Laboratory Biosafety

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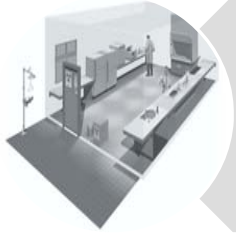
Laboratory biosafety is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

Source: WHO Laboratory Biosafety Manual 2004

Containment principles, technologies and practices

Biohazards and Risk Groups



Laboratory Facilities by Biosafety Levels

Good Microbiological Techniques

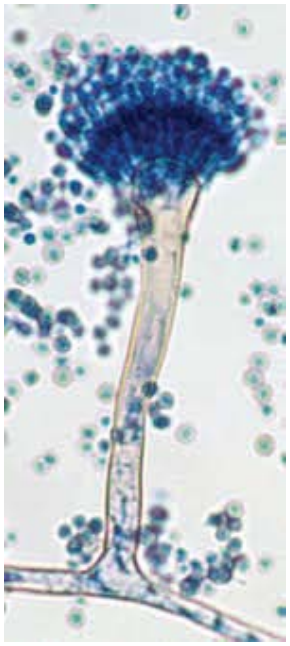
Biohazard

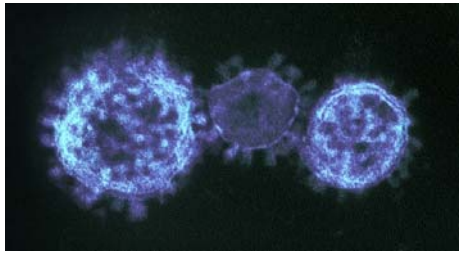
Definition

Biohazards are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals, or the environment.

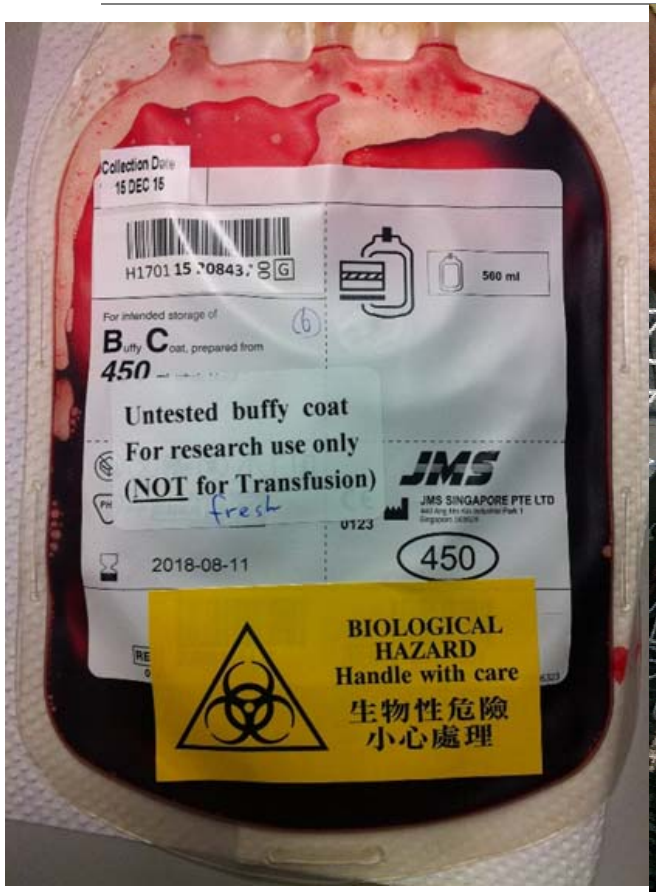
Infectious Agents

- Bacteria
- Fungi
- Virus
- Protozoans
- Arthropod vectors
- Prion





Biological Materials



Risk Group

Classification of Microbiological Agents by Risk Group

Microbiological agents are categorized based on:

- Pathogenicity of the organism
- Mode of transmission and host range
- Availability of effective preventive measures (e.g., vaccines)
- Availability of effective treatment (e.g., antibiotics)

Classification of Microbiological Agents by Risk Group

Risk Group 1 (no or low individual and community risk)

- A microorganism that is unlikely to cause human or animal disease.

Risk Group 2 (moderate individual risk, low community risk)

- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.
- Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk)

- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another.
- Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly.
- Effective treatment and preventive measures are not usually available.

Examples

RG1:

- *E. coli* K-12
- *Saccharomyces cerevisiae.*

RG2:

- *Salmonella sp.*
- *Legionella pneumophila*
- Enteropathogenic *E. coli strains,*
- *Staphylococcus aureus*

RG3:

- *Mycobacterium tuberculosis*
- *Human immunodeficiency virus*

RG4:

- Ebola Virus
- Herpes Simiae Virus
- Yellow Fever Virus
- Venezuela Equine Encephalitis Virus

For More Information on Safety Classification of Microbes…

Risk Group Classifications for Infectious Agents (American Biological Safety Association)

https://my.absa.org/tiki-index.php?page=Riskgroups

Material Safety Data Sheets for Infectious Agents (Health Canada)

http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

Biosafety Level (BSL)

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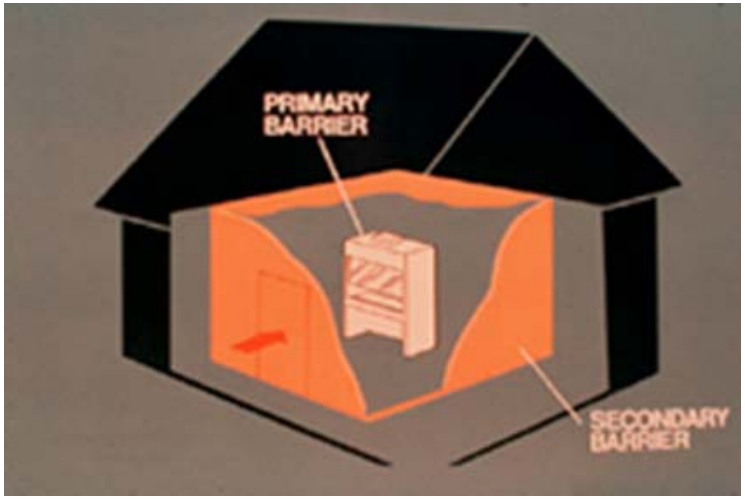
Biosafety level designations are based on a composite of

- Design features, construction and containment facilities (Secondary Barrier)
- Safety equipment (Primary Barrier)
- Practices and operational procedures



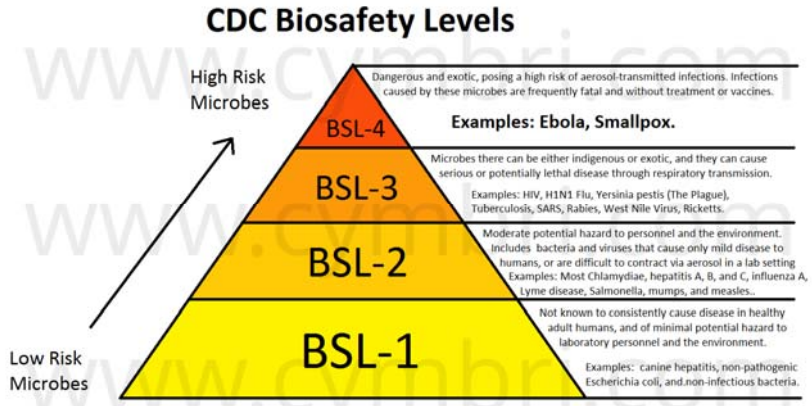


Table 2. ***Relation of risk groups to biosafety levels, practices and equipment***

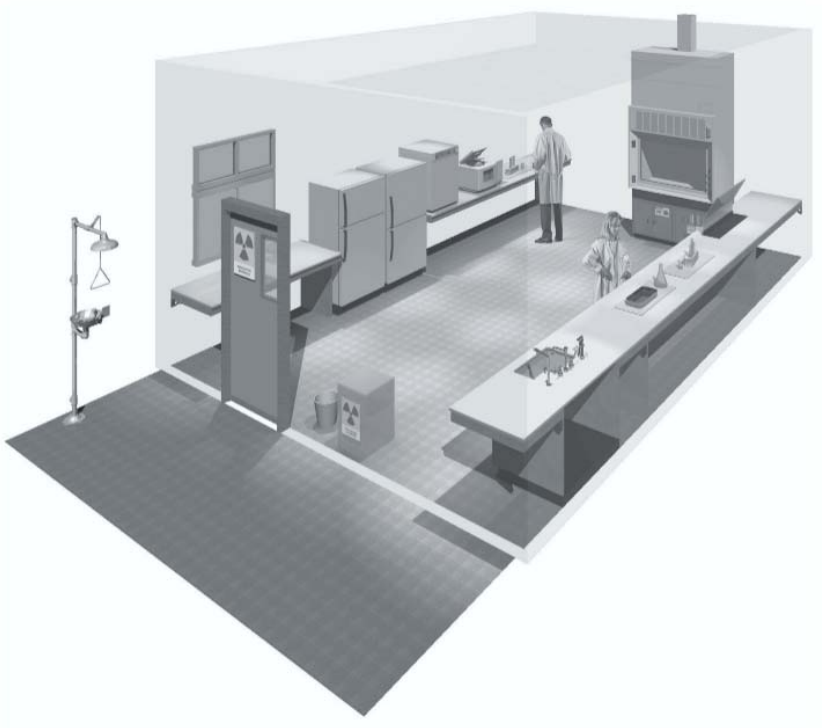
RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

Facility Requirements - BSL 1

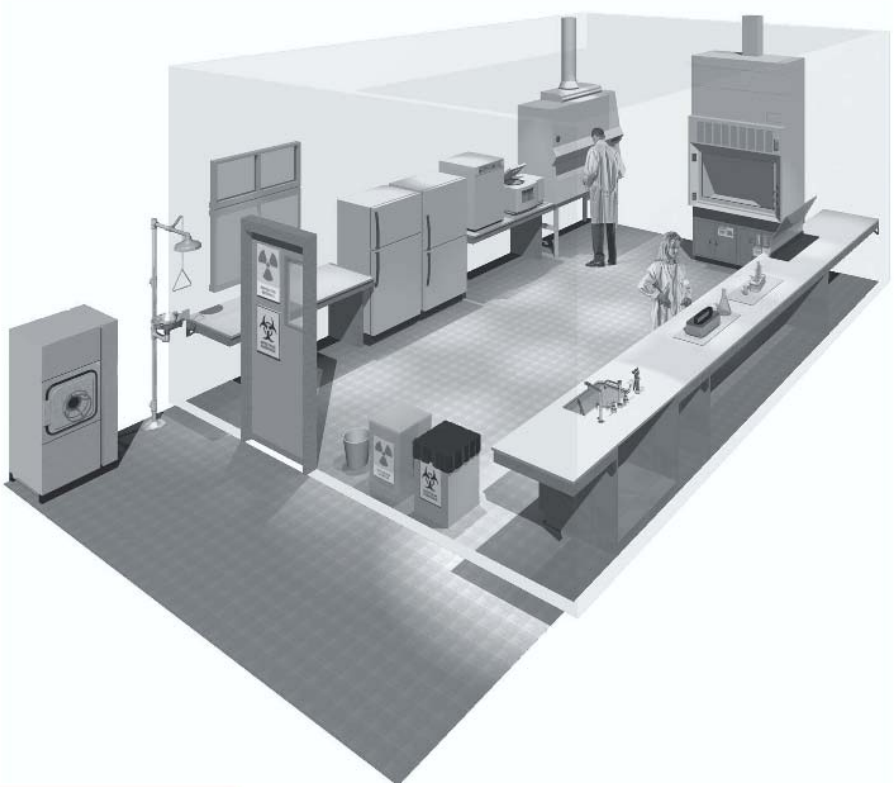
Table 3. ***Summary of biosafety level requirements***

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



Facility Requirements BSL 2

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

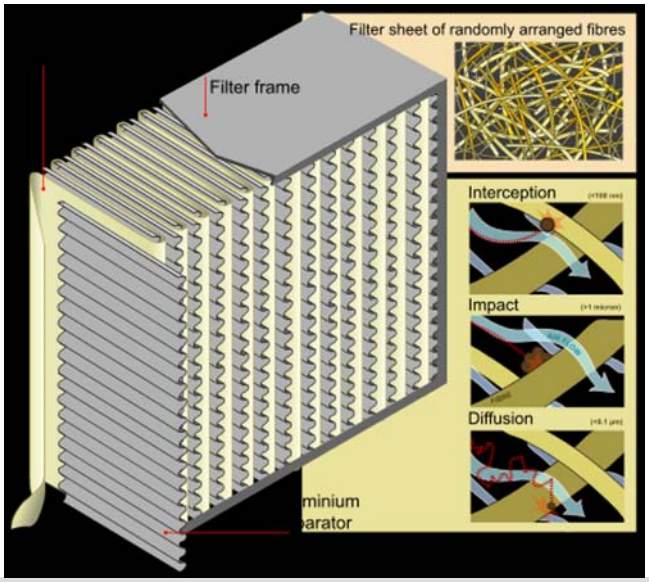


Essential Safety Equipment

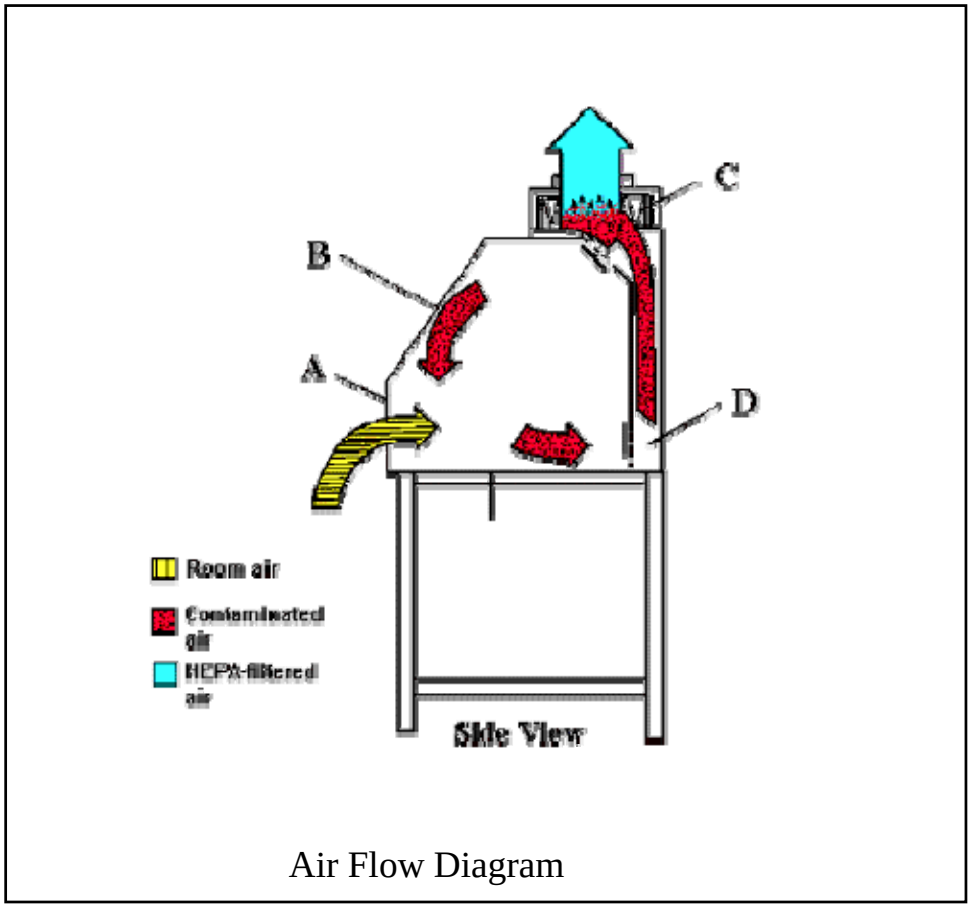
Biological Safety Cabinet (BSC): A leak tight box containing HEPA Filters and a motor/blower system to provide controlled air movement through the box and filters.

HEPA Filter: Minimum particulate removal of 99.97% for thermally generated DOP smoke particles or equivalent with a diameter of 0.3 micron

Source: Labmate

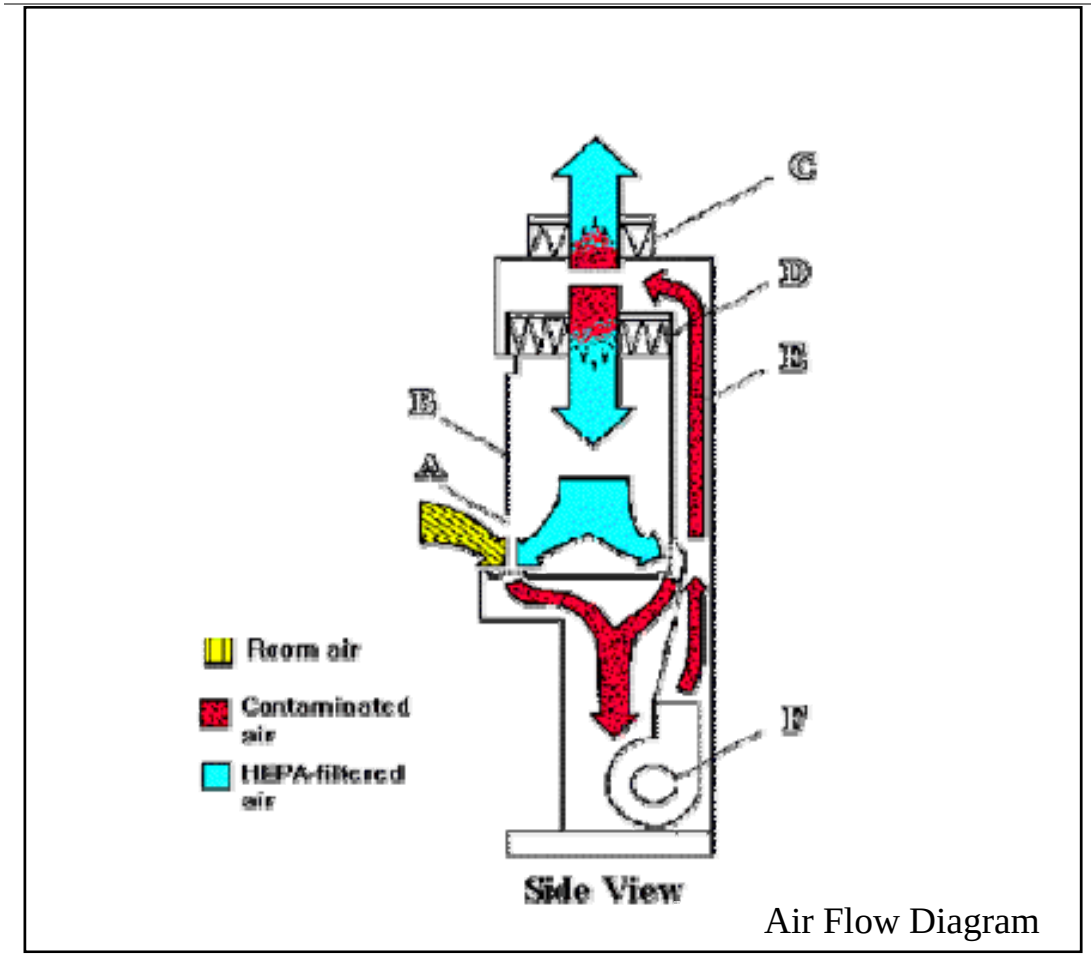


BSC- Class I



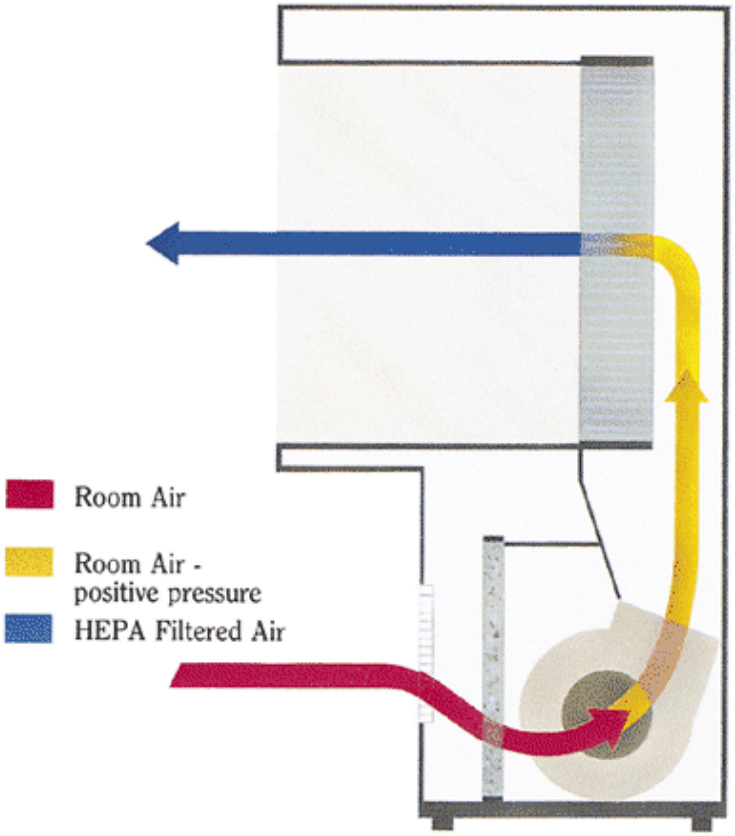
Air Flow Diagram

BSC – Class II



Air Flow Diagram

Laminar Flow Clean Bench looks like a BSC but…



Protect the product (work) ONLY!

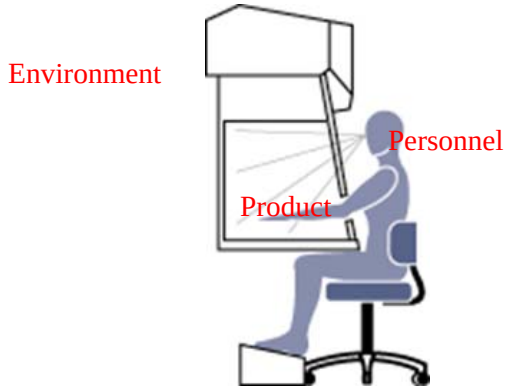
Offer no protection for the worker

Not for handling of infectious materials

Use as a clean bench or workstation for clean work such as culture media and sterile solution preparation

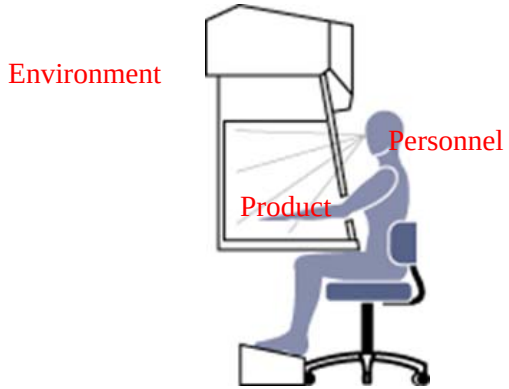
Product, Personnel and Environmental Protections

Cabinet Type	Product	Personnel	Environment
Class I BSC			
Class II BSC			
Class III BSC			
Clean Bench			
Fume Hood			



Product, Personnel and Environmental Protections

Cabinet Type	Product	Personnel	Environment
Class I BSC	No	Yes	Yes
Class II BSC	Yes	Yes	Yes
Class III BSC	Yes	Yes	Yes
Clean Bench	Yes	No	No
Fume Hood	No	Yes	No



Standard Microbiological Practices

- No eating, drinking, smoking or application of cosmetics
- No mouth pipetting
- Minimize splashing or aerosol generation
- Wash hands after handling viable organisms and before leaving lab
- Decontaminate work surface daily
- Decontaminate wastes prior to disposal
- Insect and rodent control

Routes of Exposure

- Ingestion from contaminated hands and utensils
- Inhalation of Infectious aerosols or dust
- Skin penetration (cuts, bites, scratches)
- Mucous membranes exposure to infectious droplets
- Percutaneous self-inoculation (injection or incision)

Laboratory-Acquired West Nile Virus Infections --- United States, 2002

Case 1. In August 2002, a microbiologist working in a U.S. laboratory was performing a necropsy on a blue jay submitted as part of a state's WNV surveillance program. The microbiologist worked in a Class II laminar flow biosafety cabinet under biosafety level 2 (BSL-2) conditions and lacerated a thumb while using a scalpel to remove the bird's brain. The wound, a superficial cut over the dorsal surface of the interphalangeal joint, was cleansed and bandaged. Four days after injury, the microbiologist had acute symptoms of headache, myalgias, and malaise followed by chills, sweats, dysesthesias, recurring hot flashes, swelling of the post-auricular lymph nodes, and anorexia. Serial serum samples taken from the patient and submitted to CDC for WNV serologic testing revealed evidence of an acute WNV infection. The brain of the blue jay tested positive at CDC for WNV RNA by real-time polymerase chain reaction (TaqMan®) using two primer/probe sets.

Case 2. In October 2002, a microbiologist working in a U.S. laboratory who was harvesting WNV-infected mouse brains in a Class II laminar flow biosafety cabinet under BSL-3 conditions punctured a finger with a contaminated needle. The wound was cleansed and bandaged. The microbiologist's body temperature was measured several times each day, and 3 days after injury, the microbiologist had upper respiratory infection (URI) symptoms without fever or chills. The next day, URI symptoms continued with malaise, fatigue, chills, and a low-grade fever (100.9º F [38.3º C]). Serial serum samples taken and submitted to CDC for WNV serologic testing revealed evidence of an acute WNV infection.

Personal Protective Equipment for Biohazards

Hand protection--gloves (selection, proper use)

Eye/face protection--glasses, goggles or face shields

Respiratory Protection

- Masks
- Respirators with HEPA filter cartridges
- Supply air respirators

Body protection--lab coats

Face and Eye Protection

Air-purifying Respirators

Full-face

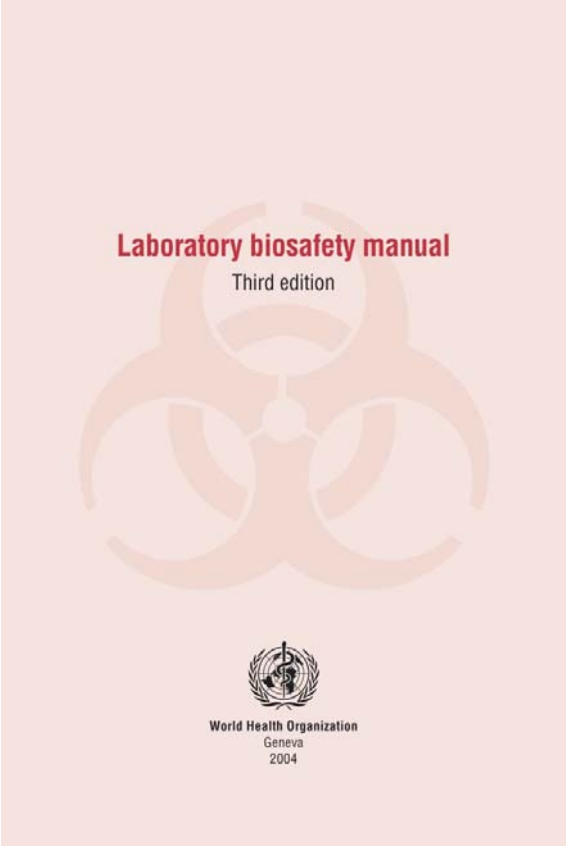
Powered Air-purifying Respirator

Particulate Filtering Facepiece Respirators

Mask versus Respirator

Mask	Respirator
Comfortable	Breathing resistance
No sealing	Sealing to face
Only good for large particles	Can filter small particles
No physical requirements	Requires lung function test, fit testing

References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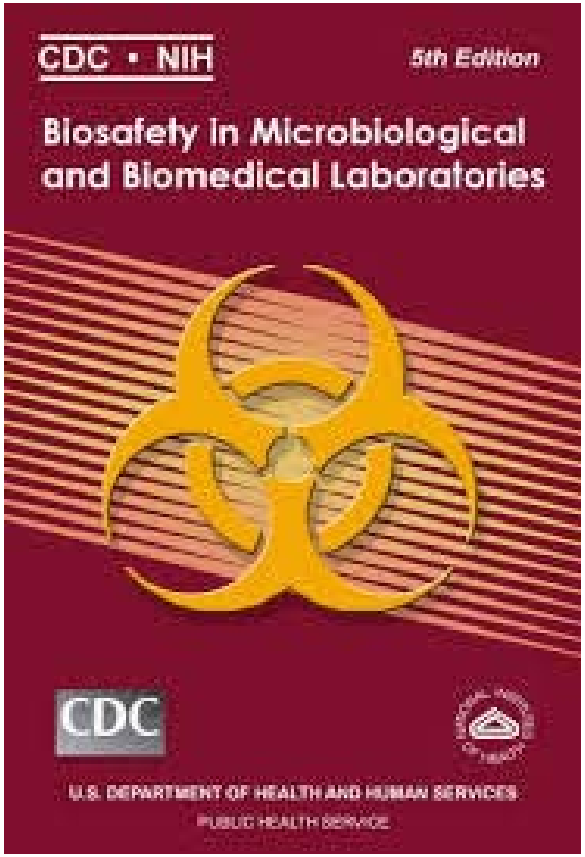
Geneva
2004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Sabia Virus in Centrifuge Incident

A few years ago the newly discovered arenavirus, Sabia, was being studied by a visiting senior virologist, who was purifying it from one liter of tissue culture fluid. At the time of the incident, the virologist was working alone in the biosafety level-three laboratory (negative pressure with HEPA-filtered exhaust system). The researcher used a high-speed centrifuge with a large angle head centrifuge rotor with six new plastic bottles in the purification process. During the centrifugation cycle there was a nasty rattling sound that indicated a problem. As soon as the unit stopped, he opened the chamber and removed the bottles from the rotor and placed them in a biological safety cabinet. Only then did he realize that one of the plastic bottles had leaked about 100 ml of infectious tissue culture into the centrifuge rotor. (Subsequently a hairline crack was found.)

The researcher was probably exposed to aerosolized Sabia virus when he opened the chamber. He immediately disinfected the centrifuge rotor, the defective bottle and the centrifuge using 10% Clorox solution. He cleaned the spilled material from the centrifuge while wearing a gown, surgical mask, and gloves. After disinfecting the centrifuge and bottles, he continued to work in the laboratory for three to four more hours. He did not inform anyone or report the incident.

About ten days later he went to the doctor with a four-day history of fever, malaise, backache, stiff neck, and myalgias that he attributed to another cause. The diagnosis of Sabia Virus was made and confirmed. Although Sabia virus infection is potentially fatal, the researcher recovered after two weeks of treatment.

Lesson Learned

Vacate room and wait at least 30 minutes for infectious aerosols to settle before attempting a clean-up

Use sealed rotors or safety cups when centrifuging blood, pathogens or other potentially infectious materials.

Written emergency procedures should be clearly defined for potential accidents/incidents that can occur in the lab.

All personnel should be trained in emergency procedures when using hazardous materials.

Thank you !