



## Biosafety in Microbiological and Biomedical Laboratories

3<sup>rd</sup> Edition  
JY Richmond & RW McKinney (eds.)

5<sup>th</sup> National Symposium on Biosafety:  
A Rational Basis for Biocontainment

1



## BMBL Introduction

### 1941 - Meyer and Eddie

- 74 lab associated brucellosis infections in US

### 1949 - Sulkin and Pike

- 222 viral infections (21 fatal)
- Only 27% related to known accidents

1



## BMBL Introduction

### 1951,1965, 1976 - Sulkin and Pike

Surveys for lab-associated infections

- More than 5,000 labs
- Cumulative total of 3,921 cases cited
- Most commonly reported:
  - ◆ Hepatitis
  - ◆ Brucellosis
  - ◆ Tuberculosis
  - ◆ Tularemia
  - ◆ Typhoid
  - ◆ Venezuelan Equine Encephalitis

2



## BMBL Introduction

### 1951,1965, 1976 - Sulkin and Pike (cont.)

Surveys for lab-associated infections

- Fewer than 20% associated with known accidents
- Exposure to infectious aerosols plausible (but unconfirmed) for >80% of reported cases

3



## Principles of Biosafety Introduction

### Biosafety Levels 1-3

Guidelines to describe combinations of:

- Laboratory Practices and Techniques
  - ◆ Standard Practices
  - ◆ Special Practices
- Safety Equipment (Primary Barriers)
- Laboratory Facilities (Secondary Barriers)

4



## Principles of Biosafety Introduction

### Biosafety Levels 1-3 Provide

- Increasing levels of personnel and environmental protection
- Guidelines for working safely in microbiological and biomedical laboratories

5



## Lab Practices and Techniques

Introduction

- Knowledgeable supervisor
- Personnel
  - ◆ Aware of potential hazards
  - ◆ Proficient in practices/techniques
- Biosafety manual specific to lab

6



## Safety Equipment

(Primary Barriers) - Introduction

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

7



## 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.

8



## Biosafety Level 1

Introduction

Examples:

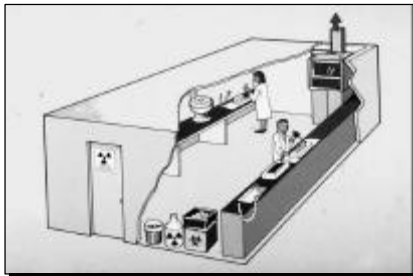
- *Bacillus subtilis*
- *Naegleria gruberi*
- Infectious canine hepatitis virus
- *E. coli*

9



## Biosafety Level 1

Laboratory Facilities (Secondary Barriers)



10



## Biosafety Level 1

Laboratory Facilities (Secondary Barriers)

- Sink for handwashing
- Work surfaces easily cleaned
- Bench tops
- Sturdy furniture
- Windows fitted with flyscreens

11



## Facility Design

(Secondary Barriers) - Introduction



Easily cleaned  
and  
decontaminated

12



## Facility Design

(Secondary Barriers) - Introduction

- Laboratory location
- Laboratory structure
- Laboratory ventilation

13



## Biosafety Level 1

Standard Microbiological Practices



Use  
mechanical  
pipetting  
devices

14



## Biosafety Level 1

Standard Microbiological Practices

- Use mechanical pipetting devices
- Wash hands
- Restrict or limit access when working
- Prohibit eating, drinking and smoking

15



## Biosafety Level 1

Standard Microbiological Practices (cont.)

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

16



## Biosafety Level 1

Safety Equipment (Primary Barriers)

Protective clothing

- Lab coat
- Gloves



17



### Biosafety Level 1

Safety Equipment (Primary Barriers)

Additionally, PPE may be needed

- Face protection
- Eye protection



18



### Biosafety Level 1

Special Practices

NONE

19



### Biosafety Level 1

Standard Microbiological Practices



Wash hands

20



### Biosafety Level 1

#### Supervision

- Scientist with general training in microbiology or related science

#### Lab Personnel

- Specific training in lab procedures

21



### Biosafety Level 2

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

22



### Biosafety Level 2

Immunization or antibiotic treatment is available

Examples:

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

23



## Biosafety Level 2

Extreme precaution with contaminated needles or sharp instruments

Examples:

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

24



## Biosafety Level 2

Laboratory Facilities (Secondary Barriers)



25



## Biosafety Level 2

### Standard Microbiological Practices

As in BSL-1

With emphasis on :

- Gloves
- Mechanical pipetting
- Attention to sharps

26



## Biosafety Level 2

Special Practices

### Needles & Sharps Precautions

**DON'T**

Break, bend, resheath or reuse syringes or needles

**DO**

Use sharps containers



27



## Biosafety Level 2

Special Practices (cont.)

### Needles & Sharps Precautions

So someone won't be injured later



28



## Biosafety Level 2

Special Practices (cont.)

### Needles & Sharps Precautions

**DON'T**

Touch broken glass with hands



29



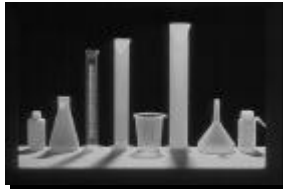
## Biosafety Level 2

Special Practices (cont.)

### Needles & Sharps Precautions

#### DO

Use plasticware



30



## Biosafety Level 2

Special Practices (cont.)



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

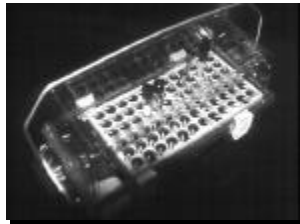
31



## Biosafety Level 2

Special Practices (cont.)

- Use leak-proof transport containers



32



## Biosafety Level 2

Special Practices (cont.)

- Immunizations
- Baseline serum samples



33



## Biosafety Level 2

Special Practices (cont.)

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

34



## Biosafety Level 2

Safety Equipment (Primary Barriers)

### BSL-1 PLUS:

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

- Aerosols and splashes
- Large volumes
- High concentrations

35

**Biosafety Level 2**  
Safety Equipment (Primary Barriers)

**Class II Biosafety Cabinets**

- Airflow

Side View

36

**Biosafety Level 2**  
Safety Equipment (Primary Barriers)

**Class II Biosafety Cabinets**

- Layout of equipment

37

**Biosafety Level 2**  
Safety Equipment (Primary Barriers)

**Class II Biosafety Cabinets**

- Technique

38

**Biosafety Level 2**  
Laboratory Facilities (Secondary Barriers)

**BSL-1 Facilities PLUS:**

- Autoclave available
- Eyewash station available

39

**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

40

**Biosafety Level 3**

Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.

41



### Biosafety Level 3

- Exposure potential to pathogens spread by aerosol
- Infection serious, possibly lethal
- Examples:
  - ◆ *M. tuberculosis*
  - ◆ St. Louis encephalitis virus
  - ◆ *Coxiella burnetii*

42



### Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

#### BSL-1 and 2 Facilities PLUS:

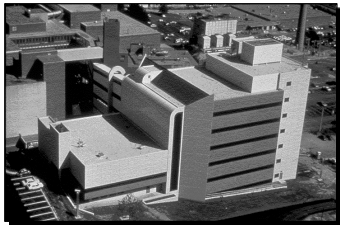
- Separate building or isolated zone
- Double door entry
- Directional inward airflow
- Single-pass air

43



### Facility Design

(Tertiary Barriers)



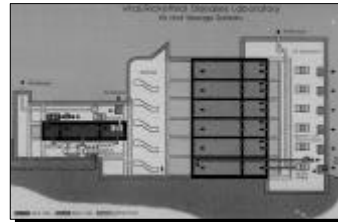
Location of CDC's MCL

44



### Facility Design

(Tertiary Barriers)



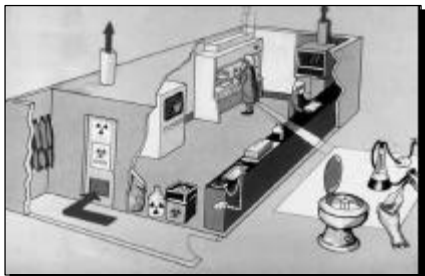
Lab structure  
Lab ventilation

45



### Biosafety Level 3

Laboratory Facilities (Secondary Barriers)



46



### Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

#### BSL-1 and 2 Facilities PLUS (cont.):

- Enclosures for aerosol generating equipment
- Room penetrations sealed
- Walls, floors and ceilings are water resistant for easy cleaning

47




**Biosafety Level 3**  
Special Practices

---

**BSL-2 Special Practices PLUS:**

- Work in certified BSC
- Use bioaerosol-containing equipment
- Decontaminate spills promptly



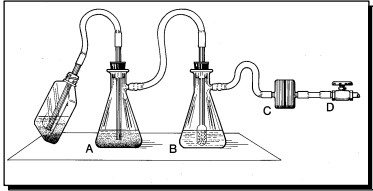
48

**Biosafety Level 3**  
Laboratory Facilities (Secondary Barriers)

---

**BSL-1 and 2 Facilities PLUS:**

- Vacuum lines protected



49

**Biosafety Level 3**

---

**Standard Microbiological Practices**  
As in BSL-1 and -2

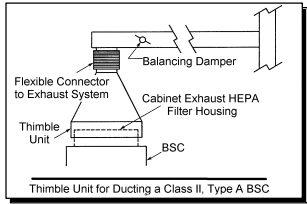
50

**Biosafety Level 3**  
Safety Equipment (Primary Barriers)

---

**BSL-1 and 2 Safety Equipment PLUS:**

- BSC class II or III to manipulate infectious material




51

**Biosafety Level 3**  
Safety Equipment (Primary Barriers)

---

**BSL-1 and 2 Safety Equipment PLUS:**

- Respiratory protection may be indicated



52

**Biosafety Level 3**  
Special Practices

---

**Supervision**

- Supervisor is a competent scientist experienced working with agents
  - ◆ Establishes criteria for entry
  - ◆ Restricts access
  - ◆ Develops policies/procedures
  - ◆ Trains lab personnel

53



## Biosafety Level 3

### Special Practices

---

#### Lab Personnel

- Strictly follow guidelines
- Demonstrate proficiency
- Receive appropriate training
- Report incidents
- Participate in medical surveillance

[ 54 ]



## Principles of Biosafety

### Summary

---

#### BSL 1-3

- Standard Practices
- Special Practices
- Safety Equipment (Primary Barriers)
- Laboratory Facilities (Secondary Barriers)
- Building (Tertiary Barriers)

[ 55 ]