

Biosafety





Principles

Definition

Biohazard

An agent of biological origin that has the capacity to produce deleterious effects on humans, i.e. microorganisms, toxins and allergens derived from those organisms; and allergens and toxins derived from higher plants and animals.



Introduction

Why Biosafety Practices?

Protection:

- workers
- “products”
- co-workers
- lab support personnel
- environment

Introduction

Chain of Infection

Reservoir of pathogen

Portal of escape

Transmission

Route of entry/infectious dose

Susceptible host

Incubation period

*Practices/
Equipment*

PPE

Immunization

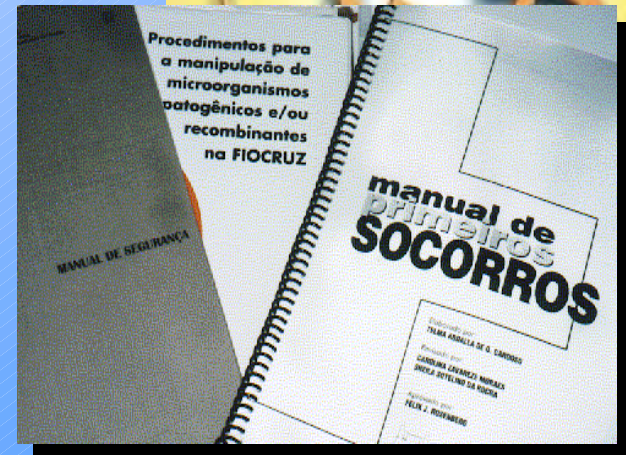
Surveillance

Risk Assessment

Principles

General Lab Requirements

- Knowledgeable supervisor
- Knowledgeable personnel
 - *Aware of potential hazards*
 - *Proficient in practices & techniques*
- Lab specific biosafety manual





Principles

General Lab Requirements

- **Biosafety Levels (BSLs)**
- **Laboratory Practice and Technique**
 - *Standard Practices*
 - *Special Practices*
- **Safety Equipment (Primary Barriers)**
- **Facility Design and Construction (Secondary Barriers)**



Principles

General Lab Requirements

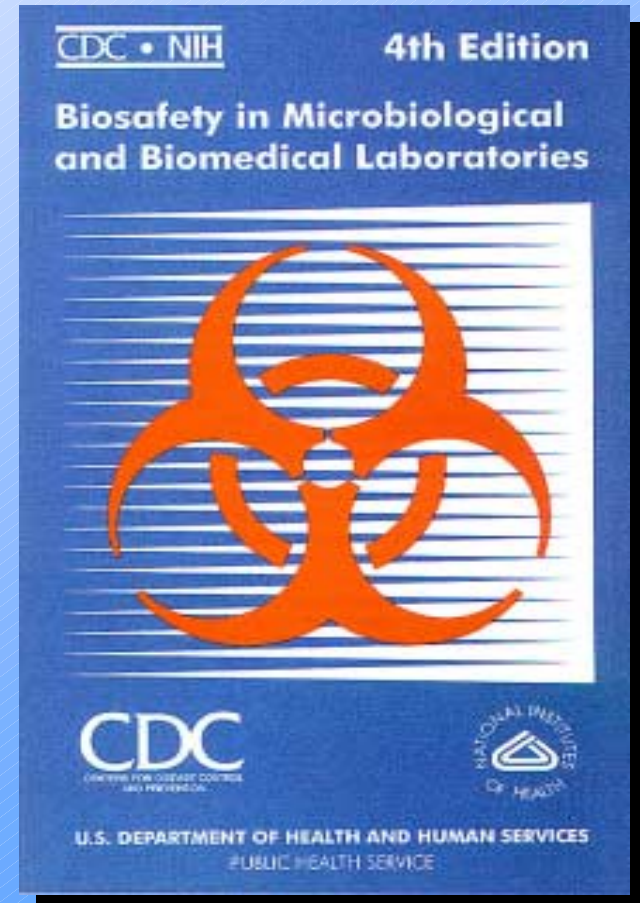
- **Biosafety cabinets (BSCs) - BSL 2/3**
- **Personal protective clothing**
 - *Gloves*
 - *Gowns*
 - *Eye and face protection*
- **Pipetting Devices**
- **Safety centrifuge cups and rotors**

Principles

Definition

Biosafety

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





Principles

Biosafety Levels

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



Biosafety Level 1

Introduction

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.



Biosafety Level 1

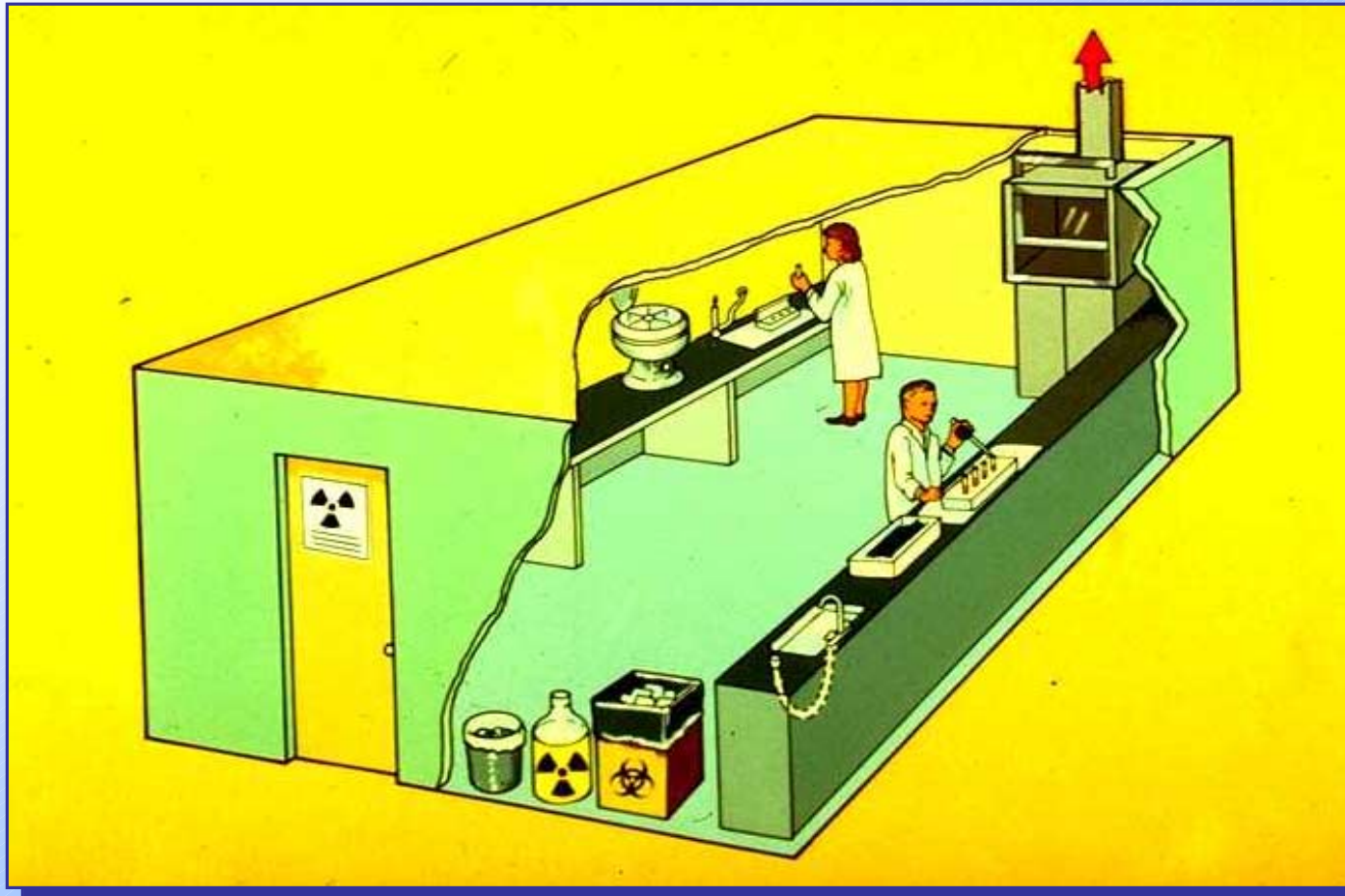
Introduction

Examples:

- *Bacillus subtilis*
- *Naegleria gruberi*
- *Infectious canine hepatitis virus*
- *E. coli*

Biosafety Level 1

Facility Design (Secondary Barrier)





Biosafety Level 1

Facility Design (Secondary Barrier)

Requirements:

- *Laboratories have doors*
- *Sink for hand washing*
- *Work surfaces easily cleaned*
- *Bench tops are impervious to water*
- *Sturdy furniture*
- *Windows fitted with flyscreens*

Biosafety Level 1

Standard Microbiological Practices

- **Restrict or limit access when working**
- **Prohibit eating, drinking and smoking**
- **Prohibit mouth pipetting**



Biosafety Level 1

Standard Microbiological Practices



***Use
mechanical
pipetting
devices***

Biosafety Level 1

Standard Microbiological Practices



Wash hands



Biosafety Level 1

Standard Microbiological Practices

- **Minimize splashes and aerosols**
- **Decontaminate work surfaces daily**
- **Decontaminate wastes**
- **Maintain insect & rodent control program**

Biosafety Level 1

Safety Equipment (Primary Barriers)

Protective clothing

- *Lab coat*
- *Gloves*



Biosafety Level 1

Safety Equipment (Primary Barriers)

Personal protective equipment

- *Face protection*
- *Eye protection*





Biosafety Level 1

Training Requirements

- **Supervisor**
 - *Scientist with general training in microbiology or related science*

- **Lab Personnel**
 - *Specific training in lab procedures*



Biosafety Level 2

Introduction

Suitable for work involving *agents of moderate potential hazard* to personnel and the environment.



Biosafety Level 2

Introduction

Examples:

- *Measles virus*
- *Salmonellae*
- *Toxoplasma spp.*
- *Hepatitis B virus*

* *Immunization or antibiotic treatment is available*



Biosafety Level 2

Introduction

Examples:

- *Bloodborne pathogens*
- *Human body fluids/particularly when visibly contaminated with blood*

*** *Extreme precaution with contaminated needles or sharp instruments***

Biosafety Level 2

Facility Design (Secondary Barriers)





Biosafety Level 2

Facility Design (Secondary Barriers)

Requirements:

- *Laboratories have lockable doors*
- *Sink for hand washing*
- *Work surfaces easily cleaned*
- *Bench tops are impervious to water*
- *Sturdy furniture*



Biosafety Level 2

Facility Design (Secondary Barriers)

Requirements (cont.):

- *Biological safety cabinets installed as needed*
- *Adequate illumination*
- *Eyewash readily available*
- *Air flows into lab without re-circulation to non-lab areas*
- *Windows fitted with flyscreens*

Biosafety Level 2

Facility Design (Secondary Barrier)



**Restricted
access when
work in
progress**

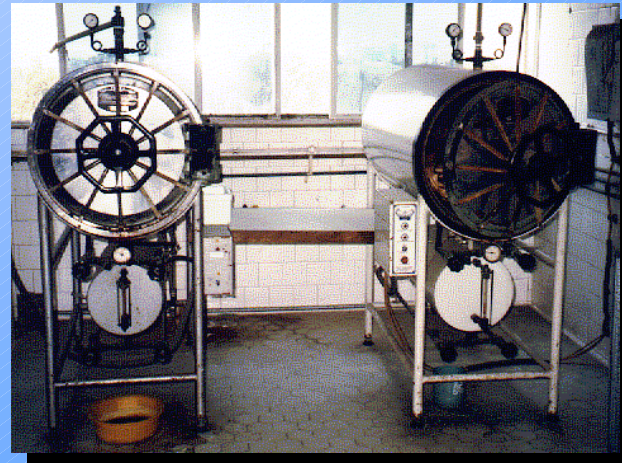
Biosafety Level 2

Laboratory Facilities (Secondary Barriers)

- **BSL-1 Facilities PLUS:**



- ***Autoclave available***
- ***Eyewash station available***





Biosafety Level 2

Standard Microbiological Practices

As in BSL-1



Biosafety Level 2

Safety Equipment (Primary Barriers)

In addition to BSL-1:

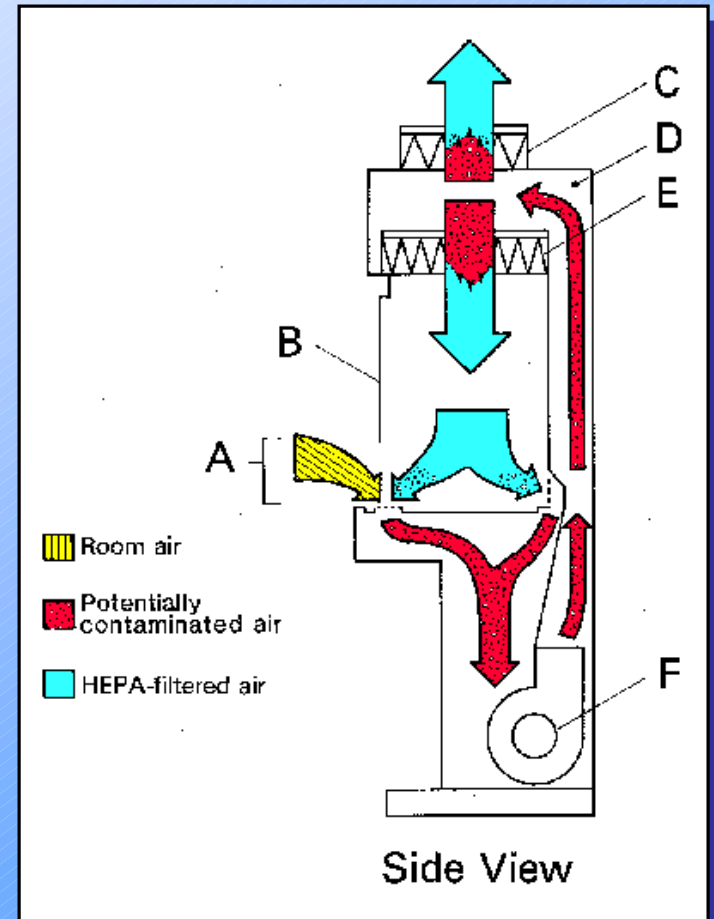
- *Use biosafety cabinets (class II) for work with infectious agents involving:*
 - **Aerosols and splashes**
 - **Large volumes**
 - **High concentrations**

Biosafety Level 2

Safety Equipment (Primary Barriers)

■ Class II Biosafety Cabinet

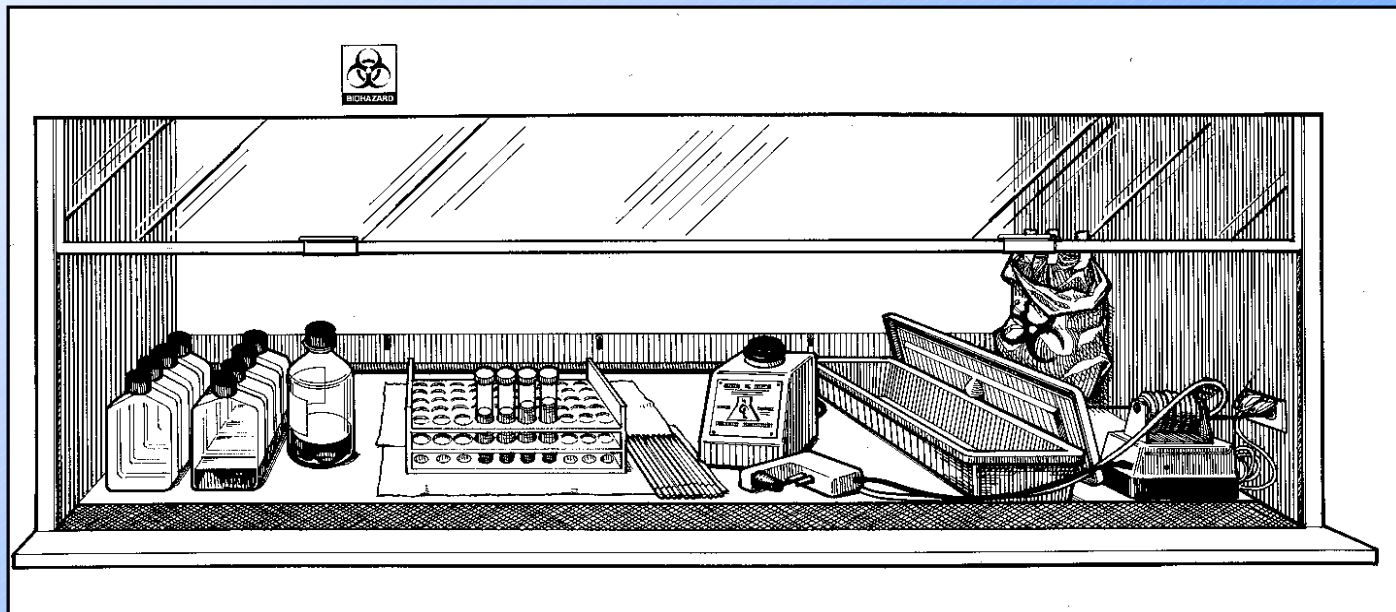
■ *Airflow*



Biosafety Level 2

Safety Equipment (Primary Barriers)

- **Class II Biosafety Cabinet**
 - *Equipment layout*



Biosafety Level 2

Safety Equipment (Primary Barriers)

- **Class II Biosafety Cabinet**

- *Technique*





Biosafety Level 2

Special Practices

Supervision

- *Supervisor is a competent scientist with increased responsibilities*
 - **Limits access if immunocompromised**
 - **Restricts access to immunized**

Lab Personnel

- *Aware of potential hazards*
- *Proficient in practices/techniques*

Biosafety Level 2

Special Practices

Needles & Sharps Precautions

- *Use sharps containers*
- *DON'T break, bend, re-sheath or reuse syringes or needles*



Biosafety Level 2

Special Practices

Needles & Sharps Precautions (cont.)

- ***DON'T place needles or sharps in office waste containers***



Biosafety Level 2

Special Practices

Needles and Sharps Precautions (cont.)

- ***DON'T touch broken glass with hands***



Biosafety Level 2

Special Practices

Needles and Sharps Precautions (cont.)

- *Use plasticware*



Biosafety Level 2

Special Practices

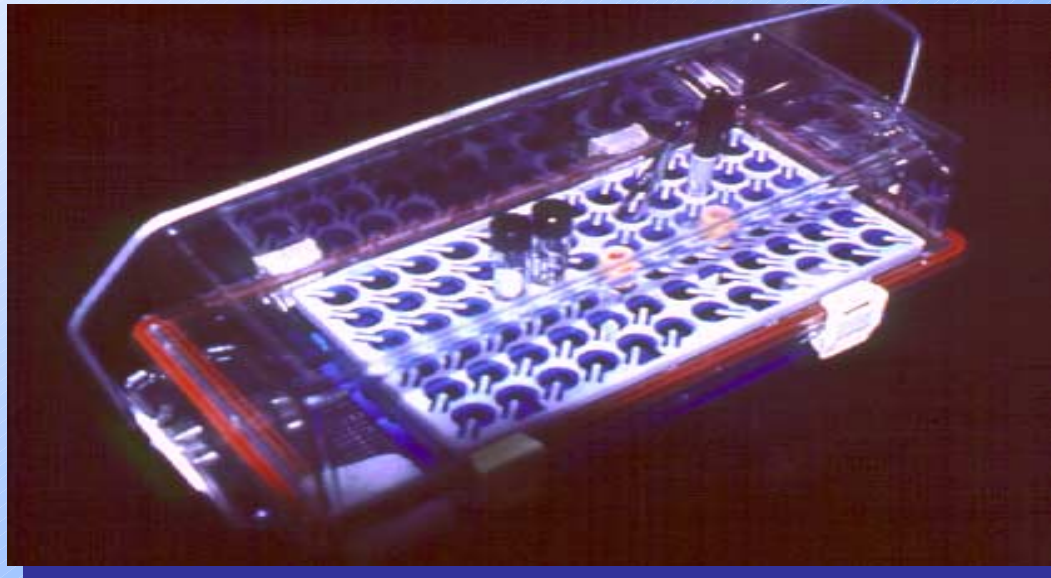
- **Policies and procedures for entry**
- **Biohazard warning signs**
- **Biosafety manual specific to lab**
- **Training with annual updates**



Biosafety Level 2

Special Practices

- **Use leak-proof transport containers**





Biosafety Level 2

Special Practices

- **Decontaminate work surfaces**
- **Report spills and accidents**
- **No animals in laboratories**

Biological Safety Cabinets

Purpose

- **Product protection**
- **Personal protection**
- **Environmental protection**





Biological Safety Cabinets

Types

A. Class I

- *inward airflow protects worker*
- *exhaust to outside (w/wo HEPA filter)*

B. Class II

- *worker, product, environmental protection*
- *“sterile” work area*
- *use for work with aerosol-transmissible micro-organisms*
- *use also for tissue culture/ virology*

C. Class III

- *totally enclosed, ventilated, air-tight*
- *suitable for work with BSL3/4 agents*



Biological Safety Cabinets

Types

Class II

- *Type A* *30% exhausted to room*
- *Type B3* *30% exhausted to outside*
- *Type B1* *70% exhausted to outside*
- *Type B2* *100% exhausted to outside*



Biological Safety Cabinets

Component

HEPA Filter

- *“High efficiency particulate air” filter*
- *Traps particulates **only**; chemicals, fumes, vapors pass through*
- *Traps particulates 0.3u*



Biological Safety Cabinets

Operating Location

- **Isolated from other work areas**
- **Removed from high traffic areas**
- **Away from airflow ducts**
- **Away from laboratory entry doors**

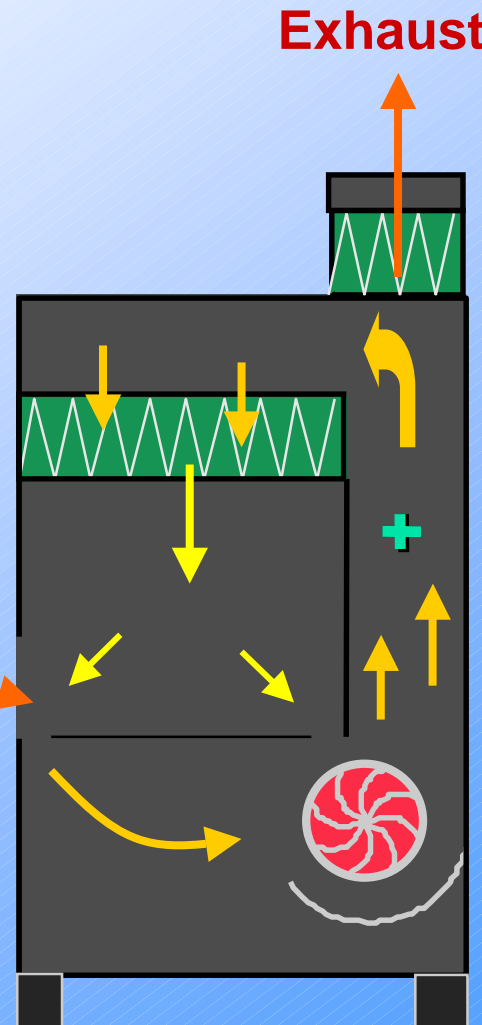
Biological Safety Cabinets

Airflow

Typical Class II



Intake
100
ft/min





Biological Safety Cabinets

Operating Procedure

- 1. Load BSC with all needed supplies.**
- 2. Turn BSC on and allow to run for 10-15 minutes.**
- 3. Check inward airflow with a piece of tissue.**
- 4. Enter straight into cabinet and perform work in a slow, methodical manner.**
- 5. At end of work, decontaminate all items to be taken out of cabinet.**
- 6. Decontaminate interior of BSC.**
- 7. Allow cabinet to run for 10-15 minutes.**
- 8. Shut off.**



Biological Safety Cabinets

Safe Operation

- **Always enter straight into cabinet - no sweeping motions**
- **Place materials well within the cabinet - not on front grill**
- **Place discard pan within cabinet**
- **Watch for disruptions of laminar air flow**
- **Decontaminate materials before removal from cabinet**



Biological Safety Cabinets

Safe Operation

- **Not designed for chemical use**
- **May use for non-volatile toxic chemicals or low-level radioactive materials**
- **May use for “minute” amounts of volatile chemicals**
- **Ensure annual certification**
- **Place all work materials into cabinet before starting**



Biological Safety Cabinets

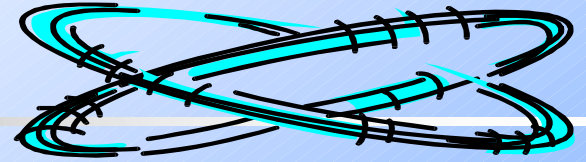
Safe Operation

CAUTIONS

- ***Chemicals may damage HEPA filter***
 - **Exposure risk - chemical/infectious agents**
- ***Volatile chemicals NOT retained by HEPA filter***
 - **Exposes personnel if not exhausted**
- ***BSC fans NOT spark proof***
 - **Chemical use may result in fire/ explosion**
 - **Never use NFPA 4 flammables**



Centrifuges



Types

Speeds (rpm)

Microcentrifuges

~15,000

Low/high speed

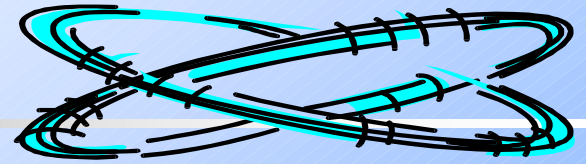
2,000 – 20,000

Ultracentrifuges

~ 120,000

Centrifuges

Hazards



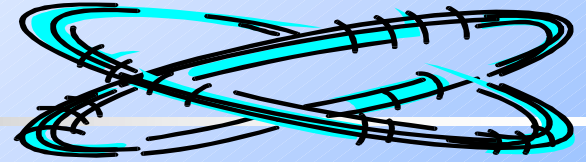
- **Mechanical failure of machine**
- **Lab equipment failure (tubes etc.)**
- **Aerosol generation**
- **Operator error**





Centrifuges

Operating Procedure

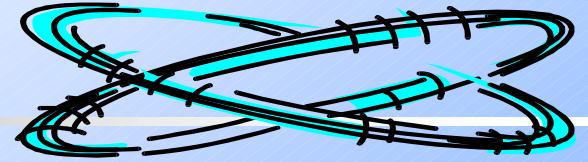


- 1. Check tubes for cracks/chips.**
- 2. Use matched sets of tubes, buckets etc.**
- 3. Tightly seal all tubes and safety cups.**
- 4. Ensure that rotor is locked to spindle and bucket seated.**
- 5. Close lid during operation.**
- 6. Allow to come to complete stop before opening.**



Centrifuges

Safe Operation



- Use safety cups whenever possible
- Disinfect weekly and after all spills or breakage's
- Lubricate O-rings and rotor threads weekly
- Do not use rotors that have been dropped
- Contact your centrifuge rep for specific information



Shipping Biological Specimens

Infectious Substance

Definition

- **Contains or has high probability of containing an infectious material...known or reasonably believed to cause disease in humans or animals**
 - *virus, prion, genetic elements*
 - *bacterium, rickettsia, parasite, fungus*
- **Contains a microbial toxin known to be pathogenic**

Shipping Biological Specimens

Infectious Substance

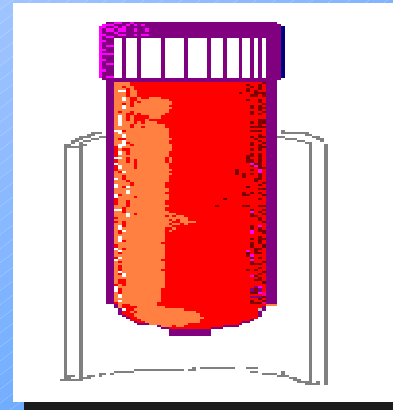
Packaging

- **Primary Container**

- *Positive seal*



- **Absorbent material**

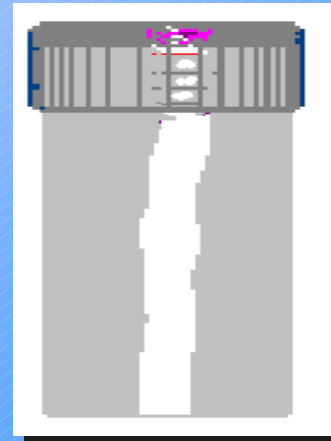
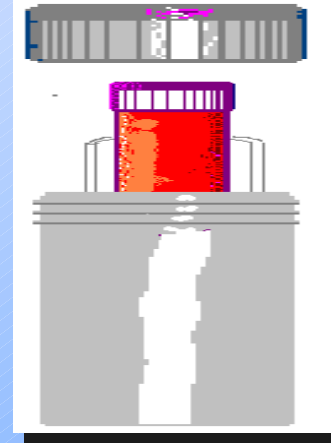


Shipping Biological Specimens

Infectious Substance

Packaging

- Secondary packaging
- Watertight/leakproof

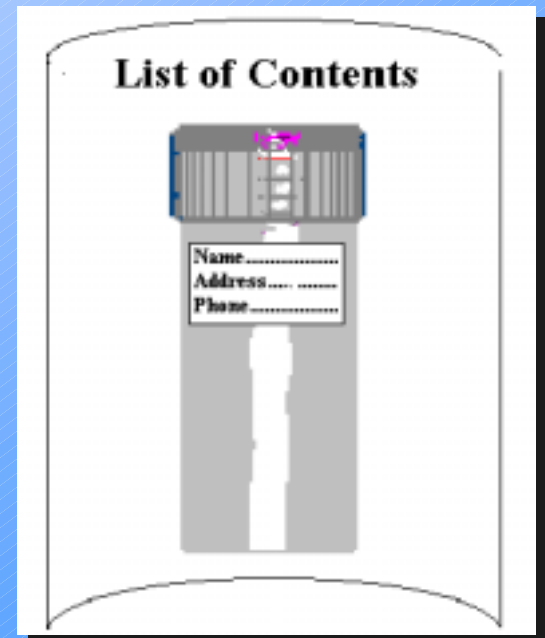


Shipping Biological Specimens

Infectious Substance

Packaging

- **Between Secondary and Outer Container**
 - *List of Contents*
 - *Shippers label*
 - Name
 - Address
 - Phone number

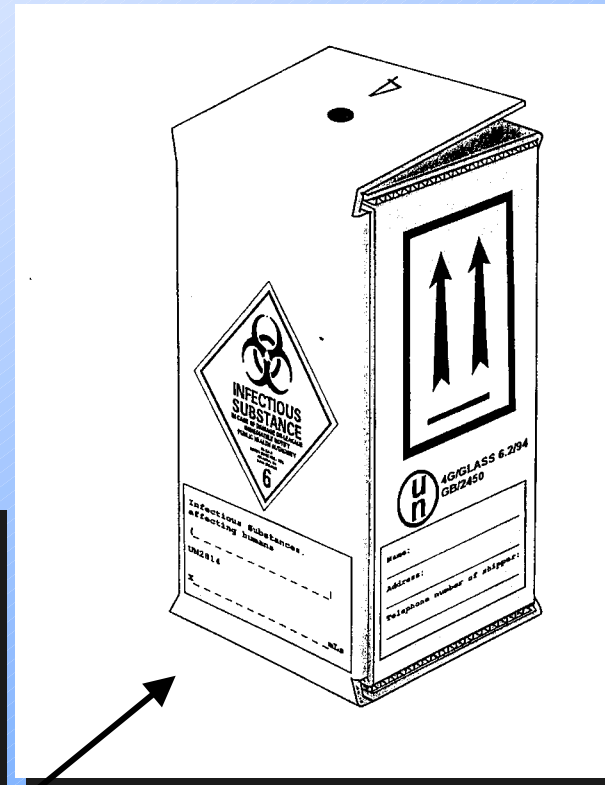
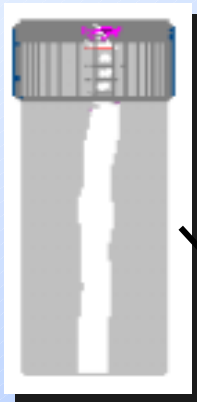


Shipping Biological Specimens

Infectious Substance

Packaging

■ Outer container



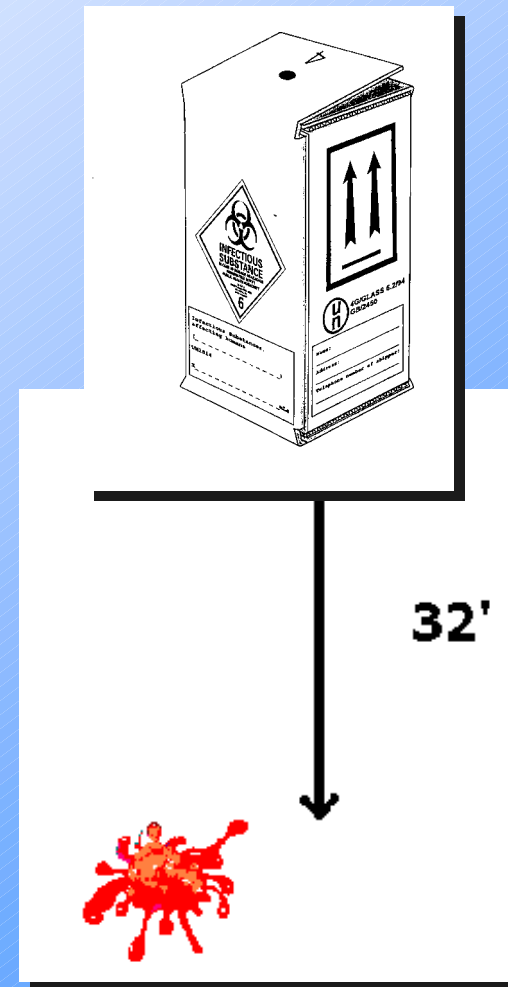
Shipping Biological Specimens

Infectious Substance

Packaging

- Performance tests
 - 49 CFR 178.609

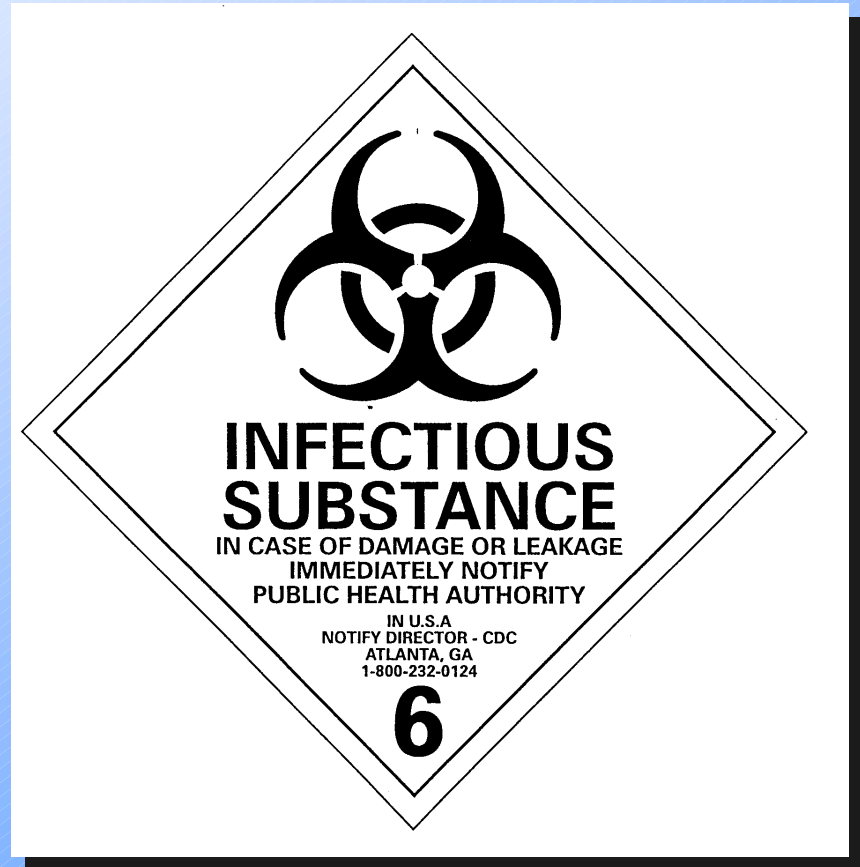
Package shall not leak



Shipping Biological Specimens

Infectious Substance

Packaging label





Decontamination

Definition

■ Disinfection

The use of a physical or chemical procedure to virtually eliminate all recognized pathogenic microorganisms but not all microbial forms (bacterial endospores) on inanimate objects.



Decontamination

Definition

■ Sterilization

The use of a physical or chemical procedure to destroy all microbial life, including large numbers of highly resistant bacterial spores.



Decontamination

Definition

■ Antisepsis

A germicide that is used on skin or living tissue for the purpose of inhibiting or destroying microorganisms.



Decontamination

Agent Selection

- **Degree of microbial killing required**
- **Nature of item/surface to be treated**
- **Ease of use**
- **Safety**
- **Cost**



Decontamination

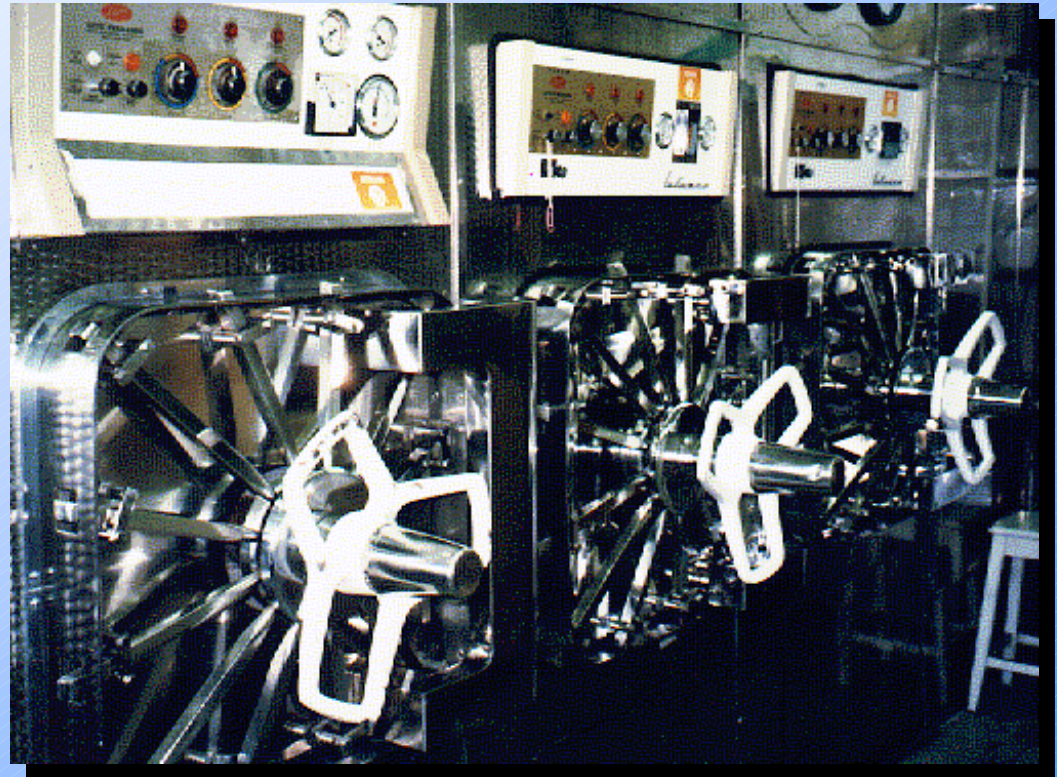
Agent Efficacy

- **Type of organism**
- **Number of organisms**
- **Amount of organic material present**
- **Type & configuration of material to be treated**
- **Type & concentration of germicide**
- **Time and temperature or exposure**
- **pH**
- **Humidity**

Decontamination

Methods

- Heat
- Chemical
- Radiation





Decontamination

Heat

■ Types

- *Moist – steam*
- *Dry*
- *Incineration*

***The most effective method of sterilization**



Decontamination

Heat

■ Steam sterilization practices

- *Ensure proper functioning of autoclave*
- *Vessels should not be capped or plugged*
- *Large loads require longer contact time*
- *Excessive amounts of liquid should not be added to load*

Decontamination

Heat

- **Steam sterilization verification**
 - *Direct assay*
 - *Thermocouples*
 - *Chemical indicators*
 - *Biological indicators*
(*Bacillus stearothermophilis*)





Decontamination

Heat

■ Dry heat sterilization

- *Denaturation of proteins: 160⁰ – 170⁰ C/2-4 hours*
- *Effective on impervious non-organic materials like glass*

Decontamination

Heat

■ Incineration

- *Method of choice for animal carcasses*
- *Requires certified incinerator*



Decontamination

Chemical

■ **Types**

- *Liquids, i.e. chlorox, hydrogen peroxide*
- *Gases, i.e. ethylene oxide*





Decontamination

Chemical

- **Agent selection - complexity**
 - *Over 14,000 registered products*
 - *Over 300 active ingredients*
 - *14 ingredients present in 92% of products*



Decontamination

Chemical

- **Agent selection - activity**
 - *HLD – high level disinfection*
 - *ILD – intermediate level disinfection*
 - *LLD – low level disinfection*



Decontamination

Chemical

- **High level disinfection - sporocides**
 - *Kills all microorganisms except high numbers of bacterial spores*
 - *Require 5-10 min. exposure*
 - *Examples: aldehydes, hydrogen peroxide, paracetic acid*



Decontamination

Chemical

■ Intermediate level disinfection - tuberculocides

- *Kills M. tuberculosis var. bovis and all vegetative bacteria, fungi, and most viruses*
- *Require minimum 20 min. exposure*
- *Examples: phenolics, iodophores, chlorine compounds, alcohols*



Decontamination

Chemical

- **Low level disinfection – hospital germicides used for housekeeping**
 - *Kills most vegetative bacteria and some fungi, but not M. tuberculosis var. bovis*
 - *Require minimum 20 min. exposure*
 - *Examples: quaternary ammonium compounds*

Decontamination

Chemical

- **General Lab Use - *Hypochlorite Solutions***
 - *Large Spills/Large Organic Load*
 - undiluted from bottle
 - *Small Spills/Virus Inactivation*
 - 10% - 1:9
 - *General Surface Disinfection*
 - 1% - 1:99





Decontamination

**Disinfectants do not replace
standard microbiological
practices or good hygiene!**



Biological Waste

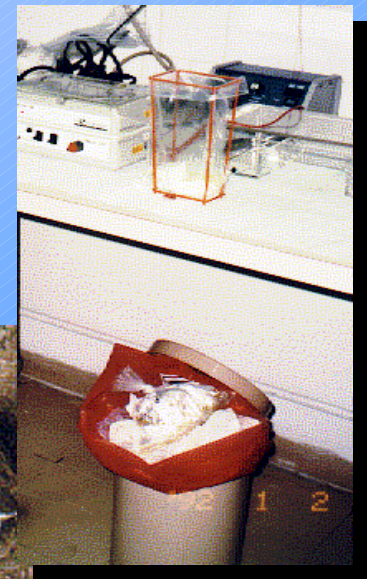
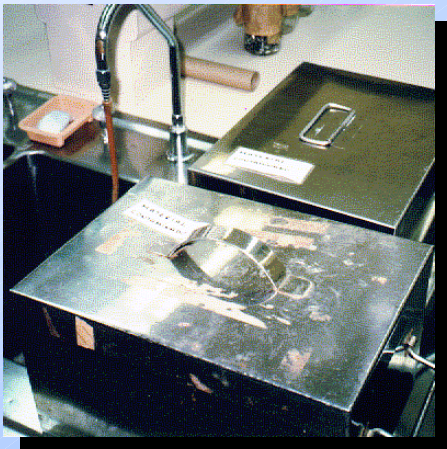
■ Types

- *cultures, stocks, isolates*
- *materials containing or contaminated with blood*
- *sharps*
- *pipettes, wrappers, tips*
- *All materials used in the lab*

Biological Waste

■ Disposal

- *puncture-proof, leak-proof, sealable receptacles*
- *avoid over-filling*
- *dispose properly*



Biological Waste

■ Disposal

- **Never** place lab waste into office waste containers
- Place sharps into “sharps” container
- Line discard containers with autoclave bag
- Decontaminate discard pans before they leave the lab:

1. Disinfect outside
2. Label
3. Tape ends with autoclave
4. Tape
5. Secure for transport to autoclave





Biological Waste

Decontamination

- **To render the object/material safe by reducing or removing the bioburden**
- **Methods**
 - *chemical ... match, contact time*
 - *physical ... Heat, steam and pressure*
 - *incineration*
 - *other choices, i.e. shredding + chemical*



Medical Surveillance

Criteria

- **Based on risk assessment**
- **Pre-placement**
 - *evaluate physical requirements*
- **Periodic review**



Medical Surveillance

Risk Assessment

- **What is the natural host?**
- **Does agent cross species barriers?**
- **Wild-type agent or attenuated?**
- **Infectious for normal healthy adult?**
- **What if adult is immunocompromised?**



Medical Surveillance

Risk Assessment

- **Mode of transmission?**
 - *contact*
 - *fomites*
 - *mucous membrane exposure*
 - *ingestion*
 - *inoculation or insect bites*
 - *inhalation*
 - *sex*



Medical Surveillance

Risk Assessment

- **Volume being manipulated?**
- **Concentration of agent?**
- **Infectious dose?**
- **Past history of lab-associated infection?**
- **Secondary spread in community?**



Medical Surveillance

Risk Assessment

- **Prophylaxis**
 - *Immunizations available?*
 - *Pharmaceuticals?*
 - *Effectiveness?*
- **Post-Exposure**
 - *Anti-microbial agents?*
 - *Pharmaceuticals?*
 - *Effectiveness?*



Emergency Response

Personal Contamination

- 1. Alert co-workers**
- 2. Clean exposed surface with soap/water, eyewash (eyes), or saline (mouth)**
- 3. Apply first aid and treat as an emergency**
- 4. Notify supervisor or security desk (after hours)**
- 5. Report to medical clinic for treatment/counseling**



Emergency Response

Surface Contamination

- 1. Alert co-workers**
- 2. Define/isolate contaminated area**
- 3. Put on appropriate PPE**
- 4. Remove glass/lumps with forceps or scoop**
- 5. Apply absorbent towel(s) to spill; remove bulk & reapply if needed**
- 6. Apply disinfectant to towel surface**
- 6. Allow adequate contact time (20")***
- 8. Remove towel, mop up; clean with alcohol or soap/water**
- 9. Properly dispose of materials**
- 10. Notify supervisor**

