International BIOLOGICAL THREAT REDUCTION

BIOSECURITY ENGAGEMENT PROGRAM

# Components of Laboratory Biosafety – Part I

***Training Seminar on Laboratory Biosecurity and Biosafety***

**Manila, Philippines**

**13 July 2006**

**And**

**Cebu, Philippines**

**18 July 2006**

**www.biosecurity.sandia.gov**

SAND No. 2005-3346C, 2004-3689C, & 2006-3688C
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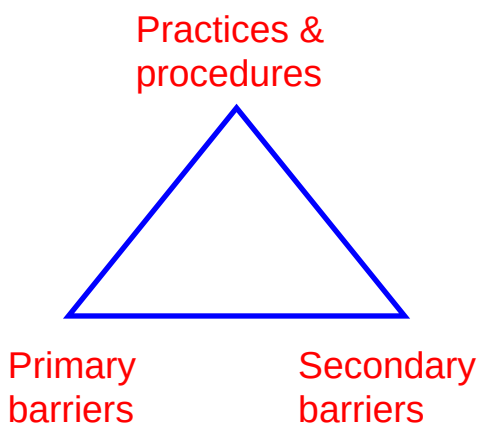
# Biocontainment

- Biocontainment is the control of biohazards through
  - Practices & procedures, including administrative controls
    - Good lab practices
    - Written SOPs for research activities, specialized equipment, etc
    - Required training
    - Access requirements
  - Primary barriers (safety equipment)
    - Biosafety cabinets (BSCs)
    - Lab equipment (pipetting devices, waste containers, safety centrifuge cups)
    - Personal protective equipment
  - Secondary barriers (engineering & architectural controls)
    - Building & room construction – the floor plan
    - HVAC issues – directional airflow, filtration
    - Waste treatment

# Biosafety Levels

- **Biosafety Levels**
  - **Four biosafety levels provide increasing degrees of protection**
  - **What's the right balance of practices & procedures, primary barriers and secondary barriers?**

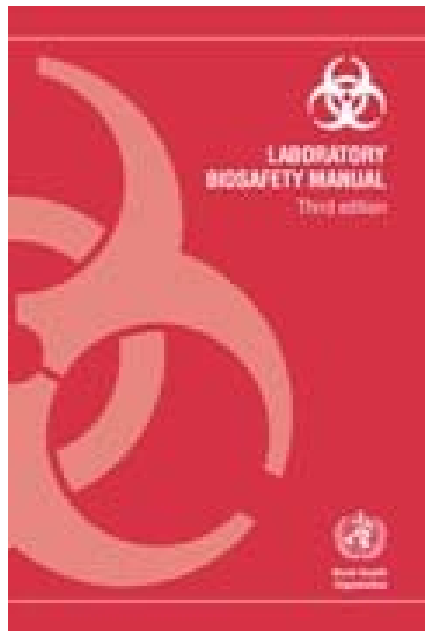
- **Applications:**
  - **Labs (BSL-1, 2, 3, 4)**
  - **(Small) Animal Containment (ABSL-1, 2, 3, 4)**
  - **Large Animal Containment ("BSL-3 AG")**
  - **Plant Containment (BSL-1P, 2P, 3P, 4P)**



# Guidance and Regulations

- **Performance-based vs. prescriptive**
  - **A performance approach generally defines what result is intended, leaving how to achieve the result up to the user.**
  - **Prescriptive approaches outline specific requirements that must be done.**

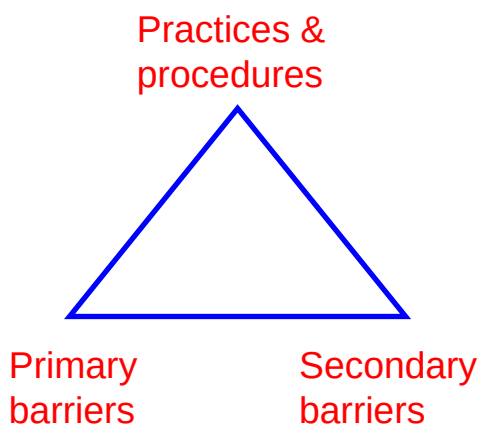
- **WHO Laboratory Biosafety Manual – 3rd edition**




  - **See CD for WHO LBM**

# Planning: Risk Assessment is Critical

- **Identify biological agents that will be used**
- **Perform risk assessment**
  - **Include an evaluation of settings**
    - **Labs**
    - **Animal housing**
    - **Patient care**
    - **Autopsy/Necropsy**
    - **Other?**
  - **And activities:**
    - **At the macro scale: research, diagnostics, health care, industry, other?**
    - **At the micro scale: routine manipulations, producing large volumes, aerosolization, animal testing, other?**
- **Determine biosafety level**
  - **What degree of biocontainment is necessary to mitigate the risk?**
    - **What is the appropriate balance of practices & procedures, primary barriers, and secondary barriers?**



# Components of Laboratory Biosafety

Engineering Controls

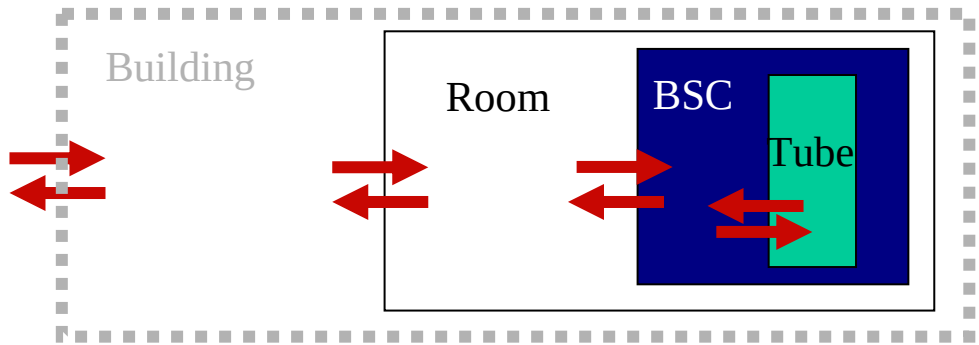
Work Practices

PPE

# Biosafety: Engineering Controls

- **Primary barriers – contain the agent at the source**
  - **Biological safety cabinet**
  - **Animal caging**
  - **Specialized lab equipment (centrifuges, fermenters, etc.)**

- **Secondary barriers – contain the agent within the room or facility *in case an agent escapes from the primary barriers***
  - **Building & Room Construction**
  - **HVAC Issues:**
    - **Directional airflow**
    - **Exhaust filtration**
  - **Other Engineering Controls:**
    - **Solid waste treatment**
    - **Wastewater treatment**



# Biosafety Levels 1 and 2

- **Biosafety Level 1**
  - **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.**
  - **Examples:**
    - ***Bacillus subtilis***
    - ***Naegleria gruberi***
    - **Infectious canine hepatitis virus**
    - ***E. coli***
- **Biosafety Level 2**
  - **Suitable for work involving agents of moderate potential hazard to personnel and the environment**
  - **Examples:**
    - **Measles virus**
    - **Salmonellae**
    - **Toxoplasma species**
    - **Hepatitis B virus**

# Biosafety Levels 3 and 4

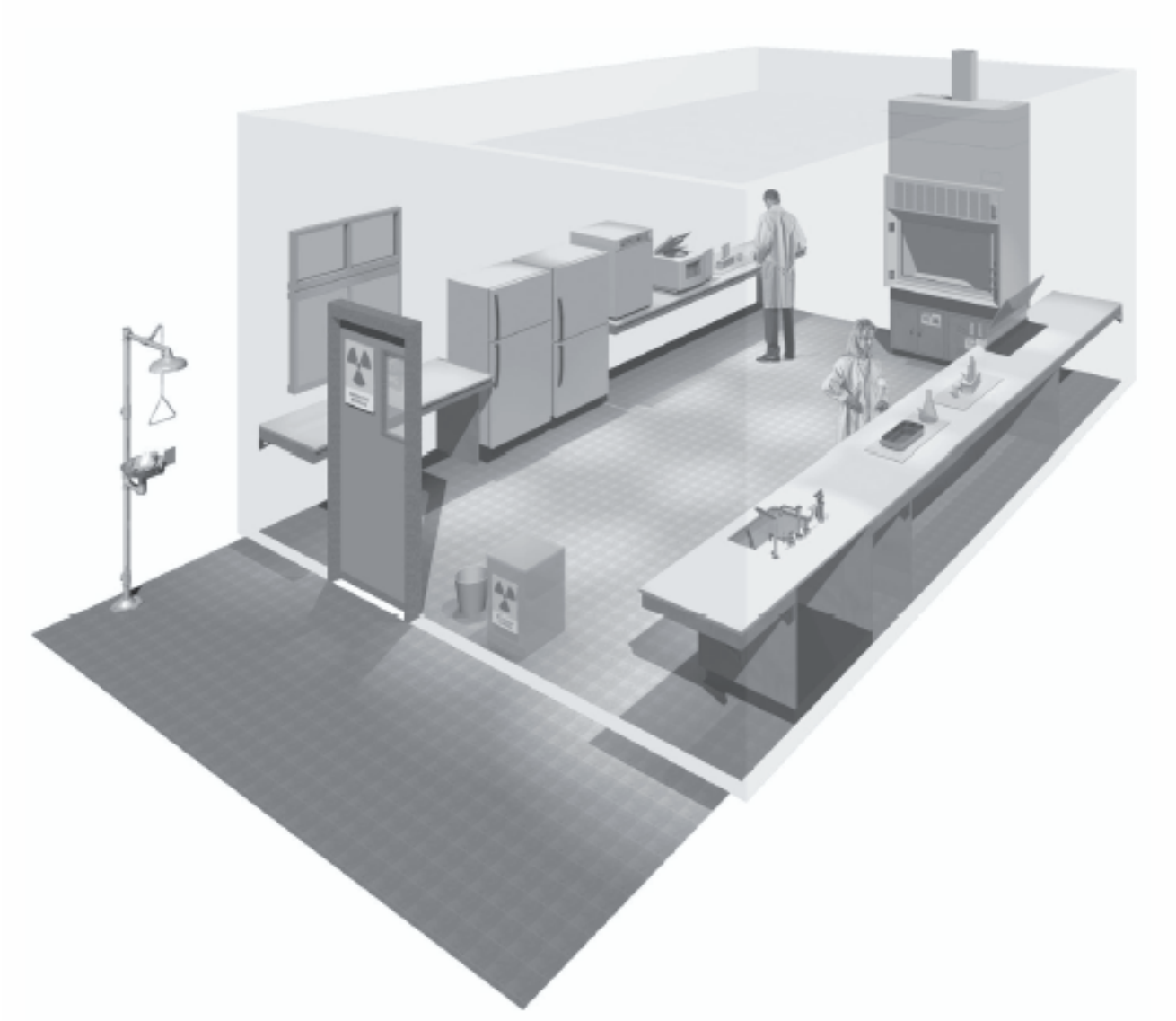
- **Biosafety Level 3**
  - **Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.**
  - **Examples:**
    - ***Mycobacterium tuberculosis***
    - **St. Louis encephalitis virus**
    - ***Coxiella burnetii***
- **Biosafety Level 4**
  - **Suitable for work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life-threatening disease.**
  - **Examples:**
    - **Ebola Zaire virus**
    - **Rift Valley Fever virus**

# Biosafety Level 1: Facility Design

- **Requirements:**
  - **Laboratories have doors**
  - **Sink for hand washing**
  - **Work surfaces easily cleaned**
  - **Bench tops are impervious to water**
  - **Sturdy furniture**
  - **Windows fitted with flyscreens**
- **Other design and construction issues:**
  - **Location – not separated**
  - **Structure – normal construction**
  - **Ventilation – no special requirements**

Easily cleaned and decontaminated

# Biosafety Level 1: Facility Design

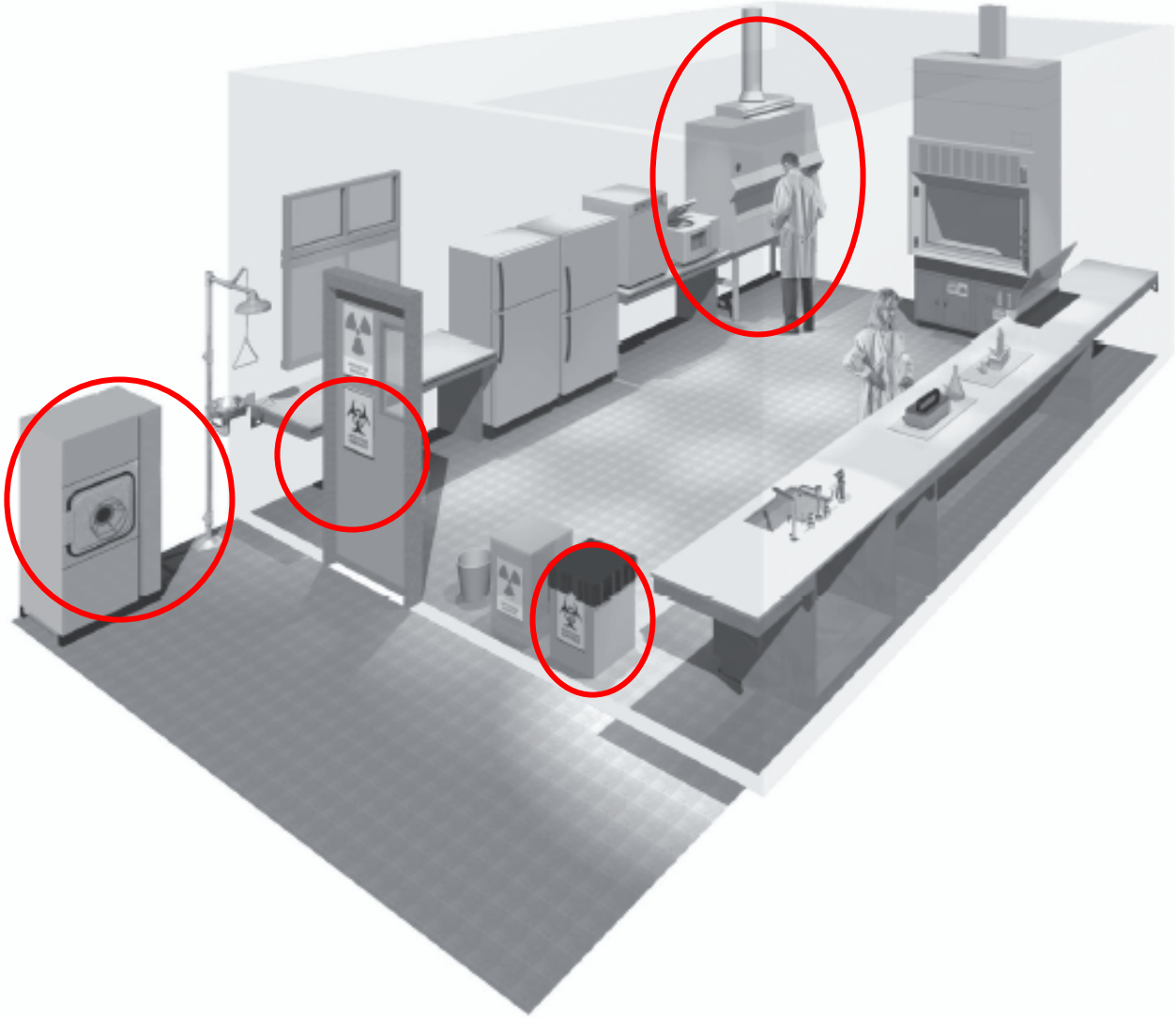


# Biosafety Level 2: Facility Design

- **Requirements:**
  - **Laboratories have lockable doors**
  - **Sink for hand washing**
  - **Work surfaces easily cleaned**
  - **Bench tops are impervious to water**
  - **Sturdy furniture**
  - **Biological safety cabinets installed as needed**
  - **Adequate illumination**
  - **Eyewash readily available**
  - **Windows fitted with flyscreens**
  - **Location - separated from public areas**
  - **Ventilation – directional**
    - **Air flows into lab without re-circulation to non-lab areas**

Restricted access
when work in progress

# Biosafety Level 2: Facility Design

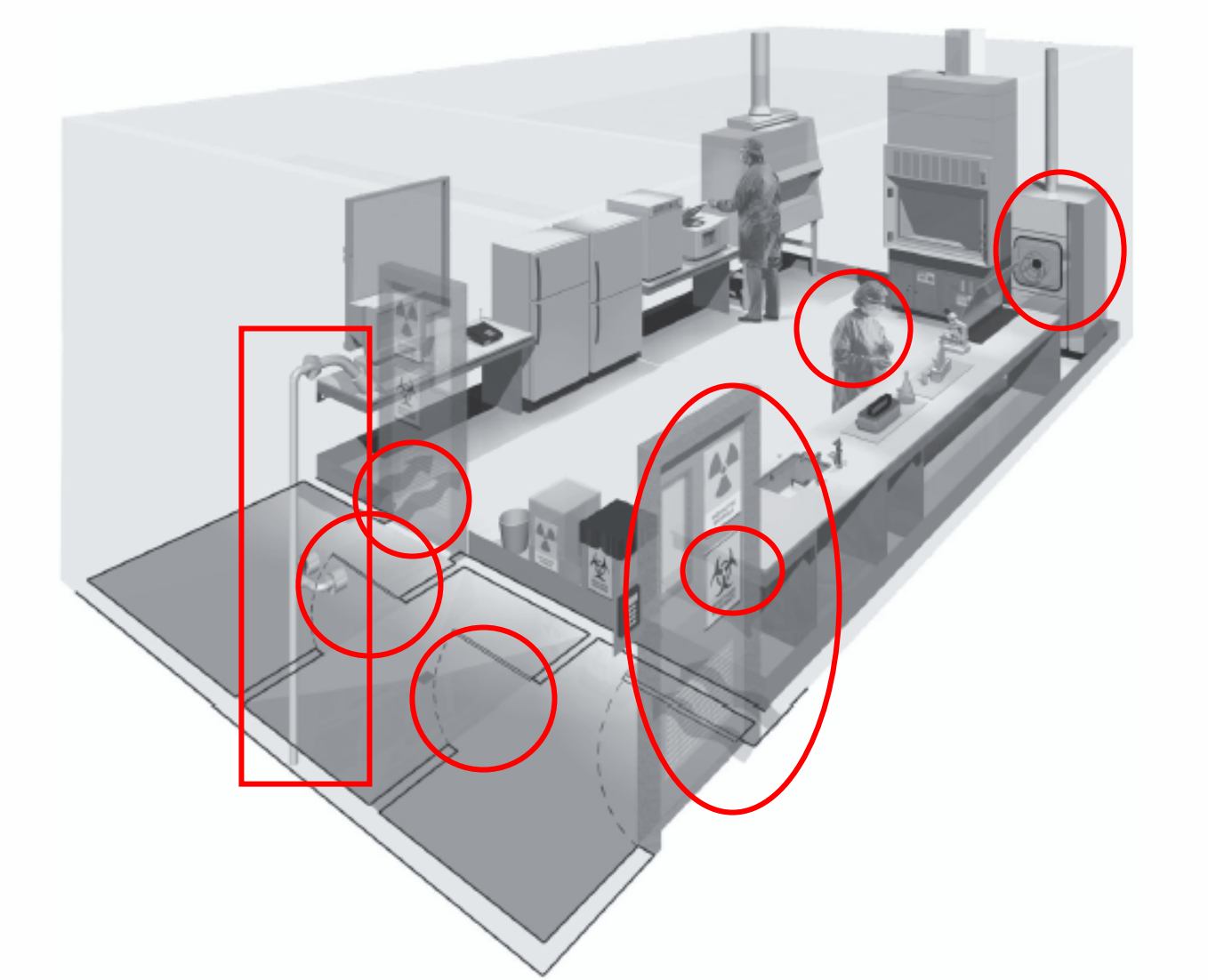


# Biosafety Level 3: Facility Design

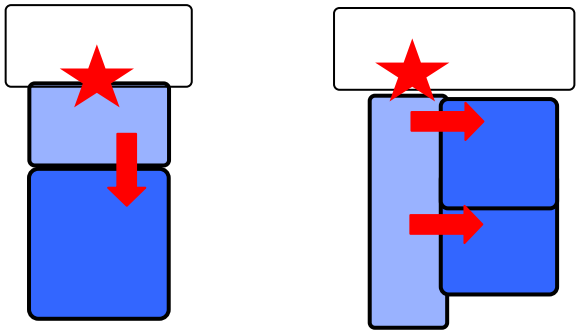
- **Requirements:**
  - **BSL-1 and 2 Facilities PLUS**
    - **Enclosures for aerosol generating equipment**
    - **Room penetrations sealed**
    - **Walls, floors and ceilings are water resistant for easy cleaning**
    - **BSC class II or III to manipulate infectious material**
    - **Separate building or isolated zone within a building**
    - **Directional inward airflow**
    - **Single-pass air; can be recirculated if HEPA filtered**
    - **Double door entry**
    - **BSCs mandatory**
- **Additional requirements depending on work and agents:**
  - **HEPA filtration of the exhaust**
  - **Effluent decontamination**
  - **Personnel showers**

*Follow the guidelines, regulations, and site-specific risk assessment for your unique situation!*

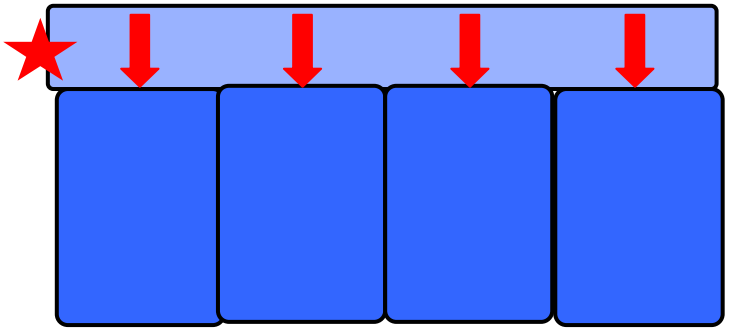
# Biosafety Level 3: Facility Design



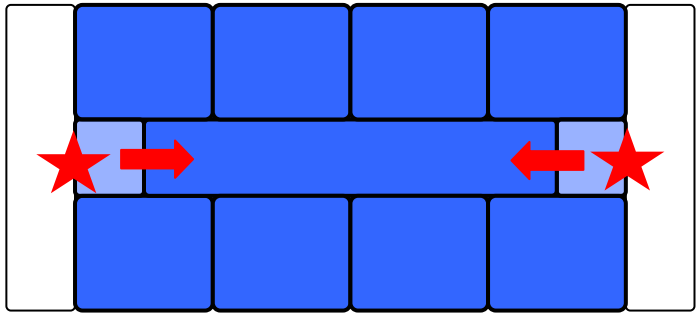
# Options for Double Door Entry



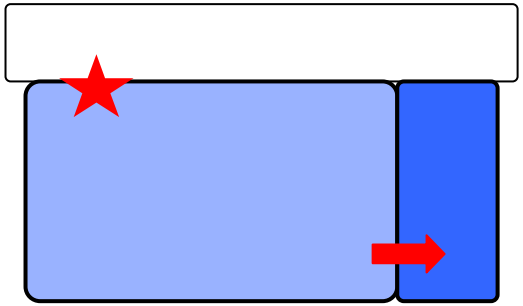
ROOM AS ACCESS ZONE



CORRIDOR AS ACCESS ZONE



SUITE CONCEPT



LAB AS ACCESS ZONE

CORRIDOR ACCESS ZONE BSL3

ENTRY DOOR 1

ENTRY DOOR 2

# *Biological Safety Cabinets (BSC)*

# Biological Safety Cabinets (BSCs)

- Primary means of containment
- Designed to provide protection for
  - Personnel
  - Product
  - Environment
- Three design types
  - Class I, Class II, and Class III

# Primary Barriers – Ventilation Equipment

| | Personnel | Product | Environment |
|---|---|---|---|
| Chemical Fume Hoods | X | | |
| Laminar Flow Clean Benches | | X | |
| Class I Biological Safety Cabinet | X | | X |
| Class II Biological Safety Cabinet | X | X | X |
| Class III Biological Safety Cabinet | X | X | X |
| Isolators | X | X | X |

# HEPA Filters

- High Efficiency Particulate Air
- Components:
  - Filter Media
    - Folded cellulose/borosilicate media
    - Very large surface area
  - Sealants
    - epoxy, polyurethane
  - Frames
    - metal
    - wood
  - Edge Sealant
    - gasket with clamp
    - gel seal with knife edge
- Minimum efficiency of 99.97% removal of 0.3 micron particles

- HEPA filters do not filter out gases and vapors, they only filter out particulates

# HEPA

# Testing and Certification

- Field test (certification) is required
  - Prior to service
  - After repairs or relocation
  - Annually
- Certification reports kept in the equipment books

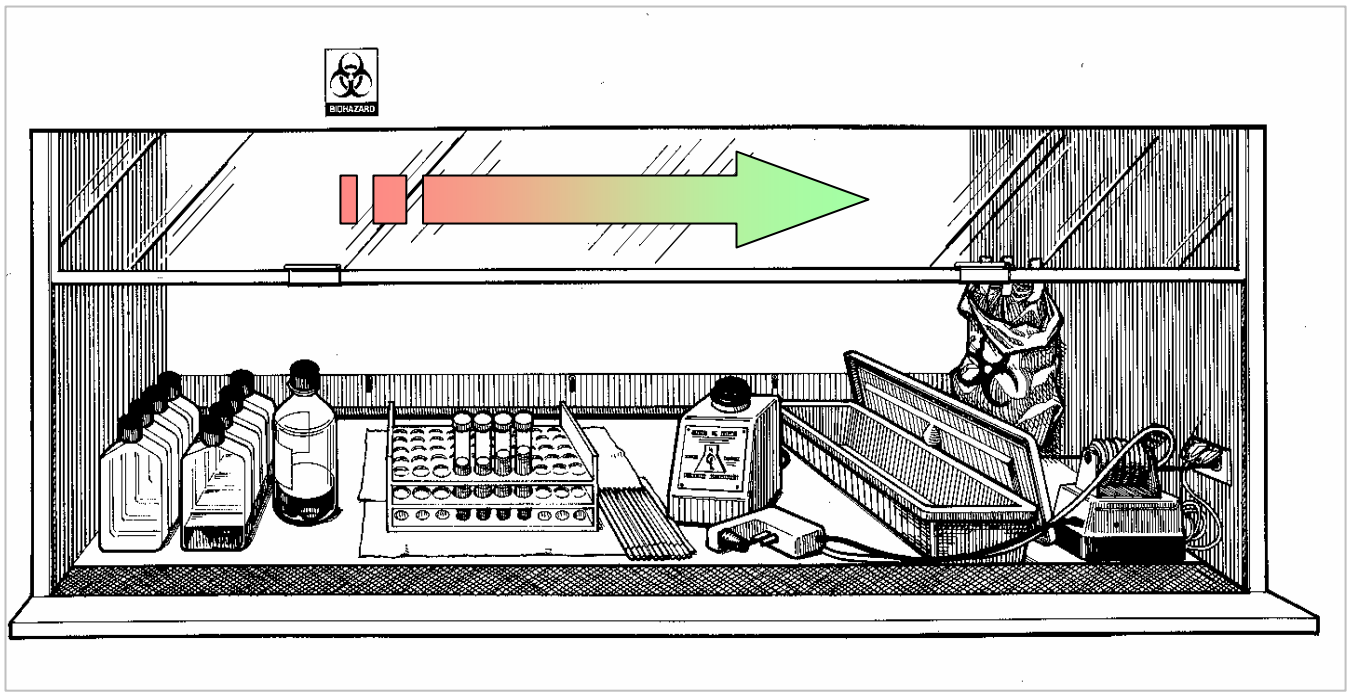
# Biosafety Cabinet Technique

- **Proper technique is critical to maintaining personnel and product protection!**

- **Elements**
  - **Decontamination**
  - **Setup**
  - **Work flow**
  - **Locations of supplies and waste containers**
  - **Movement in and out**
  - **Others**

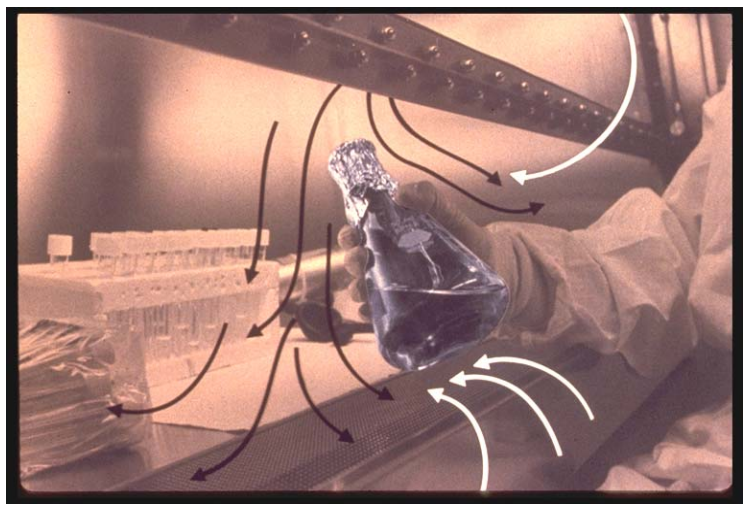
# Operation in a Class II BSC

- Class II BSC are designed to capture horizontally nebulized spores in the downward flowing air
- Workflow from clean to contaminated ("dirty")



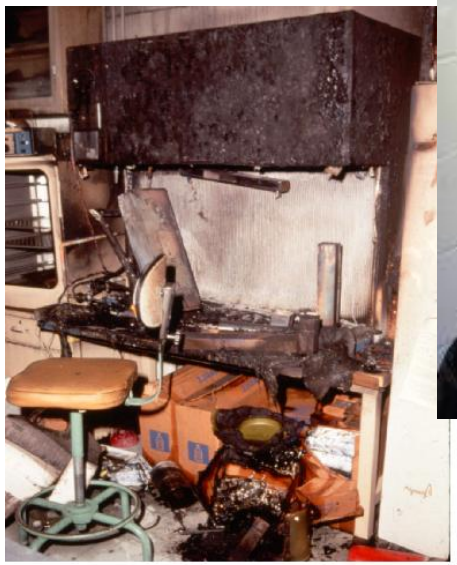
# Operation in a Class II BSC

- After setup, allow a minimum of 2 minutes for air flow to stabilize
- Place hands in BSC and wait 30 seconds before beginning work
- Refrain from removing hands from BSC during operation so as not to disturb air-curtain
- Immediately clean up small spills
- Limit foot traffic near the BSC while it is in use



# BSC Pitfalls

- Overloading
- Blocking airflow elements
- BSC location in lab
- Bunsen Burners
- UV Lamps….



- See CD for information pertaining to UV lamps

**Any comments?**

# Working in a Class III BSC

- **Advantages**
  - For work with emerging diseases, prions, and agents not found in nature
  - Can be used to separate PCR reagent and sample preparation steps to prevent contamination and augment QC
  - More forgiving of operator error
  - Closed system decreases chance of aerosol escape, accidental exposure and potential laboratory acquired illnesses
  - Chain of custody using Rapid Transfer Port containers
  - Provides superior operator protection in areas with limited reliable infrastructure

- **Disadvantages**
  - More limited range of motion than a Class II BSC.
  - Not as easy to move/remove large pieces of equipment (must remove front panel)
  - Must be able to purchase replacement gloves or bag-out supplies used in removing samples
  - Operations may take a little more time to conduct
  - Many operators are not familiar with the technology

# Examples of Class III BSCs

- **Small Class III BSC for diagnostic samples with ergonomic improvements and rapid transfer port entry**

- **Class III BSC with pass-through chamber on right and bag-out for samples on the left**

# Summary of Biosafety Level Requirements

*Table 3.* ***Summary of biosafety level requirements***

| | BIOSAFETY LEVEL | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| Isolation[a] of laboratory | No | No | Yes | Yes |
| Room sealable for decontamination | No | No | Yes | Yes |
| Ventilation: | | | | |
| — inward airflow | No | Desirable | Yes | Yes |
| — controlled ventilating system | No | Desirable | Yes | Yes |
| — HEPA-filtered air exhaust | No | No | Yes/No[b] | Yes |
| Double-door entry | No | No | Yes | Yes |
| Airlock | No | No | No | Yes |
| Airlock with shower | No | No | No | Yes |
| Anteroom | No | No | Yes | — |
| Anteroom with shower | No | No | Yes/No[c] | No |
| Effluent treatment | No | No | Yes/No[c] | Yes |
| Autoclave: | | | | |
| — on site | No | Desirable | Yes | Yes |
| — in laboratory room | No | No | Desirable | Yes |
| — double-ended | No | No | Desirable | Yes |
| Biological safety cabinets | No | Desirable | Yes | Yes |
| Personnel safety monitoring capability[d] | No | No | Desirable | Yes |

[a] Environmental and functional isolation from general traffic.
[b] Dependent on location of exhaust (see Chapter 4).
[c] Dependent on agent(s) used in the laboratory.
[d] For example, window, closed-circuit television, two-way communication.

From: WHO LBM 3rd edition