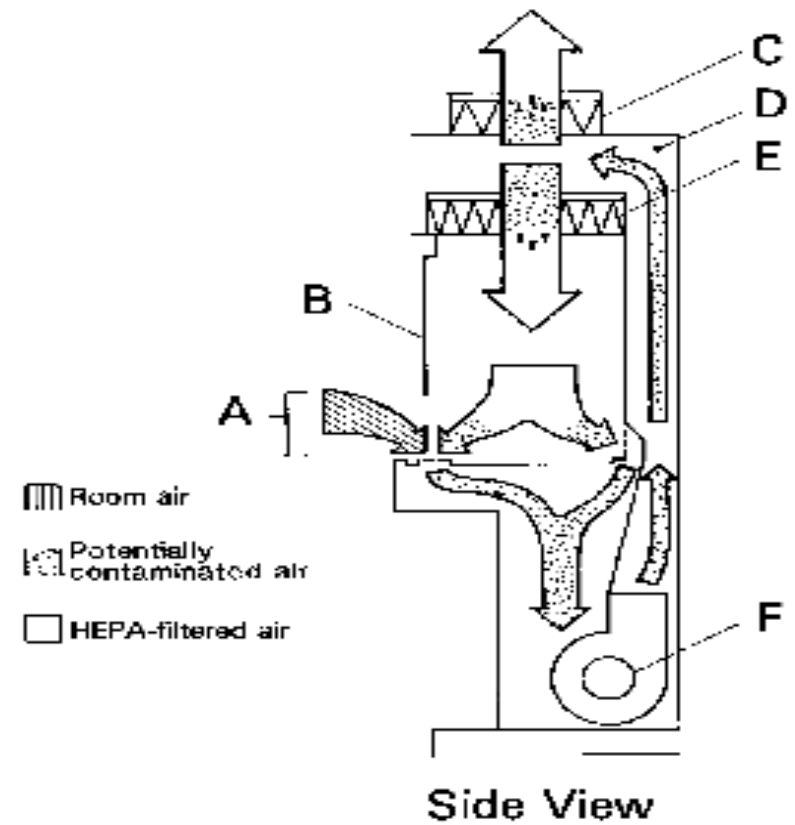


Figure 2a.  
Class II, Type A Biological Safety Cabinet.

A. front opening, B. sash, C. exhaust HEPA filter, D. rear plenum, E. supply HEPA filter, F. blower



# Principles and Practices of Biosafety- Biological Safety Cabinets (cont.)



Biological Safety Cabinet



Chemical Fume Hood

# Principles and Practices of Biosafety- Biological Safety Cabinets (cont.)

- All BSL-2 activities that can produce splashes and/or aerosols should be conducted in a BSC
- Never use gas burners or alcohol burners/flames in a BSC (i.e. loop sterilizers, Bunsen Burners, etc.)
- Handout-Proper Work Practices When Using a Biological Safety Cabinet

# Principles and Practices of Biosafety- Secondary Containment

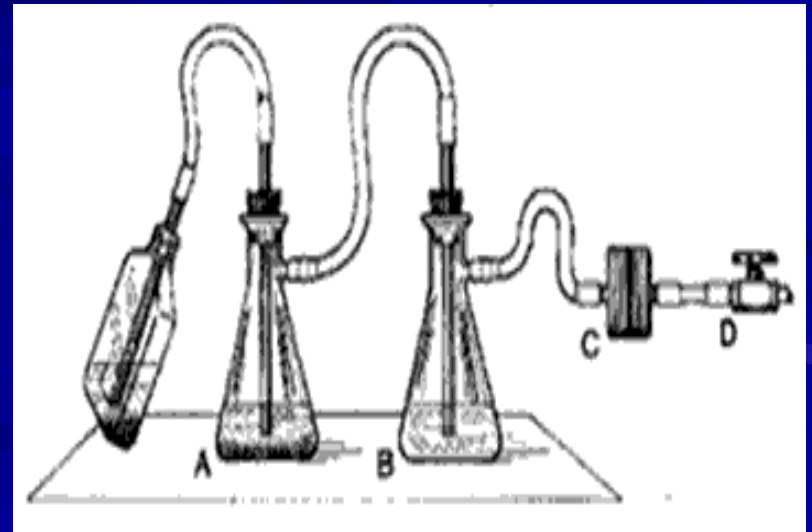
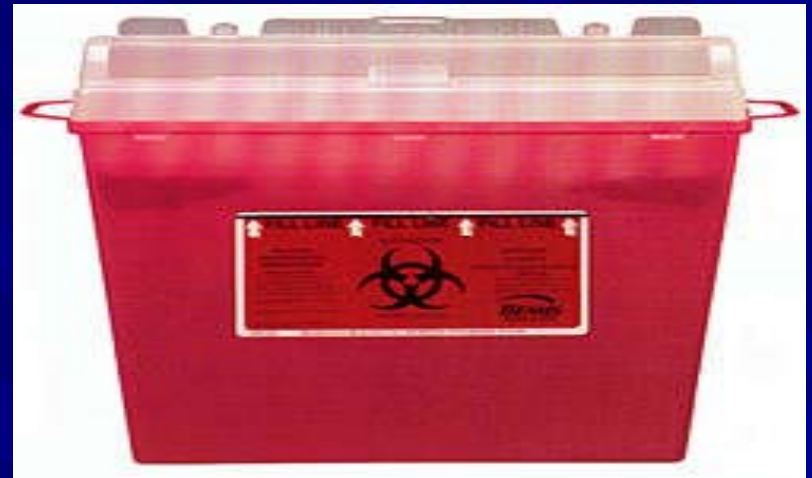
- Surround the primary barriers
  - Lab walls, floors, and ceiling
- Protects persons outside the laboratory and the environment

# Other Control Methods

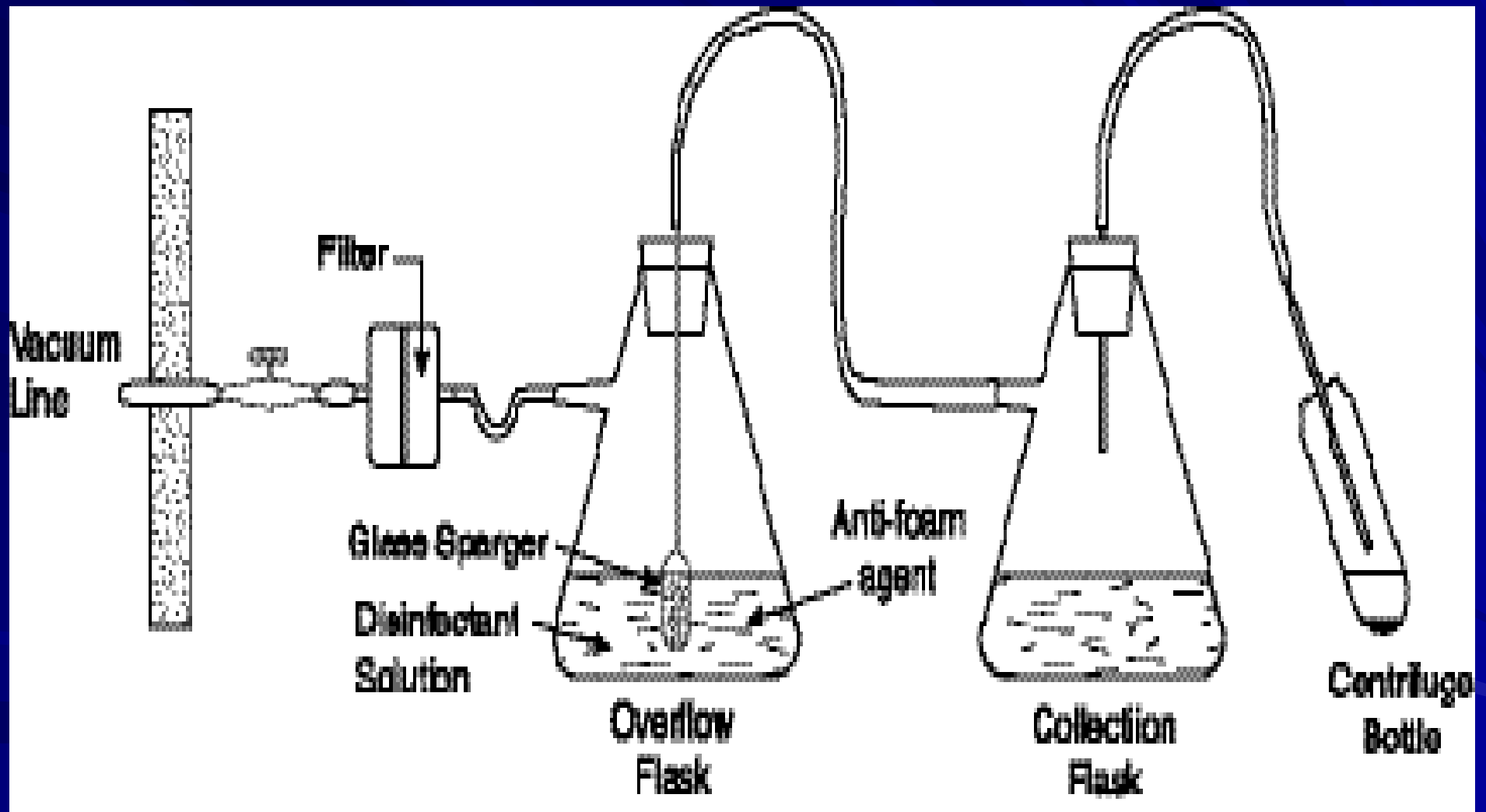
- They include:
  - additional types of engineering controls
  - work practice controls
  - personal protective equipment (PPE)
  - signs, labels, and color coding

# Engineering Controls

- Controls that isolate or remove the hazard from the workplace.
  - Sharps disposal containers, safer devices, etc.
  - Substitution-Replace needles and other sharps with other alternatives
    - Safer devices are often available and must be substituted wherever feasible
  - Suction flasks with an inline HEPA filter and disinfectant trap



# Vacuum Line Filters



# Work Practice Controls

- Reduce the likelihood of exposure by altering the manner in which a task is performed.

# Work Practice Controls-Examples

- Disinfect after a spill and after work is complete!!
- Never re-cap, bend, or shear needles
- Do not eat, drink, smoke, apply cosmetics, handle contact lenses, or store food in hazard area!
- No mouth pipetting!



# Work Practice Controls- Handwashing

- Easiest and most effective way of preventing infection
- Should be done when:
  - Gloves are removed
  - Before leaving the lab
  - After spill cleanup
  - At the end of the workday



# Proper Hand Washing Technique

- Wet hands with warm water
- Dispense soap into cupped hand
- Spread soap around hands and between fingers
- Wash hands for 15 seconds
- Rinse hands thoroughly with warm water
- Dry hands thoroughly with paper towels
- Remember, contamination can remain under fingernails!

# Personal Protective Equipment (PPE)



# Personal Protective Equipment (PPE)

- **Personal Protective Equipment** is specialized clothing or equipment worn by an employee for protection against a hazard
  - General work clothes (e.g., uniforms, pants, shirts or blouses) are not PPE!

# Personal Protective Equipment (PPE)

- The following PPE must be worn in ALL Laboratories:
  - Closed toed shoes
  - Disposable gloves (alternatives to latex must be provided)
  - Lab coat, gown or smock
  - Eye and/or face protection when not working in a BSC
- Demonstration-Proper Glove Removal

# Personal Protective Equipment (PPE)- Eye and Face Protection

- Needed if there is danger of splashing or particle generation, when for example:
  - Cleaning up a spill
  - Manipulating vials of blood or other potentially infectious material
  - Pipetting

# Personal Protective Equipment (PPE)- Final Thought

- You should never rely on personal protective equipment!

# Signs, Labels, and Color Coding

- Universal Biohazard warning label is required on:
  - All containers of biohazard materials
  - Biohazard waste containers
  - Doors/areas where biohazards are used
  - Refrigerators, freezers or other equipment used to store biohazard material



# Waste Disposal Procedures

- Waste must be segregated into two streams:
  - Sharps Waste
  - “Non-sharps” Waste

# Sharps Waste

- Disposable glass pipettes
- Needles and syringes
- Razor blades
- Scalpels
- Plastic pipette tips
- Microtome blades
- Glass microscope slides and cover slips

# “Non-Sharps” Waste

- Carcasses, body parts and bedding of animals exposed to pathogens
- Cell culture flasks etc.
- Cultures, stocks of infectious agents and associated biologicals
- Gloves, lab coats, masks, and aprons
- Human blood, blood products, and body fluids
- Materials from spill clean-ups
- Microorganisms constructed using recombinant DNA
- Pathological Waste: human/animal tissue and anatomical parts
- Specimen containers

# Waste Disposal Procedures

## ■ Sharps Waste:

- Place directly into a sharps container:
- Never place sharps into the ordinary trash!
- Do not recap needles,

## ■ “Non-Sharps” Waste:

- Place directly into a double **red** bag-lined biohazard box

## ■ Call or email me to arrange waste removal

# Decontamination

- Decontamination eliminates or reduces microbial contamination to a safe level.
- Three (3) forms:
  - Sterilization-Kills all microorganisms including their spores-Autoclave
  - Disinfection-Kills pathogenic organisms, but not necessarily their spores-Chemicals
  - Antisepsis-Prevents or arrests the growth or action of microorganisms-Alcohol

# Liquid Disinfectants

- Anything that comes into contact with biohazardous material must be cleaned and disinfected before reuse
- All spills of biohazardous materials must be disinfected with an appropriate and fresh disinfectant-
  - 1:10 dilution of HOUSEHOLD BLEACH is the disinfectant of choice
- All disinfectants require 10-20 minutes of contact time to be effective
- Anything that will be transported out of the lab must be disinfected (i.e. animal cages, laboratory equipment, etc.)

# Activity Level of Disinfectants

Disinfectant	Activity Level
Chlorine Compounds	Intermediate
Alcohols	Intermediate
Phenolic Compounds	Intermediate to Low
Iodophor Compounds	Intermediate to Low
QUATS	Low

# What Does 99% Effective Mean?

- If there are 1 million organisms we want to destroy:
  - A disinfectant that is 99% effective would destroy 990,000 organisms (10,000 organisms would remain)
  - 99.9% effective would destroy 999,000 (1000 organisms would remain)
  - 99.99% effective would destroy 999,900 (100 organisms would remain)
  - 99.999% effective would destroy 999,990 (10 organisms would remain)
  - 99.9999% effective would destroy 999,999 (1 organism would remain)

# Spill Response

- How you handle a spill depends upon:
  - How hazardous the substance
  - How big the spill
  - How much aerosol is generated
  - It's location: inside/outside BSC/Fume hood
  - Is there broken glass involved
- Always wear the PPE required!

# Spills Outside a BSC

- **Stop, notify others and isolate the area!**
- **Put on appropriate PPE (lab coat, gloves, eye and face protection)**
- **Remove glass/lumps with forceps or scoop if applicable and place into a rigid, puncture resistant container**
- **Small spills-Place paper towels soaked in bleach directly on the spill and let soak for 20 minutes**
- **Wipe up area and discard towels in biohazard waste container**
- **Continue wiping area with paper towels soaked in bleach until the spill area is completely cleaned**
- **Discard all materials in biohazard waste container**
- **Wash hands thoroughly**

# Spills Inside a Centrifuge

- Put on appropriate PPE
- Leave lid closed and allow aerosols to settle for at least one hour
- Ensure centrifuge is off and affix a sign to indicate that a spill has taken place
- Move to a BSC if possible
- Remove glass/lumps with tongs or forceps
- Pour or spray an appropriate disinfectant onto centrifuge, rotors and buckets and allow 20 minutes of contact time
- Drain the disinfectant
- Wipe down all parts with disinfectant-soaked paper towels
- Rinse the parts of centrifuge with water
- Discard all waste in a biological waste container
- Remove PPE and wash hands thoroughly

# Exposure Incident Procedures

- **Wash affected areas with soap and water; mucous membranes should be flushed with water for 15 minutes!**
- **Immediately notify your supervisor**
- **Contact Campus Police (8-2323) to arrange escort to Presbyterian University Hospital Emergency Department**
- **Be sure any spilled material is contained.**