Biosafety
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

Risk Assessment

PPE

Practices/Equipment

Immunization

Surveillance

2.1
**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)
Biosafety cabinets (BSCs) - BSL 2/3
Personal protective clothing
- Gloves
- Gowns
- Eye and face protection
Pipetting Devices
Safety centrifuge cups and rotors
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.
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
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
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
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
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**
Needles & Sharps Precautions

- *Use sharps containers*
- *DON’T break, bend, re-sheath or reuse syringes or needles*
Needles & Sharps Precautions (cont.)

- DON’T place needles or sharps in office waste containers
Needles and Sharps Precautions (cont.)

- DON’T touch broken glass with hands
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 microorganisms
- 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

Exhaust

Intake 100 ft/min
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.
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
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

<table>
<thead>
<tr>
<th>Types</th>
<th>Speeds (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcentrifuges</td>
<td>~15,000</td>
</tr>
<tr>
<td>Low/high speed</td>
<td>2,000 – 20,000</td>
</tr>
<tr>
<td>Ultracentrifuges</td>
<td>~120,000</td>
</tr>
</tbody>
</table>
Centrifuges

Hazards

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

INFECTIONOUS SUBSTANCE
IN CASE OF DAMAGE OR LEAKAGE
IMMEDIATELY NOTIFY
PUBLIC HEALTH AUTHORITY

IN U.S.A
NOTIFY DIRECTOR - CDC
ATLANTA, GA
1-800-232-0124

2.9
**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.*
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*
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
Steam sterilization verification

- Direct assay
- Thermocouples
- Chemical indicators
- Biological indicators (*Bacillus stearothermophilis*)
Dry heat sterilization

- **Denaturation of proteins:** $160^0 - 170^0 \text{ C/2-4 hours}$
- **Effective on impervious non-organic materials like glass**
Decontamination

Incineration

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

Chemical

Types

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

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

- **HLD** – high level disinfection
- **ILD** – intermediate level disinfection
- **LLD** – low level disinfection
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*
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: quartenary 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
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?
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