

Biosafety



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Principles

Definition

Biohazard

- *Infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals, plants or the environment.*
- *Biohazardous materials include organisms and viruses infectious to humans, animals or plants; biologically active agents (such as toxins of biological origin); human cell lines and recombinant DNA.*

Introduction

Harding and Byers, 2006. Epidemiology of laboratory-associated infections, in Biological safety: principles and practices, 4th ed. ASM Press.

- Reviewed 270 publications, from 1979-2004
- Total of 1,448 cases and 36 deaths
- Clinical and research labs accounted for ~76%
- Few related to actual accidents; most acquired by simply working in the lab or exposure to infected animals.

Introduction

Characteristics of persons who have fewer accidents

- ✓ Adherence to safety regulations (Buy-in)
- ✓ Healthy respect for infectious agents
- ✓ “defensive” work habits
- ✓ Ability to recognize a potentially hazardous situation

Introduction

Persons involved in laboratory accidents

- ✗ Low opinions of safety programs
- ✗ Risk takers
- ✗ Work too fast
- ✗ Decreased awareness of the infectious risks of the agents handled.

*men and younger employees (17-24) are involved more often than women and older employees (45-64)

Principles

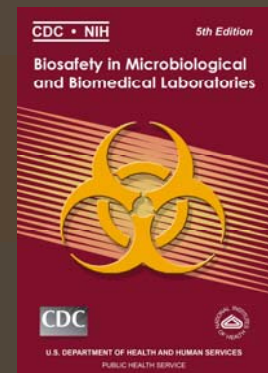
Definition

Biosafety

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

BMBL

1984; CDC, NIH, HHS



Introduction

Why Biosafety Practices?

Protection:

- workers
- “products”
- co-workers
- lab support personnel
- Environment
- family

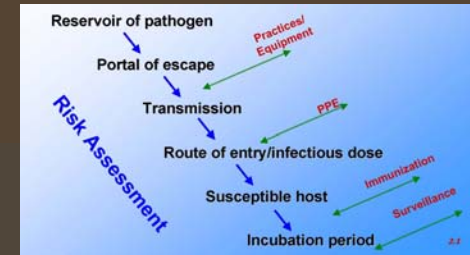
Introduction

Principles of Biosafety: Risk Assessment and Containment

Risk assessment:

Hazards of the agent

Hazards of the lab
procedures



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

Biosafety Levels

- **BSL 1** – agents not known to cause disease.
- **BSL 2** – agents associated with human disease; not transmitted by aerosols in nature.
- **BSL 3** – indigenous/exotic agents associated with potential for aerosol transmission.
- **BSL 4** – dangerous/exotic agents of life threatening nature.

Principles

- Risk groups often correlate, but don't equate, with biosafety levels.
 - If the agent isn't listed in the BMBL or NIH Guidelines as a RG 2, 3 or 4 agent, it isn't automatically implied that it's a RG1 agent.

Biosafety Level 1

Introduction

Suitable for work involving well-characterized agents *not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard* to laboratory personnel and the environment.

Biosafety Level 1

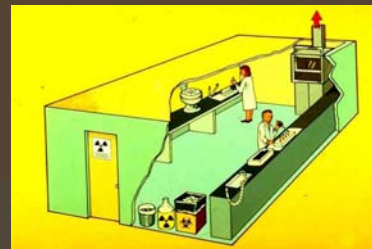
Introduction

Examples:

- *Bacillus subtilis*
- *Saccharomyces cerevisiae*
- Non pathogenic *E. coli* (K-12)

Biosafety Level 1

Facility Design

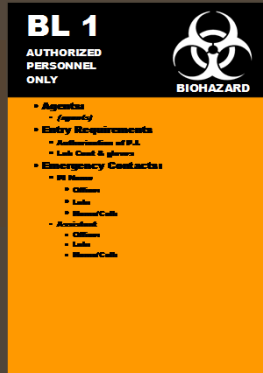


Basic level of containment

- Laboratories have doors and sinks for hand washing; exterior windows fitted with flyscreens or sealed shut;
- Work takes place on the open bench; benchtops are impervious to water and resistant to chemicals;
- Sturdy furniture; no cloth-covered chairs or carpets;
- Work surfaces easily cleaned; and
- No special requirements with regards to location, structure or ventilation.

Biosafety Level 1

Standard Microbiological Practices



Door Entry Sign

Biosafety Level 1

Standard Microbiological Practices

- Restrict or limit access when working
- Prohibited:
 - eating, drinking and smoking
 - storing food
 - applying makeup or contact lenses
 - mouth pipetting



Biosafety Level 1

Safety Equipment (Primary Barriers)

Personal Protective Equipment (PPE)

- Lab coat
- Gloves
- Eye protection



★ Please remember to remove all PPE ★
before exiting the lab.

Usage of gloves

- Change gloves when contaminated or compromised
- Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory
- Do not wash or reuse disposable gloves
- Dispose of used gloves with contaminated laboratory waste

Biosafety Level 1

Standard Microbiological Practices

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

Properly dispose sharps

Needles & Sharp Precautions

➤ Use approved sharps containers



➤ DON'T break, bend, re-sheath or reuse syringes or needles



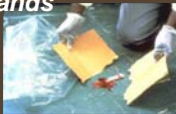
➤ DON'T place needles or sharps in office waste containers



Biosafety Level 1

Needles & Sharp Precautions, cont.

➤ Please DON'T touch broken glass with hands



➤ Please place broken glass in an appropriate container



➤ Whenever possible, please use plastic



Biosafety Level 1

Standard Microbiological Practices

Wash hands



- After working with potentially hazardous materials
- Before leaving laboratory
- Do it right!

Biosafety Level 1



Special Practices

None required

Biosafety Level 1



Training Requirements

- **The laboratory principal investigator (PI)**
 - *Ensure that lab personnel receive appropriate training regarding duties, necessary precautions to prevent exposures and environmental release.*
- **Lab Personnel**
 - *Must receive annual updates or additional training when procedural or policy changes occur.*

Biosafety Level 2



Introduction

Suitable for work involving the broad spectrum of moderate risk agents that are present in the community and associated with disease of varying severity—these agents pose moderate risk to personnel and the environment.

Biosafety Level 2



Introduction

Examples of RG2 agents*:

- *Measles, Mumps, RSV, EBV*
- *Salmonella, Listeria, Staphylococcus, Streptococcus*
- *Toxoplasma, Babesia, Schistosoma, Trypanosoma*
- *Human tissues, cell lines, primary cells and body fluids (particularly if visibly contaminated with blood)*

*Immunization or antibiotic treatment is available

Biosafety Level 2

- *Transmission from laboratory procedures with a RG2 agent may occur even if the disease caused by that agent is not transmitted by aerosol in the community.*
 - *Why?*
 - *Because in the laboratory one typically uses higher concentrations of organisms and procedures that may generate aerosols.*

Biosafety Level 2

All the BL1 requirements plus:

- *Laboratories must have lockable, self-closing doors;*
- *Biological safety cabinets installed;*
- *Eyewash readily available;*
- *Directional (inward) airflow into the lab;*
- *Vacuum lines protected with HEPA filters;*
- *Autoclave available.*

Biosafety Level 2

Special Practices

- **Occupational Health Enrollment/ Immunizations;**
- **No plants or animals (not associated with research) permitted in the lab;**
- **Use leak-proof transport containers;**



Biosafety Level 2

Please consult with Environmental Health and Services for shipment of infectious and/or recombinant materials.

<http://ehsd.tamu.edu/HazardousMaterialShipping.aspx>

Biosafety Level 2

Special Practices, continued

- ***Most procedures* with infectious biohazards should be conducted inside a BSC***

****exception: centrifugation, so long as aerosol tight rotors or safety cups are used which are loaded and unloaded inside the BSC.***

Biosafety Level 2

Devices that may create aerosols:

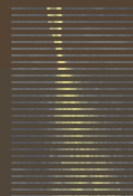
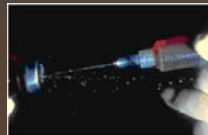
- Blenders and vortexers
- Cell sorters
- Centrifuges
- homogenizers
- Needles and syringes
- Pipets
- Pressurized vessels
- Vacuum and aspirating equipment



Biosafety Level 2

Procedures that may produce aerosols:

- Blowing out pipettes;
- Dropping culture containers;
- Animal necropsy or intranasal inoculation of animals;
- Cage cleaning and changing animal bedding;
- Pouring or stirring liquids;



Biosafety Level 2

- Carelessly removing gloves;
- Flaming inoculating needles, slides or loops;
- Inserting a hot loop into a culture;
- Pipetting;
- Opening ampoules, tubes and bottles; and
- Streaking inoculum.



Biosafety Level 2



Special Practices

• Supervision

Supervisor is a competent scientist with increased responsibilities

- Limits access if immunocompromised and/or restricts access to immunized
- Provides training to personnel

Lab Personnel

- *Aware of potential hazards*
- *Receive medical surveillance and be offered appropriate immunizations*
- *Proficient in practices/techniques*

Biosafety Level 2



Part IV
Personal Information

Signature Page

Each employee working in BSL-2 and above laboratories must complete this page.

Employees working in laboratories containing Select Agents may submit copies of training certificates instead of signature pages.

By my signature below, I certify that I have read and understood the laboratory security and emergency policies and procedures for working with _____ in laboratory building _____ and room(s) _____ under the direction of _____.

I further certify that I understand the hazards of working with _____ (the indication of infection or intoxication by this biological material), the appropriate control measures, the potential exposure and infection, how to work reduction and storage, the standard microbiological practices for the laboratory, the standard Biosafety practices required for Biosafety level _____ work, in accordance with the Biosafety in Microbiological and Biomedical Laboratories (BMBL) Guidelines and the standard operating procedures for the laboratory.

Finally, I certify that any transfer of this biological material will be done in accordance with TAMU policies and regulations and under the supervision of the TAMU Office of Environmental Health and Safety Department. In addition, I ensure that the detailed records of laboratory activity to account for all activities related to this agent will be maintained.

Signature _____	Date _____	Laboratory director/Supervisor's signature _____	Date _____
Printed/Typed Name _____	Position/Title _____	Laboratory director/Supervisor's printed/typed Name _____	

Have you completed training for this specific laboratory and materials?

☐ Yes ☐ No

Date and location of training _____

Have you enrolled in the Occupational Health & Safety Program?

☐ Yes ☐ No

Date of Occupational Health & Safety Program Enrollment _____

(Please reproduce this page as needed.)

Page _____ of _____

Page 1108

Biosafety Level 2



Special Practices

• Incidents (spills or accidents) that involve exposure

- Must be reported immediately to the laboratory supervisor
- Must be immediately evaluated and treated (OHP)
- If exposure involved recombinant DNA, reporting to NIH is also required.

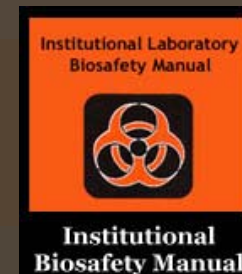
{Appendix G-II-B-2-k: "Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the IBC and NIH/OBA.}

Biosafety Level 2



Special Practices

- **Lab-specific biosafety manual**
 - Must be prepared & adopted as policy
 - Must be available and accessible
 - Involves risk assessment



Biosafety Level 2



Lab-specific biosafety manual...

1. Lab contact information (PI name, phone numbers, emergency numbers)
2. Contain Safety information for specific hazards and research materials from the BMBL
3. Serve as a training tool for personnel and include documentation of training
4. Readily available to all research personnel in the lab
5. Modified as needed to contain current Laboratory SOPs and practices
6. Copy of the current IBC Registration document
7. Copies of IBC approval letter and recent lab inspection report.

Biosafety Level 2



Facility Construction (Secondary Barriers)

Requirements:

- *Location – separated from public areas*
- *Structure – normal construction*
- *Ventilation – directional*

Biosafety Level 2



Standard Microbiological Practices



- Agents:
 - Agents
- Entry Requirements
 - Authorization of P.I.
 - Lab Coat & gloves
- Emergency Contacts:
 - PI Name
 - Office
 - Lab
 - Manager/Supervisor
- Accident:
 - Office
 - Lab
 - Manager/Supervisor

Biological Safety Cabinets



A detailed description of the BSCs and how they work is summarized in Appendix A of the BMBL.

Biological Safety Cabinets

Purpose:

- Product protection
- Personal protection
- Environmental protection



★ Must be used correctly; the BSC is not a fool proof device

Biosafety Level 2

Safety Equipment (Primary Barriers)

Use biosafety cabinets (class II) for work with biohazardous agents involving:

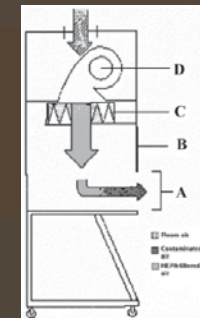
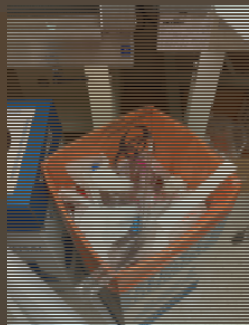
- Large volumes
- High concentrations
- Aerosols and splashes

How NOT to work

Vacuum flasks on the floor



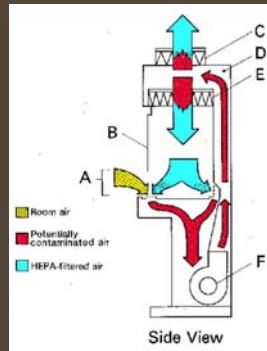
Open biohazard trash outside BSC



Biosafety Level 2

Safety Equipment (Primary Barriers)

- **Class II Biosafety Cabinet**
 - Airflow (75 - 100 ft/min)



Biological Safety Cabinets

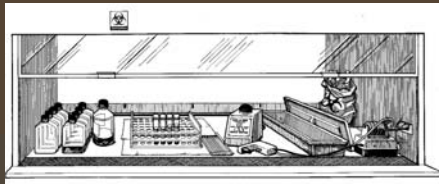
Operating Location

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

Biosafety Level 2

Safety Equipment (Primary Barriers)

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



- Only materials and equipment required for immediate use
- Perform operations at least 4 inches from inside edge of the front grille

Biological Safety Cabinets

Watch for disruptions of laminar air flow

× open flames have no place inside the BSC



BSC fans are NOT spark proof

× Chemical use may result in fire/explosion

× Never use NFPA 4 flammables

Biological Safety Cabinets

Safe Operation

- Must be certified annually, or anytime they are moved or repaired.
- UV light – not recommended

Centrifuges



Centrifuges

Hazards

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



Centrifuges

Operating Procedure

1. Inspect tubes for cracks/chips.
2. Use matched sets of tubes, buckets etc.
3. Don't overfill centrifuge tubes.
4. Tightly seal tubes and disinfect them.



**Use safety cups
or sealed rotors**



Centrifuges

Safe Operation

- Disinfect weekly and after spills
- Lubricate O-rings and rotor threads weekly
- Do not use rotors that have been dropped
- Contact your centrifuge rep for specific information

Decontamination

- critical to containment in the biosafety lab.



Sterilization vs disinfection

Decontamination

Definitions

Sterilization

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

Decontamination

Methods of sterilization

- Heat
 - Dry heat
 - Incineration
 - Moist heat - steam
- Chemical
- Radiation



Decontamination

- Dry heat sterilization
 - Effectively denatures proteins, but requires higher temperatures and more time: 160° - 170° C/2-4 hrs
 - Effective on impervious non-organic materials like glass

Decontamination

- Incineration
 - Method of choice for animal carcasses
 - Required certified incinerator
 - Reach a temperature of at least 850°C (1560°F)



Decontamination

Saturated steam + high pressure = steam sterilization

- Steam sterilization practices
 - Ensure proper functioning of autoclave
 - Temperature should reach 121°C (250°F)
 - Never cap or plug vessels
 - A small amount of liquid in the bag ensures heat transfer
 - Never put solvents, volatile or corrosive chemicals in an autoclave

Decontamination

• Verification

- Autoclave tape
- Chemical indicators
- Print out
- Biological indicators (Bacillus stearothermophilis)
– LOCATION!!!

- BL1: once/monthly
- BL2: 2X/month or every other week
- BL3: once/weekly



How NOT to dispose of autoclave trash

Biohazard sign needs to be defaced with autoclave tape; bag is placed in secondary trash bag after autoclaving then discarded

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.

Disinfection

Agent Selection

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

Chemical Disinfectants

Effectiveness...

- Specific for the organisms
- Amount of organic material present
- Type & concentration of germicide
- Contact time
 - Temperature, pH, humidity

Follow the manufacturer's guidelines.

Summary of Practical Disinfectants

Alcohols	Disrupts cell membranes, solubilize lipids and denatures proteins; all purpose disinfectant; not effective against bacterial spores, 70-80%
Phenols	Causes membrane damage; effective against vegetative bacteria, fungi, and lipid viruses; retains activity in the presence of organic material; Lysol, Pine-Sol, Amphy, Vesphene, 1-5%
Quaternary Ammonium Compounds	Disrupts cell membranes, denatures proteins; ineffective against <i>M. tb</i> , viruses, and spores; activity reduced in the presence of soaps, or soap residues.
Chlorine	Oxidizing action; broad spectrum, inexpensive, fast acting; loses potency; 10%
Iodophors	Penetrates cell wall, disrupts protein and nucleic acid structure and synthesis; broad spectrum; e.g. Wescodyne

Decontamination

Chemical

- General Lab Use – **Hypochlorite Solutions**
 - Spills/Large Organic Load
 - undiluted from bottle
 - General Surface Disinfection
 - 10.0% - 1:10 dilution
 - Should be made fresh daily



Biosafety

"Equipping a laboratory with the finest safety devices does not insure against all possible laboratory infections. Equipment is no substitute for safe technique..."

» Reitman and Wedum, 1956

"The most important element of containment is strict adherence to standard microbiological practices and techniques..."

» BMBL, 5th edition (p. 22)

"Just because you always did it that way, doesn't make it right."



Thank you for attending today

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