International
BIOLOGICAL THREAT REDUCTION

BIOSECURITY ENGAGEMENT PROGRAM

Biosafety Cabinets

Laboratory Biosecurity and Biosafety

for BSL3 Laboratories

Bogor, Indonesia

21 June 2006

www.biosecurity.sandia.gov

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company,
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under contract DE-AC04-94AL85000.

Sandia National Laboratories

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

Testing and Certification

- National Sanitation Foundation (NSF) International Standard/American National Standard (Sept 29, 2004)
- Standard No. 49 - 2004
  - Performance Criteria
  - Minimum Requirements for Design, Manufacture, and Testing
  - NSF 49 Seal
- Testing is done by the manufacturer prior to shipping of cabinet

Testing and Certification

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

Testing and Certification

- **Field test (certification) performance criteria:**
  - **Down-flow air velocity and volume**
  - **In-flow air velocity**
  - **Air flow smoke pattern**
  - **HEPA filter leak test**
  - **Cabinet leak test**

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

How does a HEPA filter work?

- Inertial Impaction
- Interception
- Diffusion
- Electrostatic forces

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

Inertial Impaction

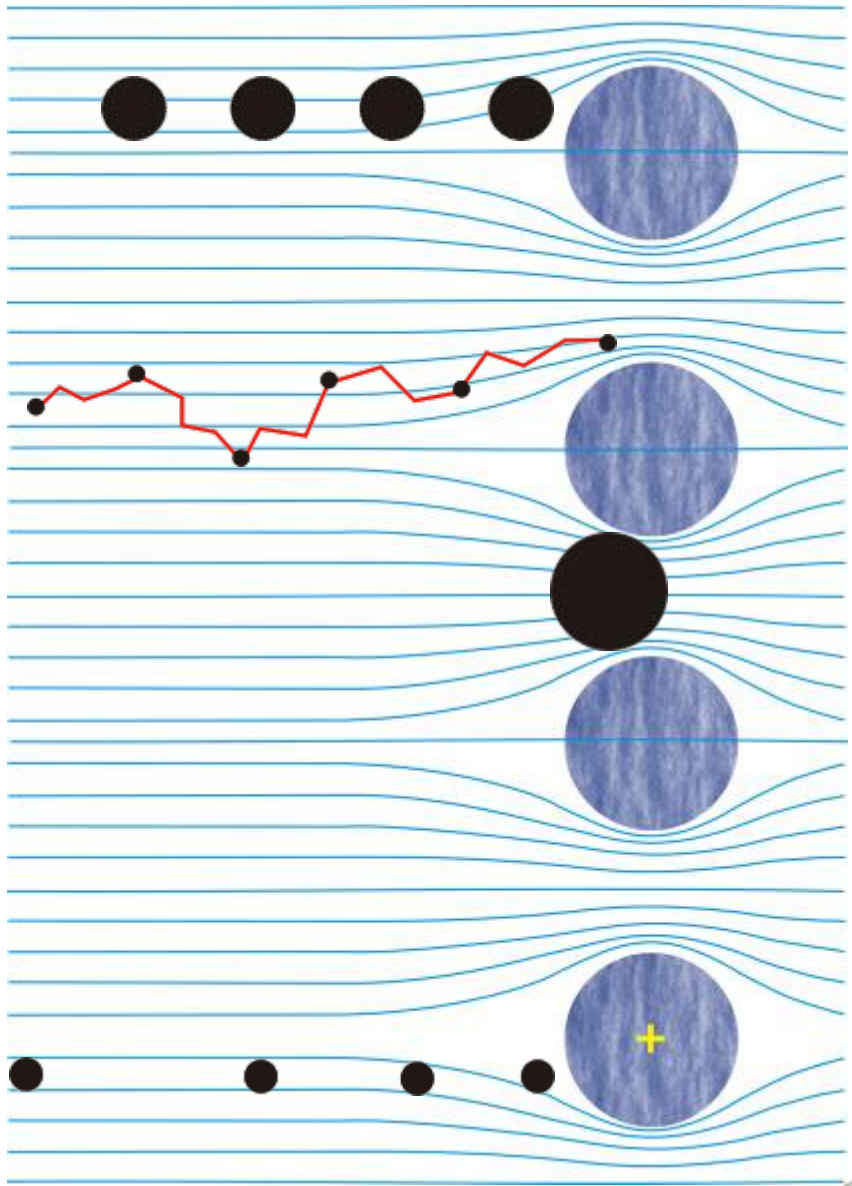
Diffusion

(Brownian motion)

Interception

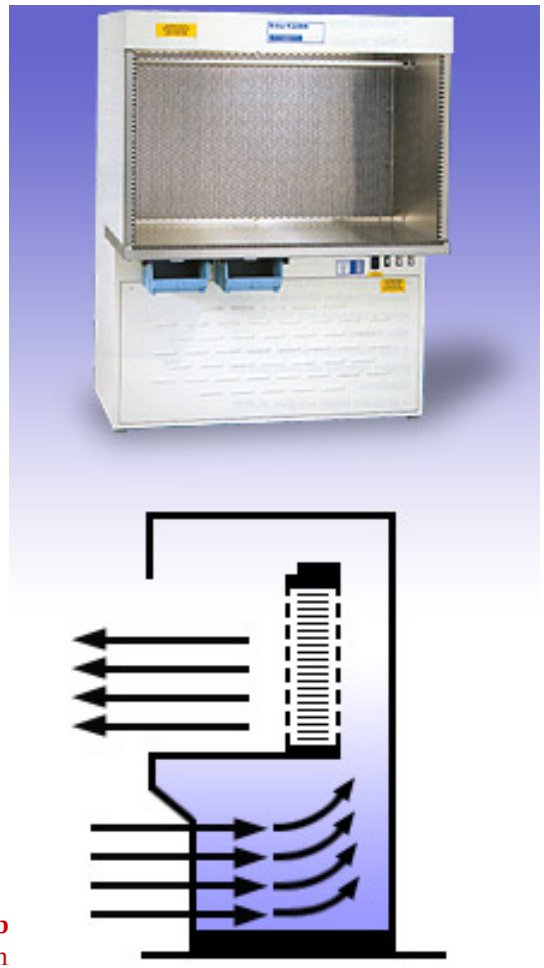
(straining)

Electrostatic



Any comments?

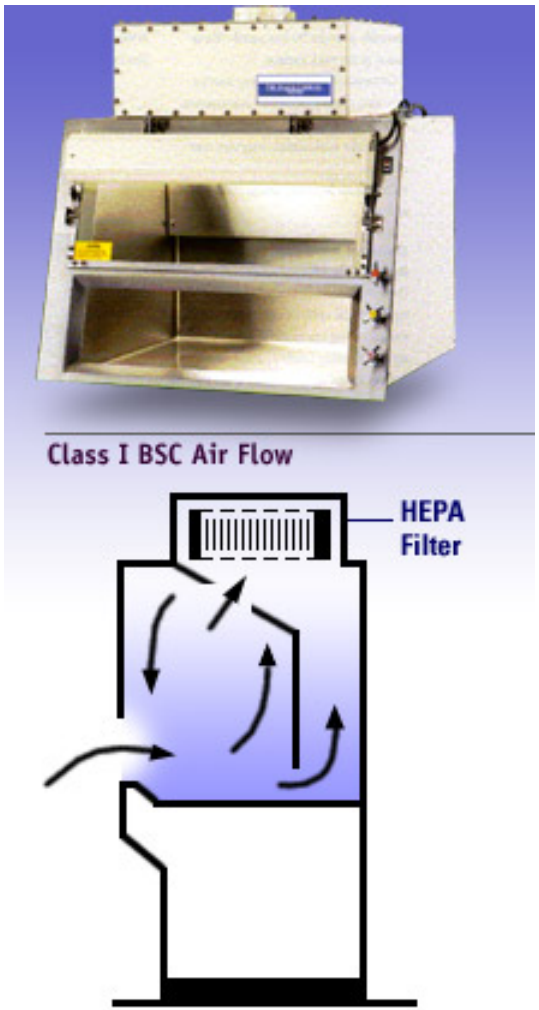
Clean Bench



- Provide product protection only
- Product protection is provided by creating a unidirectional airflow generated through a HEPA filter
- Discharge air goes directly into workroom
- Applications
  - Any application where the product is not hazardous but must be kept contaminant free
  - Preparation of non-hazardous intravenous mixtures and media
  - Particulate free assembly of sterile equipment and electronic devices

Source:
Biological Safety Cab
"A Web-based Train Program"
Eagleson Institute
www.eagleson.org

Class I Cabinet



- 75 fpm face velocity
- Provides personnel and environmental protection
- No product protection
- Requires an exhaust blower to pull the air through - usually to the outdoors
- Applications
  - Housing centrifuges, fermenters
  - Cage dumping in an animal lab
  - Aerating cultures

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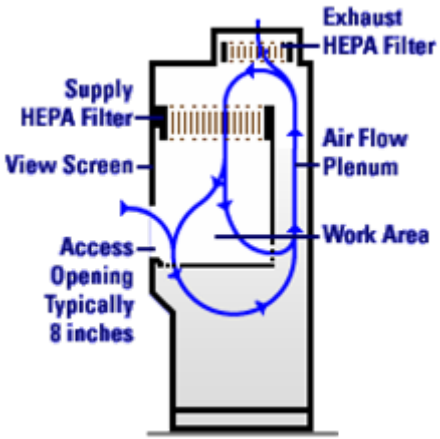
Class II Cabinets



- Ventilated cabinet
- Provides personnel, product, and environmental protection
- Open front with inward airflow for personnel protection
- Downward HEPA filtered laminar airflow for product protection
- HEPA filtered exhaust air for environmental protection

Source:
Biological Safety Cabinets
"A Web-based Training Program"
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Class II A1 Cabinet

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Biological Safety Cabinets
"A Web-based Training Program"
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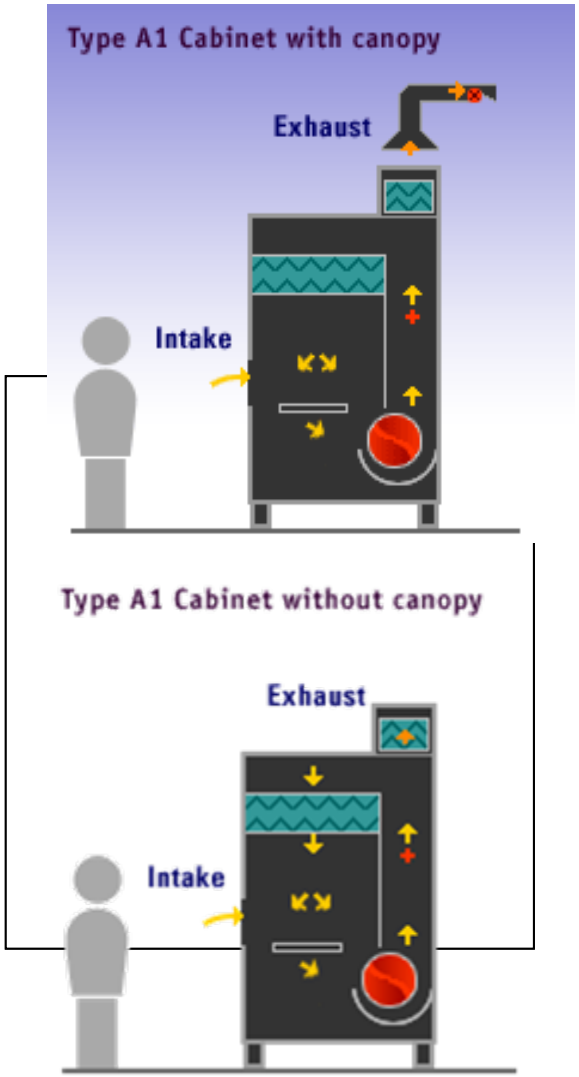
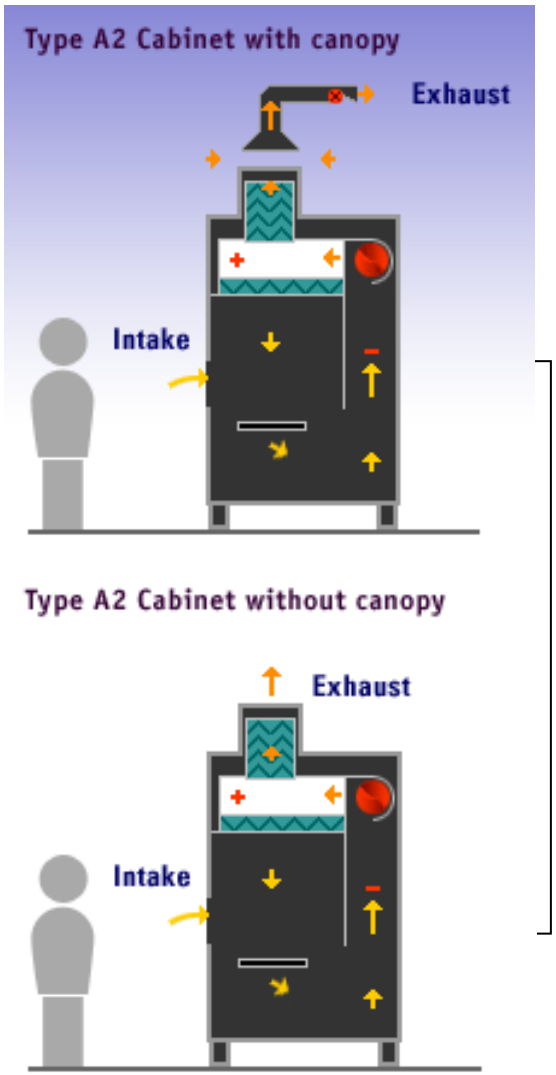


- **75 fpm face velocity**
- **70% recirculated air, 30% exhausted (thru HEPA)**
- **Exhaust to room or thimble connected to external exhaust duct**
- **Potentially contaminated ducts and plenums under positive pressure to the room**
- **Not suitable for work with volatile toxic chemical and volatile radionuclides**

Class II A2 Cabinet



- 100 fpm face velocity
- 70% recirculated air, 30% exhausted (thru HEPA)
- Exhaust to room or thimble connected to external exhaust duct
- Potentially contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums
- May be used for work with <u>minute</u> quantities of volatile toxic chemicals and tracer amounts of radionuclides if they are exhausted through properly functioning exhaust canopies

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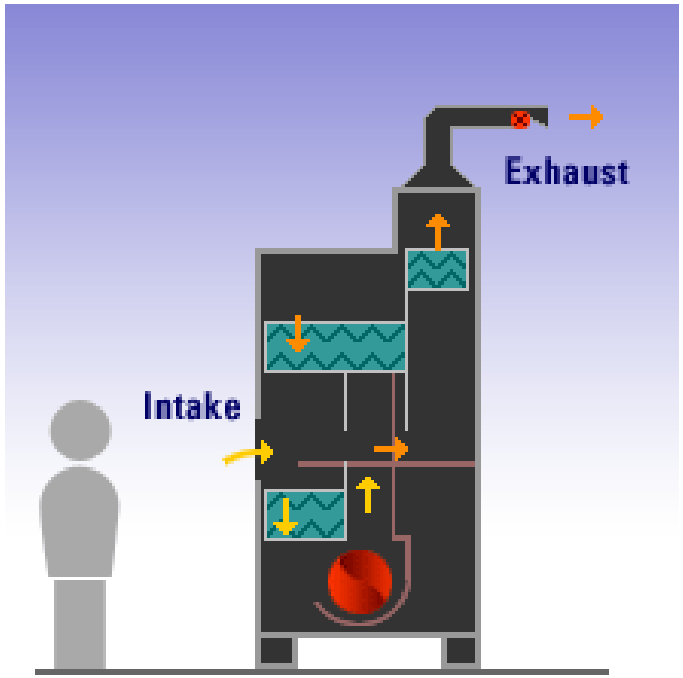
Class II B1 Cabinet



- 100 fpm face velocity
- 30% recirculated air, 70% exhausted (thru HEPA)
- Air in the back of the cabinet is exhausted to the outdoors through a dedicated exhaust plenum and the air in the front is recirculated
- Must be hard ducted to the outside for the cabinet to function
- All biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums
- Minute quantities of volatile toxic chemicals and tracer amounts of radionuclide permitted if work is done in the direct exhausted portion of the cabinet

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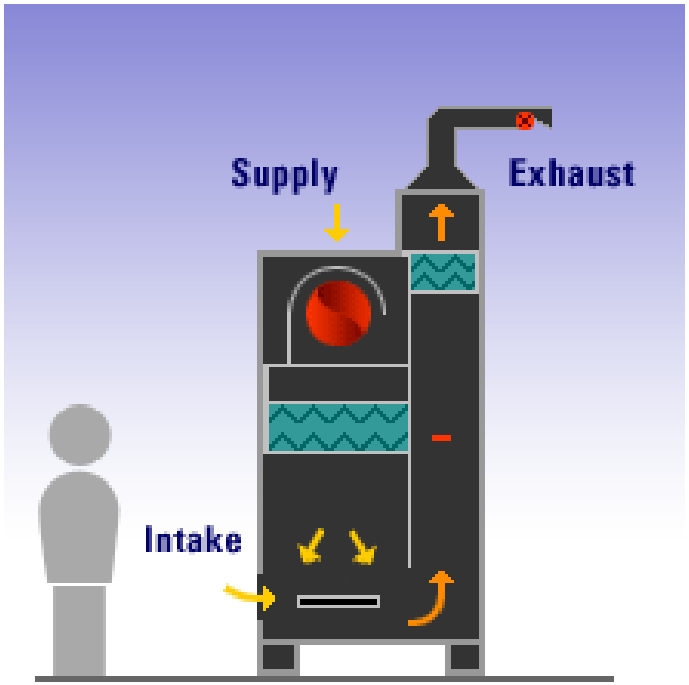
Class II B2 Cabinet



- 100 fpm face velocity
- Exhaust 100% of the air to the outside after filtration through a HEPA filter
- Must be hard ducted to the outside
- Sometimes called "Total Exhaust"
- All contaminated ducts and plenums under negative pressure, or surrounded by (directly exhausted non-recirculated through the work area) negative pressure ducts and plenums
- May be used for work with volatile toxic chemicals and radionuclides required as an adjunct to microbiological studies

Source:
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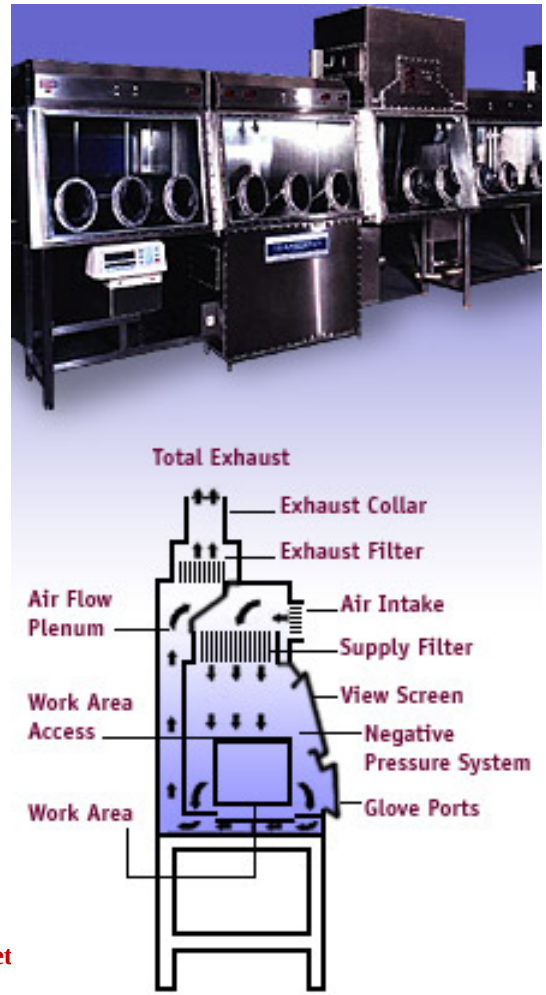
Class II (Laminar Flow) Biosafety Cabinetry NSF/ANS 49 - 2002

NSF International Standard 49-1992	NSF International Standard 49-2002
Class II Type A	Class II Type A1
Class II Type A/B3	Class II Type A2
Class II Type B1	Class II Type B1
Class II Type B2	Class II Type B2

Class III Cabinet



- Gas-tight absolute containment enclosure
- Product, personnel and environmental protection
- Minimum air intake velocity through a glove port is 100 fpm, if the glove is cut or removed
- Air must be exhausted to the outdoors
- Integral equipment allows the safe introduction and removal of materials
- Exhaust air is treated by double HEPA filtration or HEPA filtration and incineration
- Supply air is HEPA filtered
- Operation in cabinet performed through rubber gloves
- Operates under negative pressure of at least 0.5" w.g. (124 Pa)

Source:
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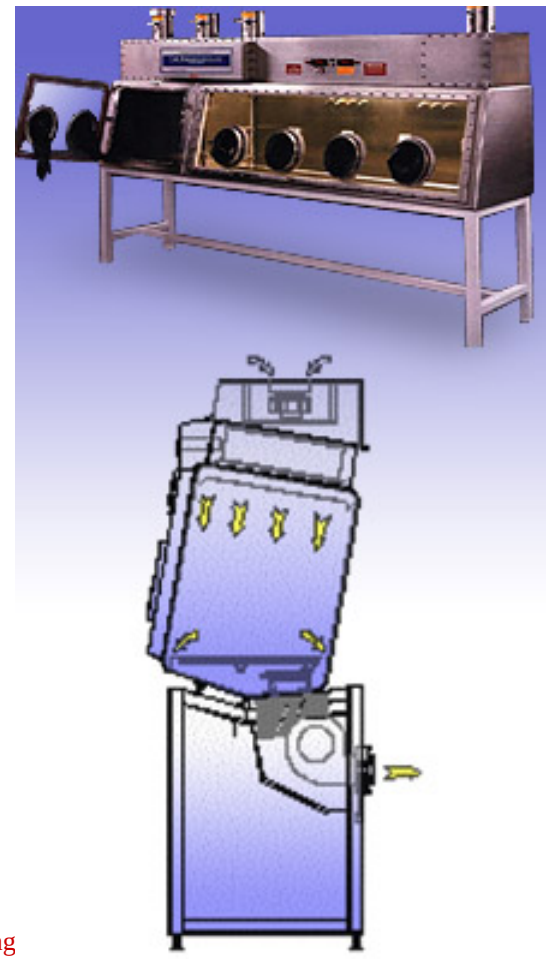
"A Web-based Training Program"
Eagleson Institute
www.eagleson.org

Class III cabinets & isolators

National Institute of Infectious Diseases, Japan

Isolators



- Designed to produce products in a clean environment
- Air is HEPA filtered as it goes into and out of the unit
- Isolators provide protection specific to each design
- Not for use in BSL-3 or BSL-4 labs
- Applications
  - Pharmaceutical quality control testing
  - Super clean manufacturing without creating clean room
  - Pharmaceutical manufacturing of potent compounds

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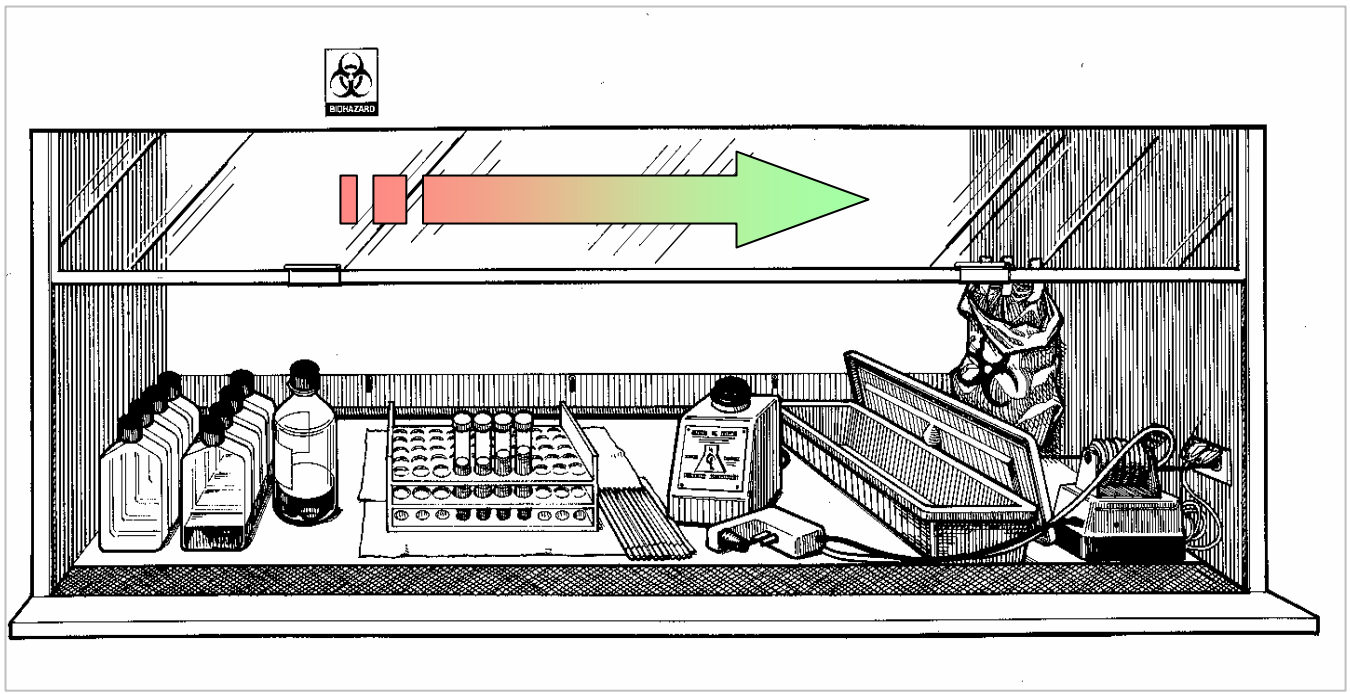
Biosafety Cabinet Technique

- **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**

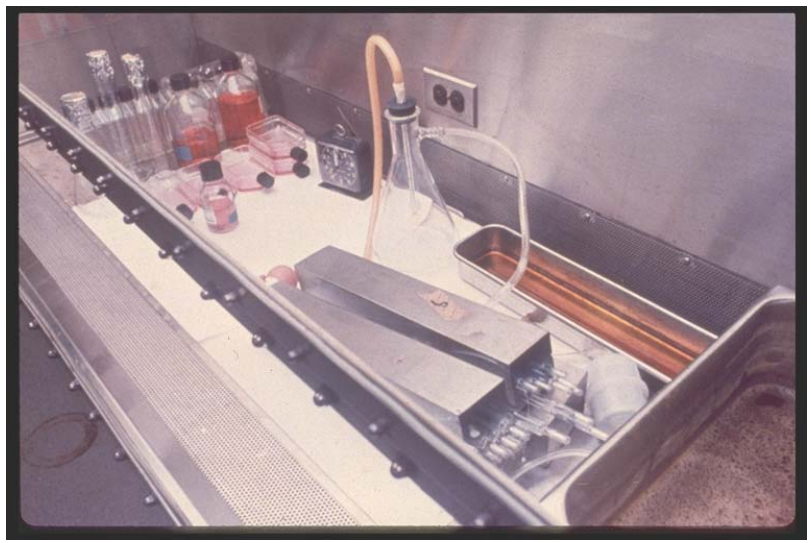
Operation in a BSC

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



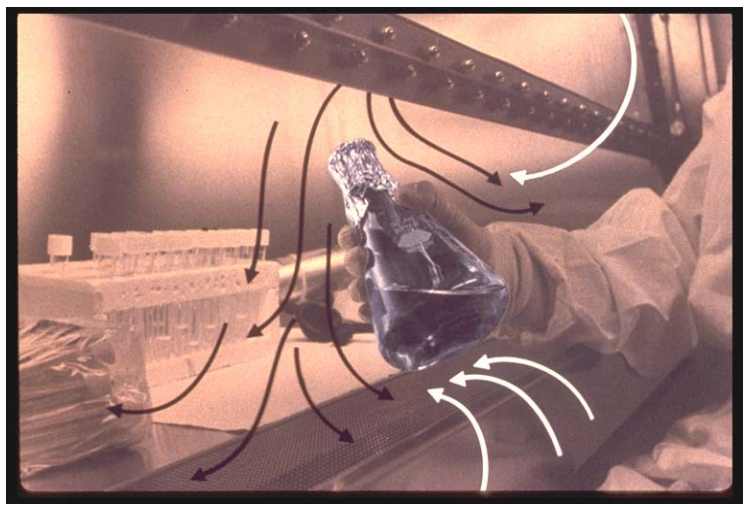
Operation in a BSC

- Plan your work
- Decontaminate BSC prior to use
- Place absorbent pad on work surface
- Set up work area with all necessary items
  - Centrifuge
  - Pipettes
  - Waste containers
  - Disinfectant



Operation in a 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



Operation in a BSC

- Surface decontaminate items before removal from the BSC
- Decontaminate cabinet following use:
  - Work surface
  - Sides
  - Back
  - Sash interior glass

Operation in a BSC

Explosion in a Cabinet

Operation in a BSC

BSC Airflow

Operation in a BSC

Improper Procedures

Particles from Hands

BSC Pitfalls

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

  - See CD for information pertaining to UV lamps

Any comments?

Summary

- Discussed the need for and the process of certification for BSC
- Discussed types of BSC
- Discussed the procedures for safe operation, while using a BSC