Biosafety at MUSC

- *Unit 4*
  - Specific Requirements for Biosafety Levels 1 to 3
    - PPE
    - Biological Safety Cabinets (BSCs)
    - Risks associated with centrifugation and proper protocols

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.

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

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.

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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*

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 1

Standard Microbiological Practices

Use mechanical pipetting devices

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Biosafety Level 1

Standard Microbiological Practices

Wash hands

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For more information: http://www.washup.org

Biosafety Level 1

Standard Microbiological Practices

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

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Biosafety Level 1

Safety Equipment (Primary Barriers)

Protective clothing

- *Lab coat*
- *Gloves*

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Biosafety Level 1

Safety Equipment (Primary Barriers)

Personal protective equipment

- *Face protection*
- *Eye protection*

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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*

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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*

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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*

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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

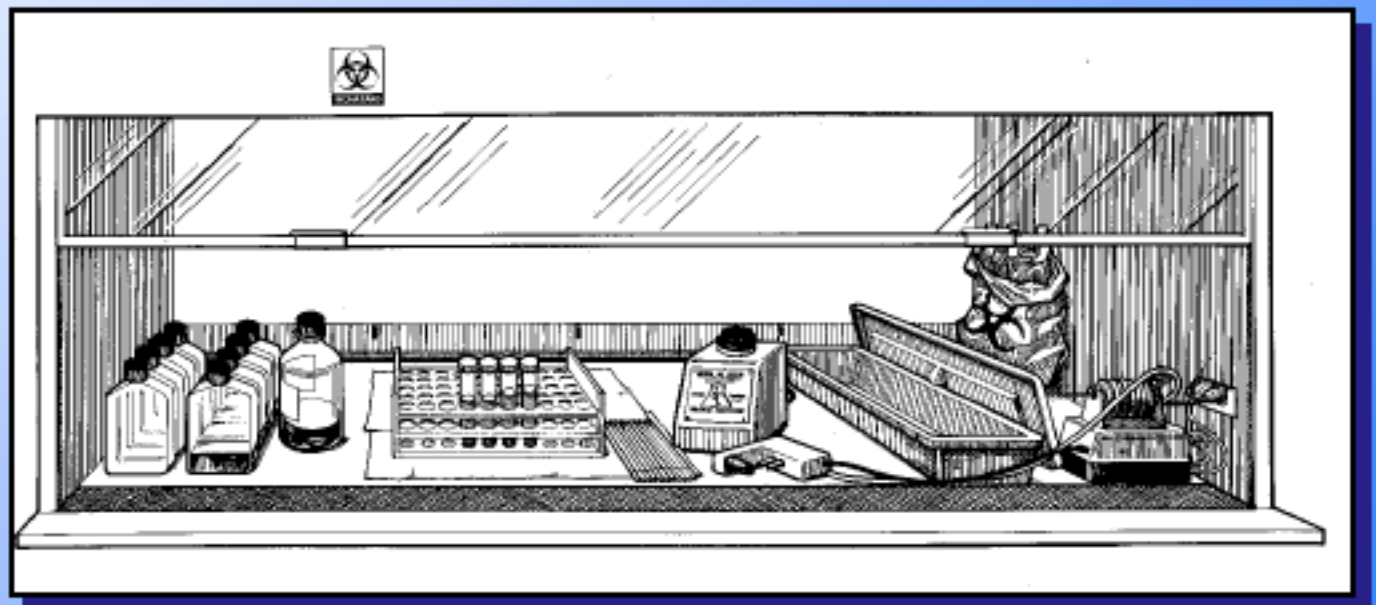
http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Safety Equipment (Primary Barriers)

Class II Biosafety Cabinet

- *Equipment layout*

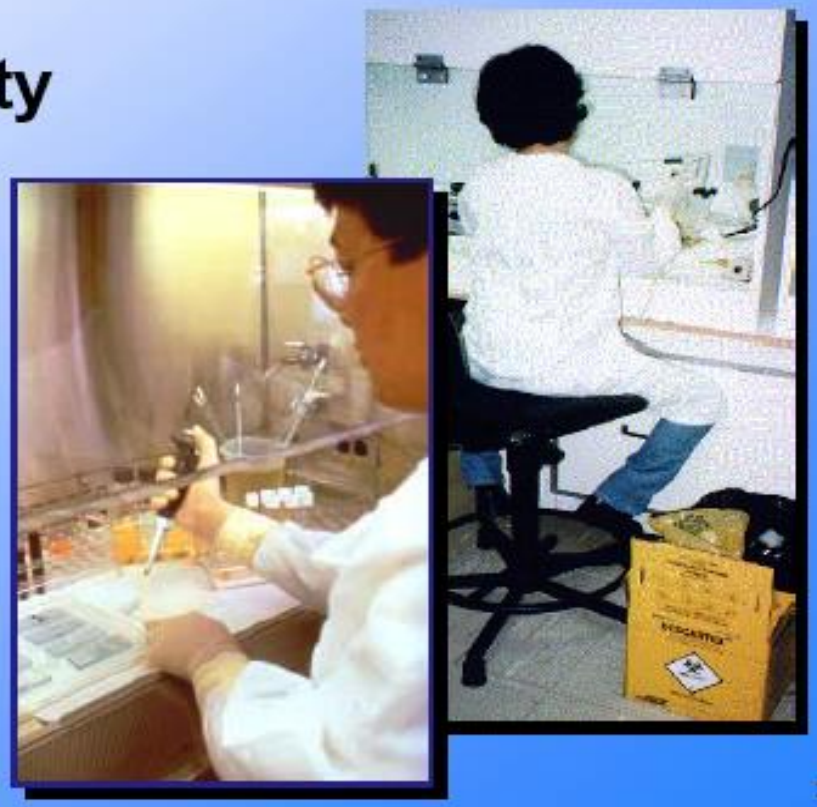


http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Safety Equipment (Primary Barriers)

- Class II Biosafety Cabinet
  - *Technique*



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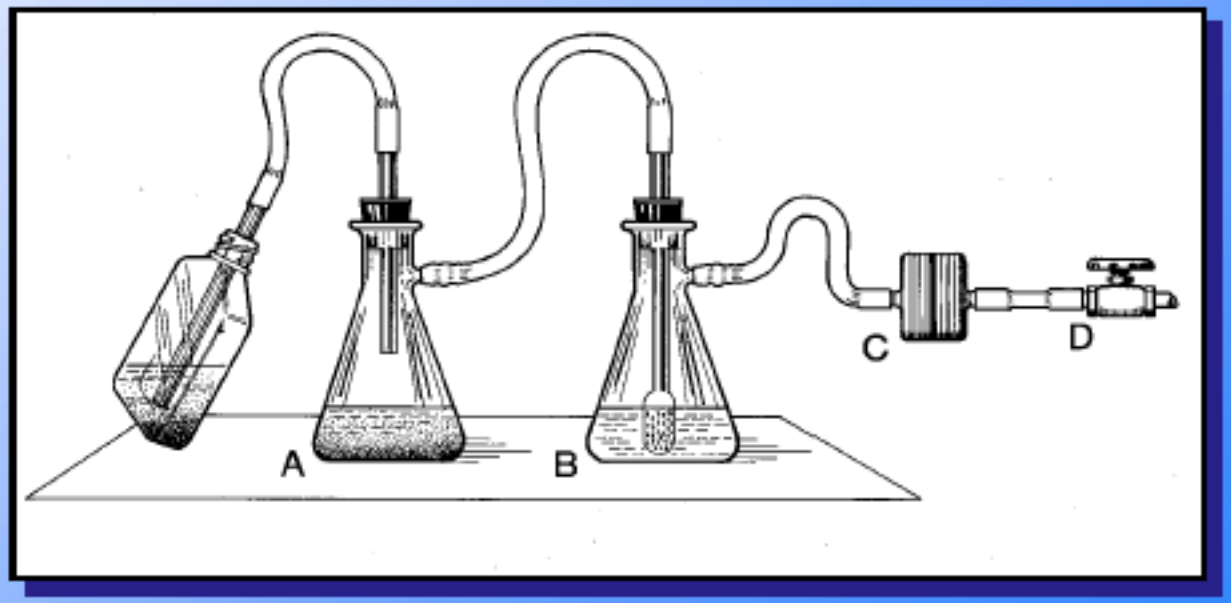
Biosafety Level 2

Safety Equipment (Primary Barriers)

- *Vacuum lines protected with liquid disinfectant traps or HEPA filters*



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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*

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Biosafety Level 2

Special Practices

Needles & Sharps Precautions

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

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Special Practices

Needles & Sharps Precautions (cont.)

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

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Special Practices

Needles and Sharps Precautions (cont.)

- *DON'T touch broken glass with hands*

2.4

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Special Practices

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

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Special Practices

- Use leak-proof transport containers

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Biosafety Level 2

Special Practices

- Immunizations
- Baseline serum samples

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Biosafety Level 2

Special Practices

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

2.4

http://www.cdc.gov/od/ohs/pdffiles/ Module%202%20-%20Biosafety.pdf

Human Tissue and Cell Culture

- **Human blood, blood products, body fluids and tissues are listed as potentially Hazardous Biological Materials**
  - **Biosafety Level 2 Practices and procedures MUST be followed when handling**
    - **Blood**
    - **Blood Products**
    - **Body fluids**
    - **Tissues**
    - **Under no circumstance shall anyone work with cells derived from themselves or from first degree relatives since the host immune systems may not provide adequate protection**
    - **See Green book for greater detail**

Human Tissue and Cell Culture

- **Cell Culture**
  - **When a cell culture contains an etiologic agent, oncogenic virus or amphotropic packaging system the cell line must be classified at the same level as that recommended for the agent.**

Human Tissue and Cell Culture

- **Cell Culture**
  - **The following cell lines are at Biosafety Level 2 or higher**
    - **All cell lines of human/primate origin**
    - **Any cell lines derived from lymphoid or tumor tissue**
    - **All cell lines exposed to or transformed by any oncogenic virus**
    - **All cell lines exposed to or transformed by amphotropic packaging systems**
    - **All clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy)**
    - **All cell lines new to the laboratory (until proven to be free of all adventitious agents)**
    - **All mycoplasma-containing cell lines**

Personal Protective Equipment

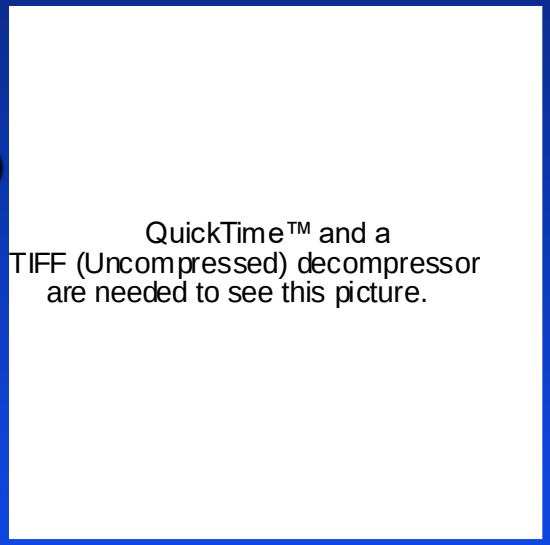
- **PPE is used to protect you from contact with biohazardous materials**

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Graphic credit: http://www-ehs.ucsd.edu/bio11.htm

Personal Protective Equipment

- **If the science you are conducting requires you to wear a N95 Respirator (*Mycobacterium tuberculosis*) Occupational Health and Safety must FIT-TEST you prior to you starting work!**



Biological Safety Cabinets

Purpose

- Product protection
- Personal protection
- Environmental protection

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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*

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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*

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Biological Safety Cabinets

Component

HEPA Filter

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

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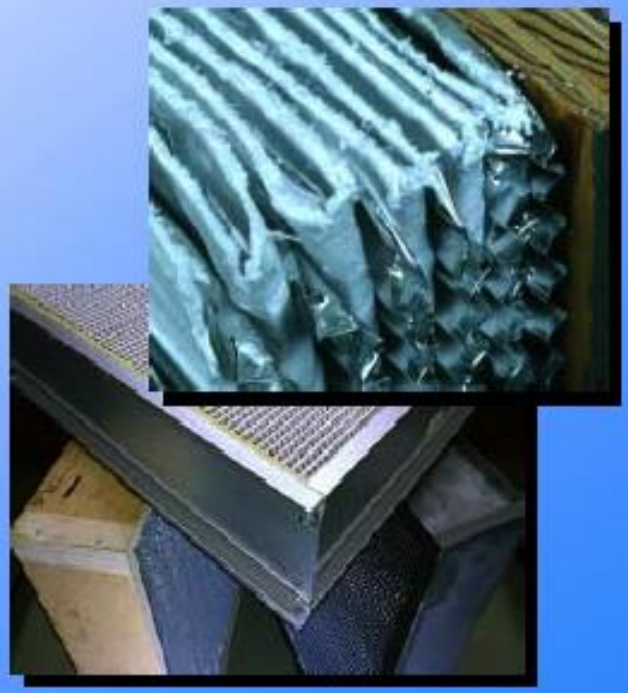
Biological Safety Cabinets

Component

HEPA Filter

- *Metal or wood framed*
- *Continuous sheet of flat filter medium with aluminum separators*
- *Gasket sealed*
- *Adhesive bond between filter pack and frame*



Biological Safety Cabinets

Operating Location

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

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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.

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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

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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

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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

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Centrifuges

Types	Speeds (rpm)
Microcentrifuges	~15,000
Low/high speed	2,000 – 20,000
Ultracentrifuges	~ 120,000

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Centrifuges

Hazards

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

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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.

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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

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Laboratory Housekeeping

- Good housekeeping is essential for
  - Reducing risks
  - Protecting the integrity of biological experiments
- Keep the laboratory neat and free of clutter.

Biosafety at MUSC

- *Questions Unit 4*
  - Specific Requirements for Biosafety Levels 1 to 3
    - PPE
    - Biological Safety Cabinets (BSCs)
    - Risks associated with centrifugation and proper protocols
    - Cell Culture
    - Spills