BIOSAFETY

MBT-402

WHAT IS BIOSAFETY?

- Measures employed when handling biohazardous materials to avoid infecting oneself, others or the environment.
- Achieved through;
  - ✓ Administrative Controls
  - ✓ Engineering Controls
  - ✓ Personal Protective Equipment
  - ✓ Practices and Procedures

WHAT IS A BIOHAZARD?

A potential hazard to humans, animals or the environment caused by a biological organism, or by material produced by such an organism

Examples:

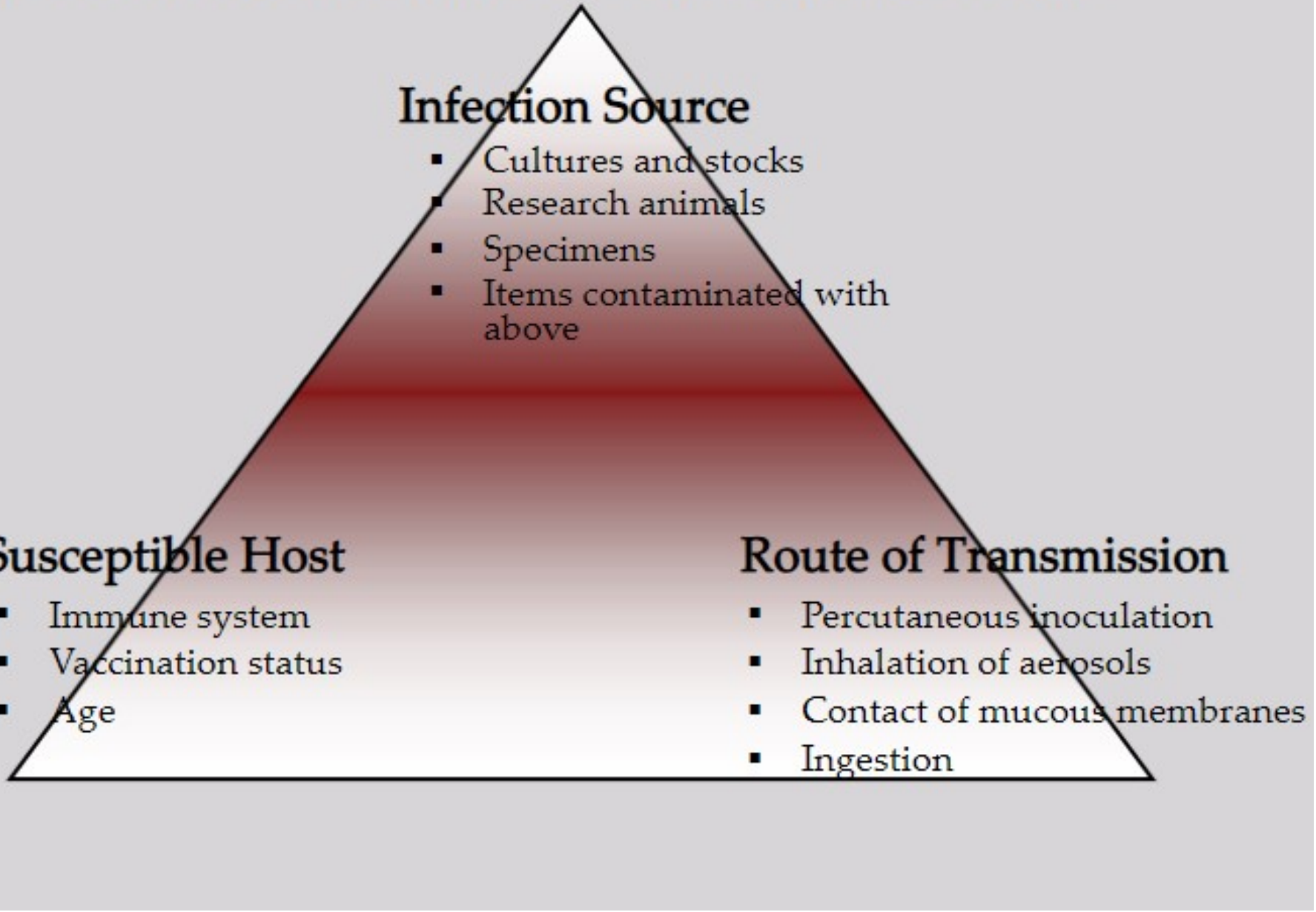
- Viruses, bacteria, fungi, and parasites and their product.
- Blood and body fluids, as well as tissues from humans and animals.
- Transformed cell lines and certain types of nucleic acids .

WHY ARE WE CONCERNED?

- Potential for acquiring a laboratory-associated infection (LAI)
- Contamination of the environment
- Contamination of research
- Public perception

LABORATORY ASSOCIATED INFECTIONS

LABORATORY ASSOCIATED INFECTIONS



LAIS

- Only 20% causative or defined event
  - ✓ 80% of which are caused by human factors
  - ✓ 20% are caused by equipment failure
- Top 4 accidents resulting in infection
  - ✓ Spillages & splashes
  - ✓ Needle and syringe
  - ✓ Sharp object, broken glass
  - ✓ Bite or scratch from animals or ectoparasites

Historical Background

•**1908**:"microbiological safety" where Winslow demonstrated a novel method of examination to enumerate bacteria present in the air

•**1941**: Additional study reviewed by Meyer and Eddie in, described laboratory-acquired brucellosis which also revealed that similar infections could pose a threat to man has no relation to lab work

•The principles of biosafety have developed together with the history of the American Biological Safety Association (ABSA).

•**1955** : As briefly described by the Federation of American Scientists, the first meeting was held in with the members of the military, as the focus addressed "The Role of Safety in the Biological Warfare Effort".

•The next meetings attendees included the US Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH), universities, laboratories, hospitals and representatives from the industries. From then, written regulations covered the shipment of biological agents, safety training and programs, with the development of biological safety level classification

1980s: Biosafety studies on the individual or group of agents became the focus in the 1980s. Some from studies focusing on specific biohazard levels of pathogens and other new strategies were developed to enhance biorisk assessment capacities, biosecurity, and biocontainment measures including the regulation of biosafety through national and international policies. Other activities such as in agriculture and biotechnology are now considering biosafety applications.

Definitions of pathogenicity and virulence

- Specifically, pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease.
- Virulence is the disease producing power of an organism, the degree of pathogenicity within a group or species.
- Pathogenicity is a qualitative term, an “all-or none” concept, whereas virulence is a term that quantifies pathogenicity.

(Shapiro-Ilan et al. / Journal of Invertebrate Pathology 88 (2005) 1–7)

Difference between pathogenicity and virulence?

- **Pathogenicity** *is an organism's capacity to cause disease*. It is context dependent; on environments; on hosts (susceptibility / resistance); number of organisms present.
- **A pathogenic organism** must be able to invade, multiply , evade host defenses, and harm the host in some way.
- **Virulence** *is the degree of disease an organism has the potential to cause*: a highly virulent pathogen can cause significant disease whereas an avirulent microorganism can cause little or no disease.
- The terms **pathogenicity** and **virulence** are loosely related. **Pathogenicity** refers to an organism's binary ability to cause disease or not under specific circumstances. **Virulence** refers to the degree of disease caused.
- Generally, **virulence** concerns the effects of the pathogenic to its natural host. **Pathogenicity** is what is observed in a given system either towards an organ or towards a target cell. The term **pathogenicity** must be thus associated with the model considered.

(http://adrien.six.online.fr/IP-HCMC2005/index.html)

Principles of Bio-safety

The term "containment" is used in describing **safe methods** for managing, handling or maintaining infectious materials /microbes in the laboratory environment. The purpose is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

(http://www.cdc.gov/od/ohs/biosfty)

Methods, Practice, Training , Safety equipment

Principles of Bio-safety

- **Classification Systems** : Because investigations into Laboratory associated infection (LAI) showed that certain micro-organisms were more likely to be involved than the others, several attempts have been made to classify human and animal pathogens according to the risks they present to the health of laboratory workers and to the human and animal community should they escape from the laboratory.

- Such categorization of risk would lead to the formulation of appropriate sets of safety precautions, called risk management.

- Biological hazards **unlike** chemical and radioactive hazards in one major significant way: biological agents can grow and multiply in the host organism Moreover, secondary infections in non-laboratory workers can occur, depending on the communicability of the infectious agent.

Risk Assessment

- **Risk assessment** constitutes the basis for the safeguards developed by different organizations, such as the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories.

- Experience shows that these established safe practices, equipment, and facility safeguards work.

- New knowledge and experiences may justify altering these safeguards. **Risk assessment**, however, must be the basis for recommended change.

BIOHAZARD CLASSIFICATION

BIOHAZARD CLASSIFICATION

- Conventional Agents
- Recombinant DNA
- Tissue Culture
- Animal Work
- Anatomical Specimens
- Unconventional Agents

BIOHAZARD CLASSIFICATION

- Organisms are categorized into a group base on the particular characteristics of each organism, such as
  - ✓ Pathogenicity
  - ✓ Infectious dose
  - ✓ Mode of transmission
  - ✓ Host Range
  - ✓ Availability of effective preventive measures
  - ✓ Availability of effective treatment

Risk Group Classification for Infectious Agents

- Infectious agents are categorized in risk groups based on their relative risk in a country. the following factors are take into consideration:

- **Pathogenicity of the organism** The outcome of the infection is dependent on the properties of the pathogen (virulence, invasiveness, toxic or allergenic effects) but also upon the host immunity status. From this point of view, pathogens fall into two basic types: **primary pathogens** that cause disease among at least a portion of normal individuals, and **opportunistic pathogens** that cause disease only in individuals who are compromised in either their innate or humoral immune defences.

- **Mode of transmission and host range**

- **Availability of effective preventive measures (**prophylaxis by vaccination or antisera; sanitary measures, e.g. food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.)

- **Availability of effective treatment** (This includes passive immunization and post-exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of the emergence of resistant strains.)

- **Other factors---** These may be influenced by existing levels of immunity, density and movement of host population presence of appropriate vectors and standards of environmental hygiene.

BIOHAZARD CLASSIFICATION

- Organisms are categorized based on the measures required for handling each organism safely in a laboratory setting, such as
  - ✓ Engineering Requirements
  - ✓ Operational Requirements
  - ✓ Technical Requirements
  - ✓ Physical Requirements

CONVENTIONAL AGENTS

Risk Group	Individual Risk	Community Risk	Containment Level	Examples
1	Low Unlikely to cause disease in healthy workers or animals	Low	**Level 1**	*E.coli, B. subtilis, S. aureus, Trichoderma reesei*
2	Moderate Rarely cause serious human or animal disease	Limited	**Level 2**	*Streptococcus& Salmonella* spp, *Measles,Adenoviruses, Hepatitis A, B& C, Influenza*
3	High May cause serious disease	Low	**Level 3**	*Bacillus anthracis, Mycobacterium tuberculosis, HIV, Yellow fever virus*
4	High Likely to cause very serious disease	High	**Level 4**	*Lassa virus, Ebola virus, Marburg virus*

RECOMBINANT DNA

- Genetic Engineering = in vitro incorporation of genetic material from one cell into another
- The level of risk depends on source of DNA, vector and host.
- The biosafety community may be able to assist the investigator in this determination.

TISSUE CULTURE

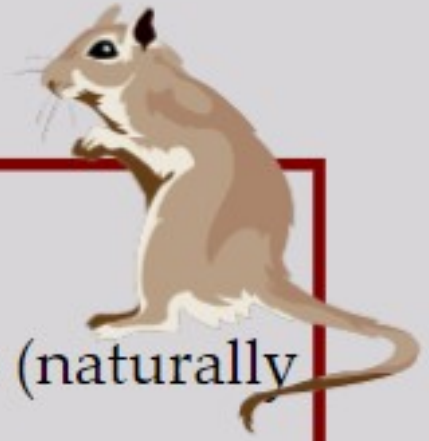
- Have the potential to contain pathogenic organisms
- In general;

Human & non-human primate, and mycoplasma-containing cell lines → **Level 2**

Others → **Level 1**

A detailed risk assessment should be undertaken when using a new cell line.

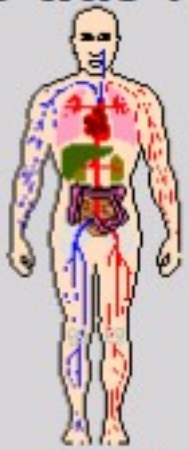
ANIMAL WORK



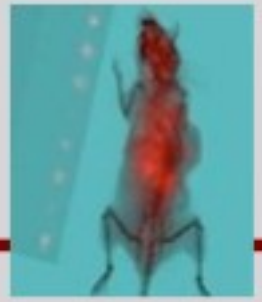
- Animals can harbour infectious organisms (naturally or introduced)
- Level dependent on type of work being conducted.
- Special Animal Care training is required for all personnel working with animals.
- All work involving animal use must receive prior approval from the Animal Care Committee

ANATOMICAL SPECIMENS

- All specimens should be considered infectious due to potential presence of infectious agents
- Important to consider the type of specimen
  - ✓blood, organs, tissues
  - ✓Spinal sample, brain tissue
  - ✓From infectious patient



- In general Level 2 but it depends on the nature of the work.



UNCONVENTIONAL PATHOGENS

- TSE prion diseases; lethal transmissible neurodegenerative conditions
  - ✓ Creutzfeld-Jakob disease, Variant C-J Disease, Mad Cow Disease, Scrapie, Chronic Wasting Disease
- Resistant to destruction by procedures that normally inactivate viruses.
- Contact regulatory authorities to assess requirements (containment, procedures, waste disposal, etc.)

Factors To Be Considered In Establishing The Appropriate Bio-safety Level.

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
5. Stability of the agent in the environment
6. Concentration of the agent and volume of concentrated material to be manipulated
7. Presence of a suitable host (human or animal)
8. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
9. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
10. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens (see Chapter 16)
11. Local availability of effective prophylaxis or therapeutic interventions.

WHO-Classification

Table 1. ***Classification of infective microorganisms 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.

Assessment of risk

- First, identify agent hazards and perform an initial assessment of risk. Consider the principal hazardous characteristics of the agent, which include its capability to infect and cause disease in a susceptible human / animal host, severity of disease, and the availability of preventive measures and effective treatments.

- Second, identify laboratory procedure hazards. The principal laboratory procedure hazards are agent concentration, suspension volume, equipment and procedures that generate small particle aerosols and larger airborne particles (droplets), and use of sharps. Procedures involving animals can present a number of hazards such as bites and scratches, exposure to zoonotic agents, and the handling of experimentally generated infectious aerosols.

- Third, make a final determination of the appropriate biosafety level and select additional precautions indicated by the risk assessment. The final selection of the appropriate biosafety level and the selection of any additional laboratory precautions require a comprehensive understanding of the practices, safety equipment, and facility safeguards

- Fourth, evaluate the proficiencies of staff regarding safe practices and the integrity of safety equipment.

- Fifth, review the risk assessment with a biosafety professional, subject experts

Containment

Primary containment

• protection of personnel

•laboratory environment from exposure to infectious agents.

Secondary containment

•the protection of the environment external to the laboratory from exposure to infectious materials.

The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Bio safety levels
- Bio containment controls

Specific controls based on –

- Infectivity of the disease
- Severity of the disease
- Source of the agent
- Route of invasion

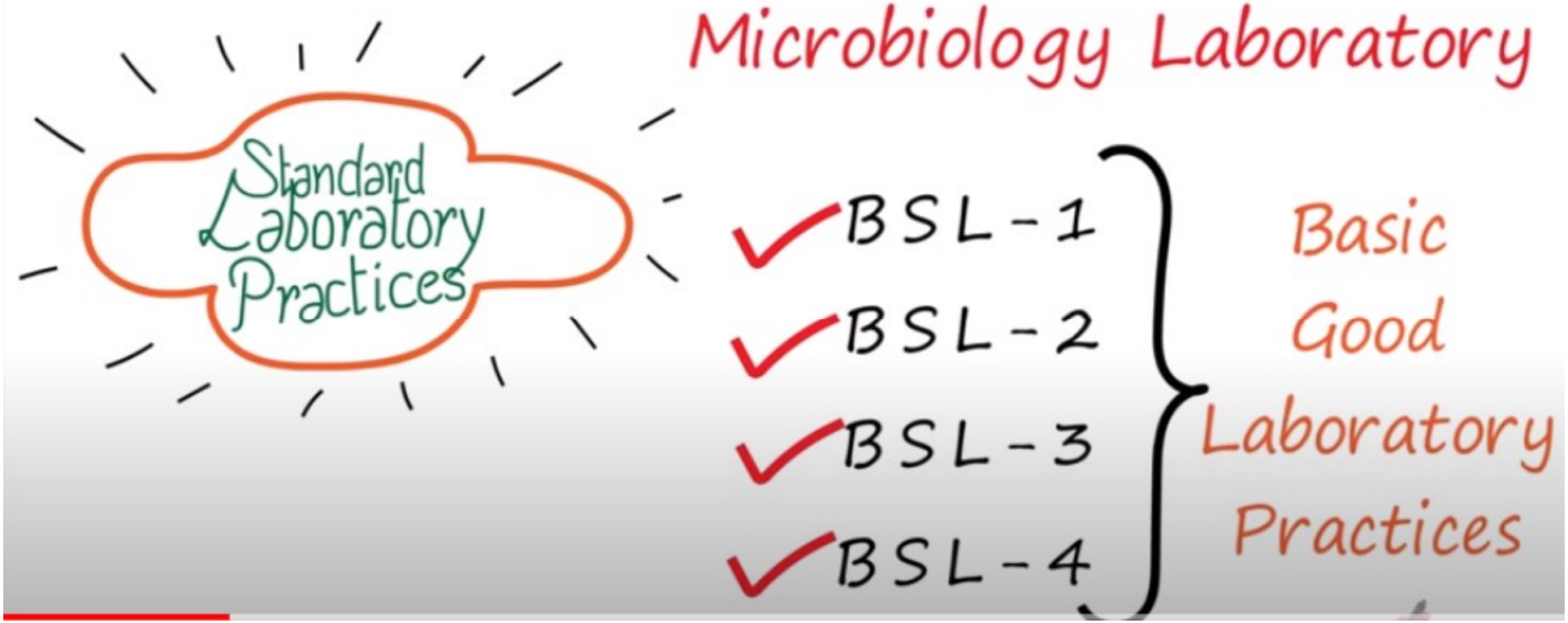
1 2 3 4

Based on the RISK

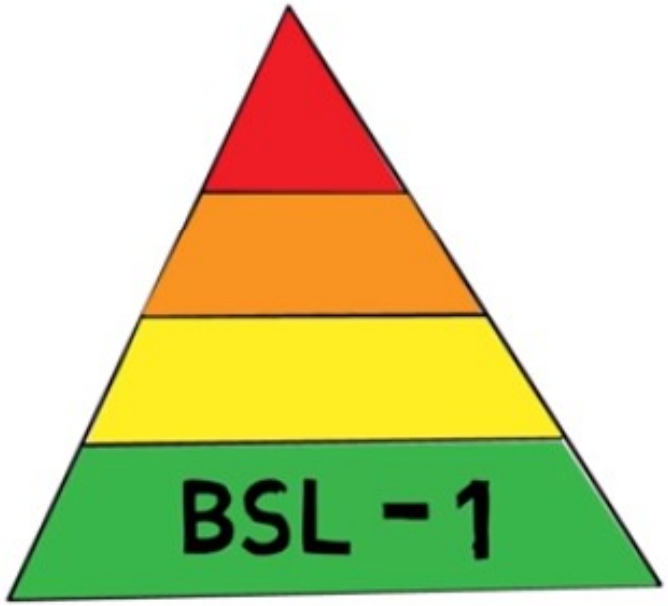
n the RISK

These play an important role in

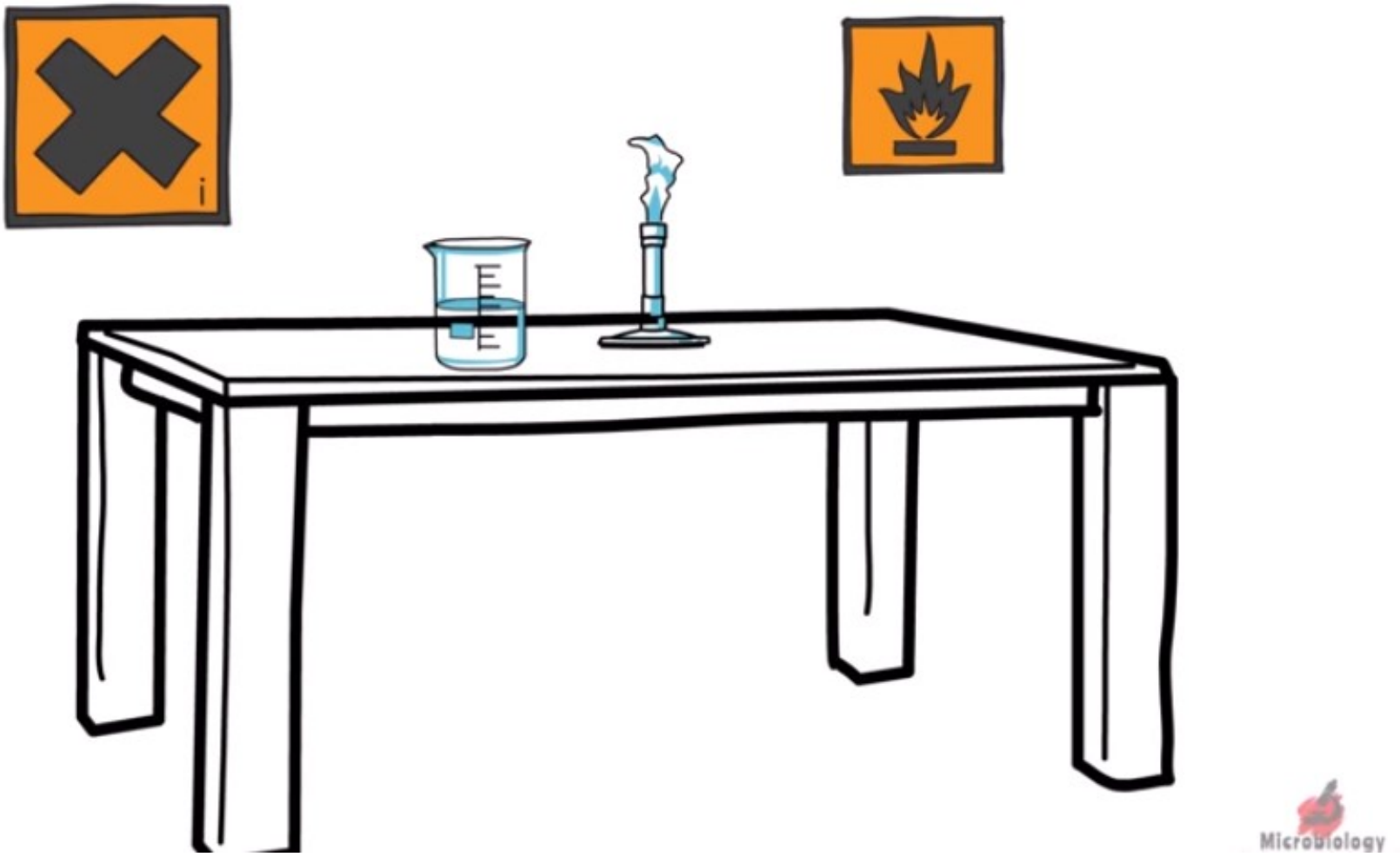
- Designing the facility
- Safety environment
- Laboratory practices


Standard Laboratory Practices
Microbiology Laboratory
BSL-1
BSL-2
BSL-3
BSL-4
Basic Good Laboratory Practices

Bio Safety Level 1



- ✓ Non-Pathogenic Microbes
- ✓ Minimum risk
- ✓ Do not need special containments

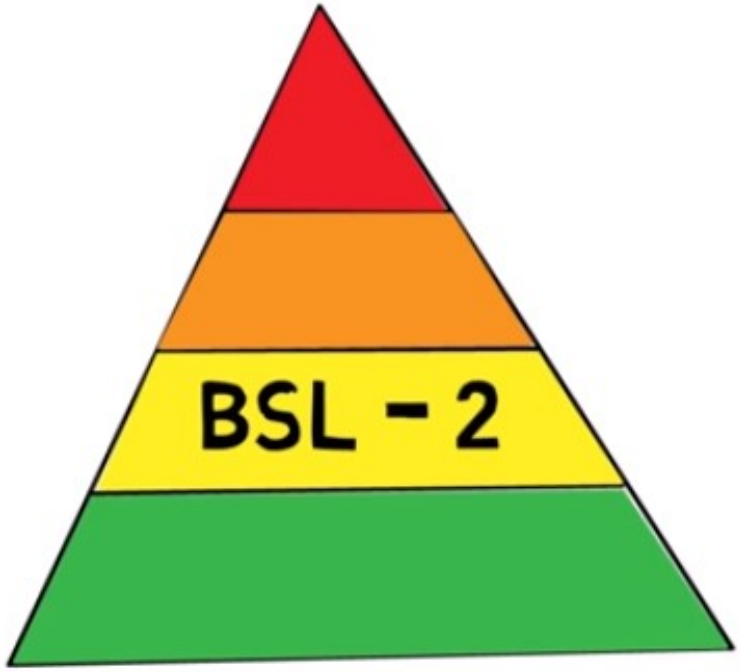

Microbiology

Example: Non-pathogenic strain of E. coli

BASIC SAFETY PRACTICES

- ✓ Hand washing
- ✓ Wearing lab coat, glove & eye protection
- ✓ Limited access to people
- ✓ No mouth pipetting
- ✓ Cleaning and decontamination of area
- ✓ Warning signs

Bio Safety Level 2

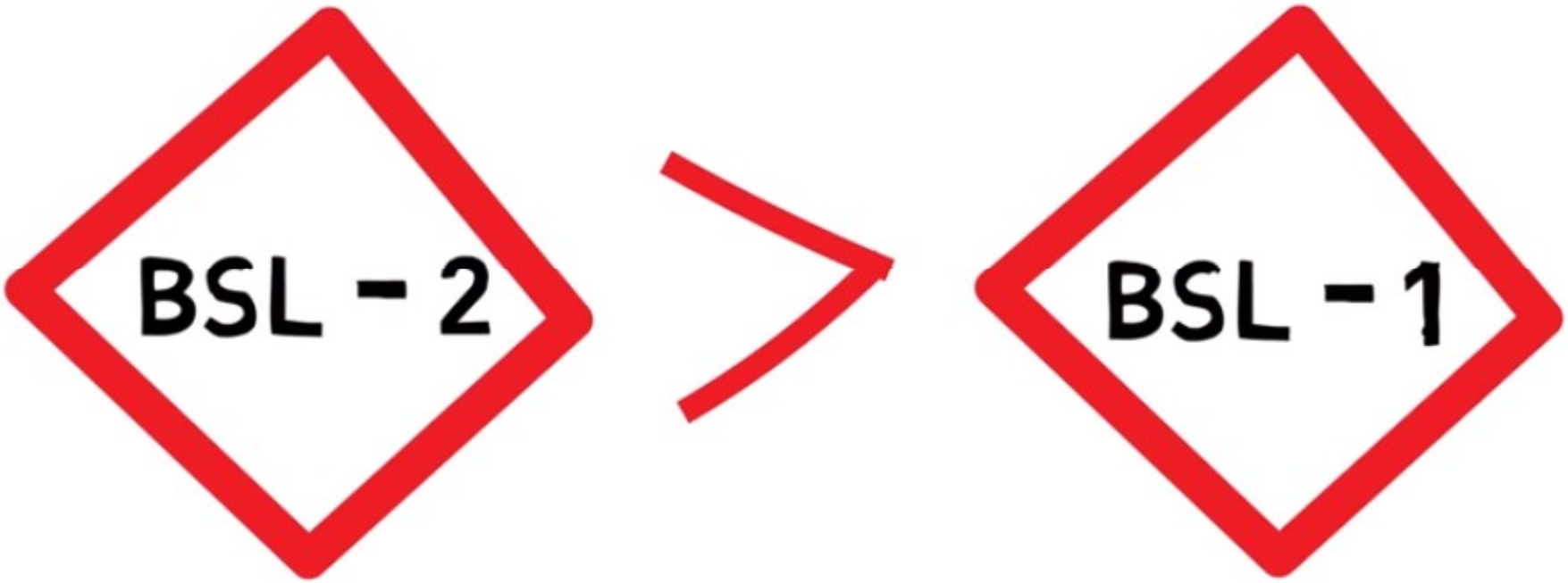


Cause diseases in Human beings

Pathogenic

Infectious

Example: Staphylococcus & Hepatitis virus

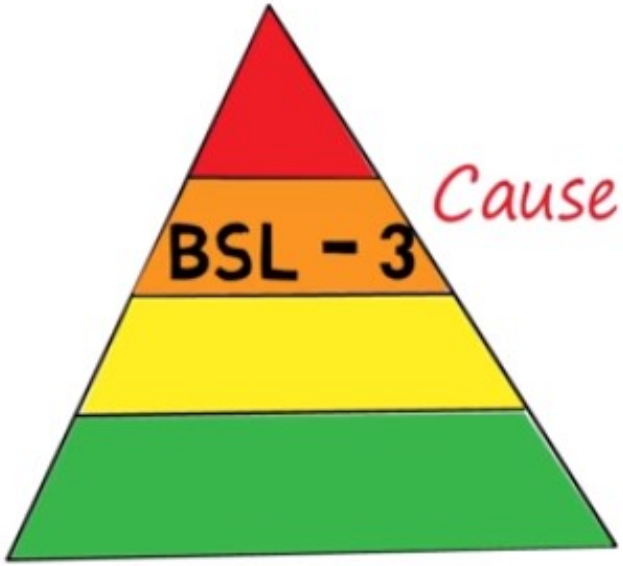

BSL - 2
BSL - 1

STANDARD PRACTICES

+

- ✓ Using Biosafety cabinet
- ✓ Trained personnel
- ✓ Availablity of Eye wash station
- ✓ Vaccination for the workers if applicable

Bio Safety Level 3



Cause Serious or Fatal diseases

Inhalation

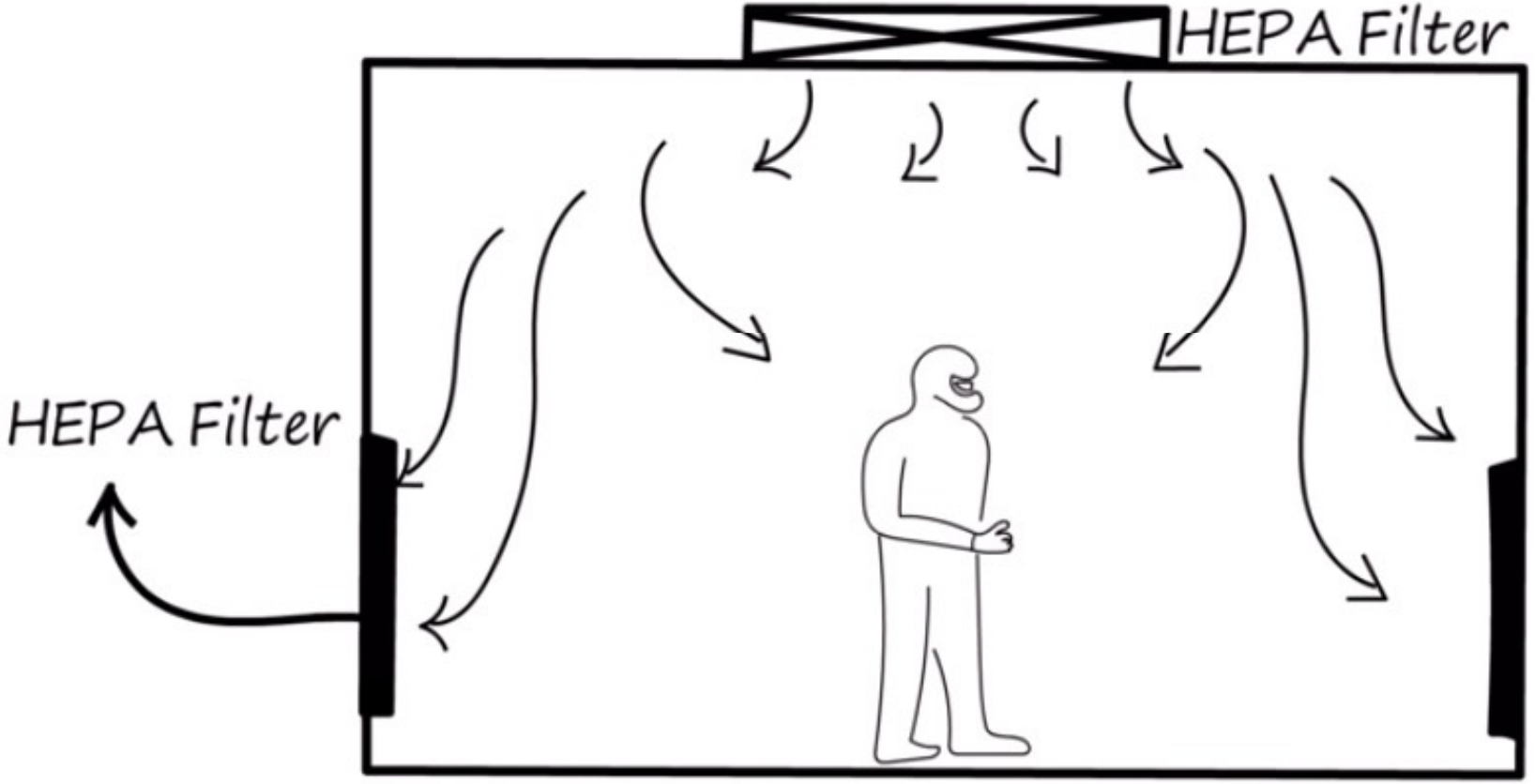
Bio Safety Level 3

Government Agencies

Medical surveillance

Example: Yellow fever & Tuberculosis bacteria

BSL - 3 FACILITY

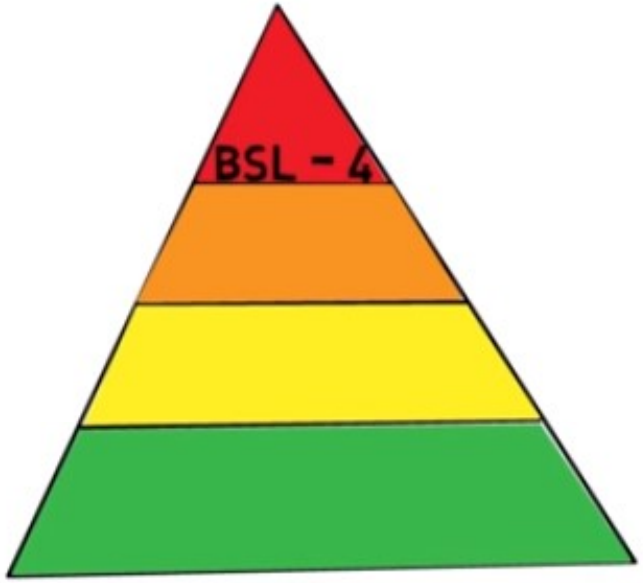


ADDITIONAL LABORATORY PRACTICES

- ✓ Periodic medical testing for workers
- ✓ Full body garment with respiratory protection
- ✓ Restricted access at all times

Bio Safety Level 4

Rare in the World



Fatal Infections

No Vaccines

Example: Ebola & Marburg viruses

LABORATORY PRACTICES

- ✓ Decontamination of materials before exiting
- ✓ Full body garment
- ✓ Taking shower after leaving the facility

Risk Groups- biosafety levels ---

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)

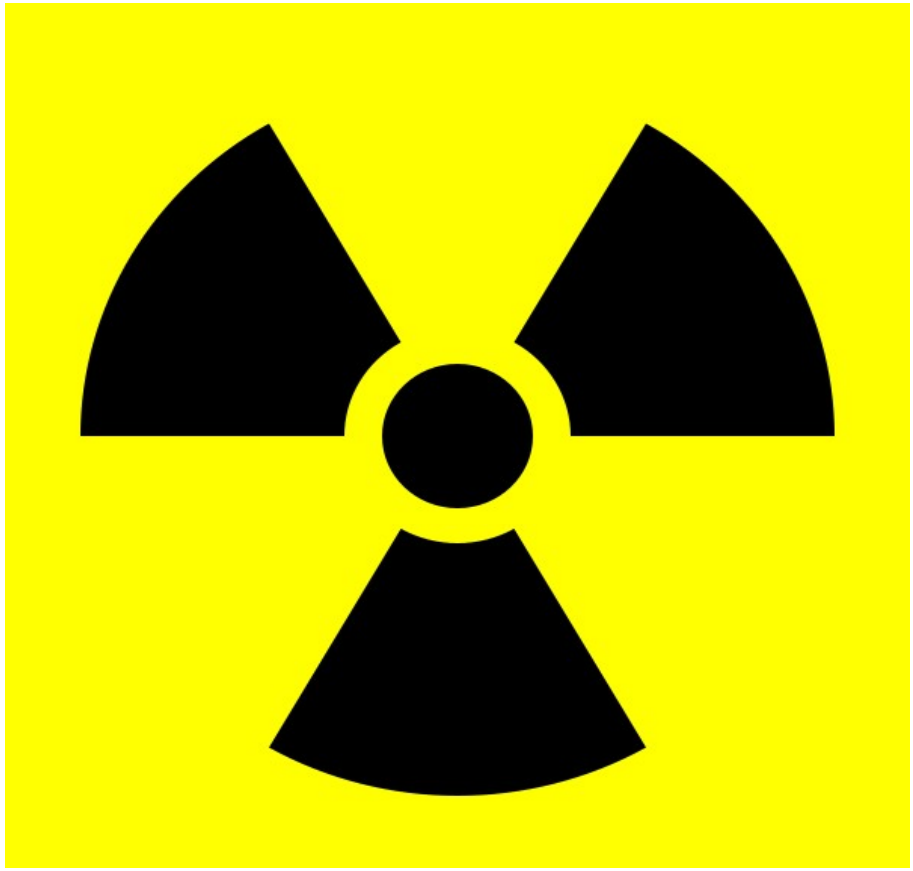
Biosafety levels

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.

1. The international biohazard warning symbol and sign (Figure 1) must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled.



Radiation warning symbol

When the risk of infection by exposure to an infectious aerosol is present:

•*multiple secondary barriers* may become necessary to prevent infectious agents from escaping into the environment.

•Lab design features include *specialized ventilation* systems to ensure *directional air flow*,

•*Air treatment* systems to decontaminate or remove agents from exhaust air,

•*Controlled access* zones,

•*Isolation of the laboratory* by Airlocks at laboratory entrances, separate buildings or modules .

Bio-safety Level

Level 1: A basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.(*Bacillus subtilis, Naegleria gruberi*)

Level 2:, equipment, facility design and construction are applicable to clinical, diagnostic, teaching, Work is done with the broad spectrum of indigenous **moderate-risk agents** that are associated with human disease of varying severity. Provided the potential for producing splashes or aerosols is low .+**Safety equipment + Secondary Barriers.** (Hepatitis B virus, HIV, salmonellae, and *Toxoplasma* spp)

Level 3: Emphasis on primary and secondary barriers to protect personnel in contiguous areas, community, and the environment. **Safety equipment + Multiple Secondary Barriers .(***M.tuberculosis, Coxiella burnetii*)

Level 4 : A **separate building** or completely **isolated zone with complex**, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment. **Safety equipment + Multiple Secondary Barriers .(No available vaccine or therapy).** Marburg or Congo-Crimean hemorrhagic fever Viruses

Containment

Therefore, the **three elements** of containment:

- laboratory practice and techniques,
- safety equipment,
- and facility design.

(The use of vaccines may provide an increased level of personal protection.)

Laboratory Practice and Technique:

- The most important element of containment is **strict adherence** to standard microbiological practices and techniques.
- Persons working must be **aware** of potential hazards, and must be **trained** and proficient in the practices and techniques required to handle such material safely.
- Hazards that will or may be encountered each laboratory **should develop or adopt a bio-safety or operations manual** that identifies, and specifies practices and procedures designed to minimize or eliminate exposures to these hazards.

Facility Design and Construction (*Secondary Barriers*)

laboratory workers' protection,

•provides a barrier to protect persons outside the laboratory,

•and protects persons or animals in the community from infectious agents which may be accidentally released from the laboratory

•The recommended secondary barrier(s) will depend on the *risk of transmission* of specific agents (Biosafety Level 1 and 2 facilities).

• **Secondary barriers** in these laboratories may include *separation of the laboratory* work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

Safety Equipment (*Primary Barriers*)

Biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials.

•BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

•Three types of biological safety cabinets (Class I, II, III) are used

Bio-safety Equipment

Laminar Flow

Laminar flow is unidirectional air moving at a steady velocity along parallel lines. Laminar flow cabinets may or may not be biological safety cabinets. Horizontal or vertical airflow.

- **safety centrifuge** cup, an enclosed container designed to prevent aerosols from being released during centrifugation .

- include items for **personal protection**, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

HEPA Filter : **H**igh **E**fficiency **P**articulate **A**ir filter. Removes particles, including microorganisms, from the air.

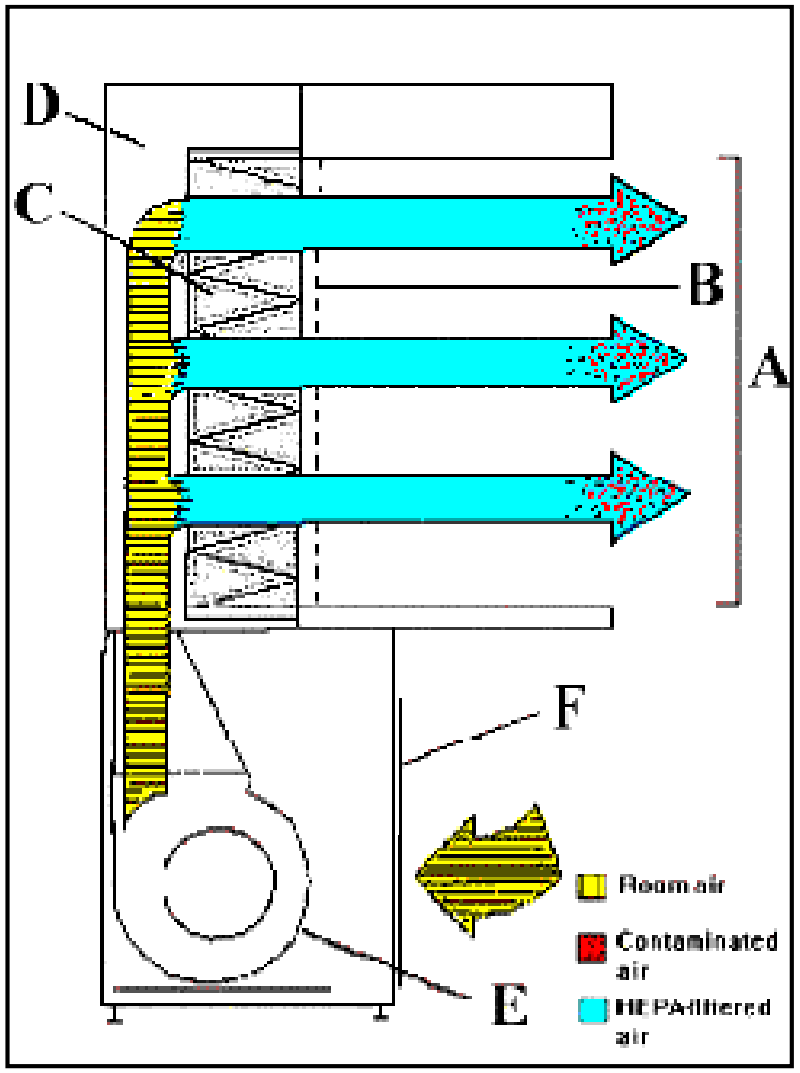
Biological safety cabinet. All biological safety cabinets use **HEPA** filters to treat **air ---** inflow/ **exhaust**. Two types:

Class I : provide worker and environmental protection, but **no** product protection. Exhaust air filtered.

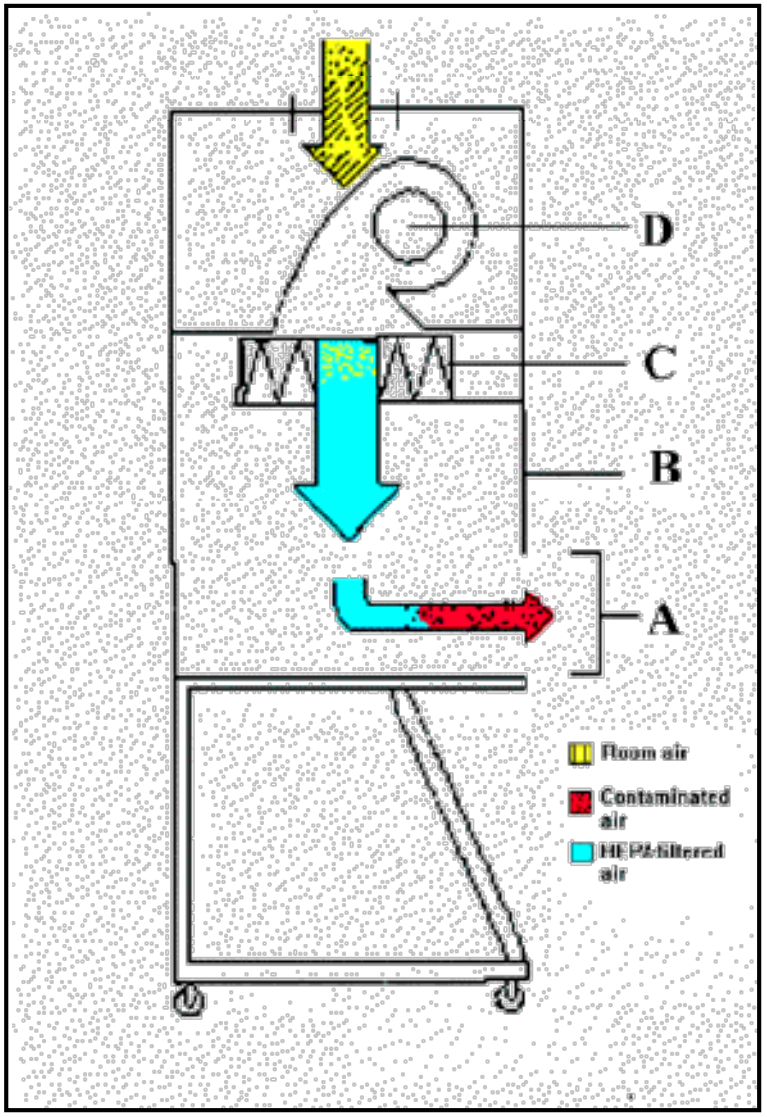
Class II : filter both exhaust and intake air to protect the worker and the environment from contamination **as well as to protect product** in the cabinet. Suitable for microorganisms assigned to bio-safety levels 1,2 and 3. .

Class III : gas-tight provides the highest attainable level of protection to personnel and the environment.

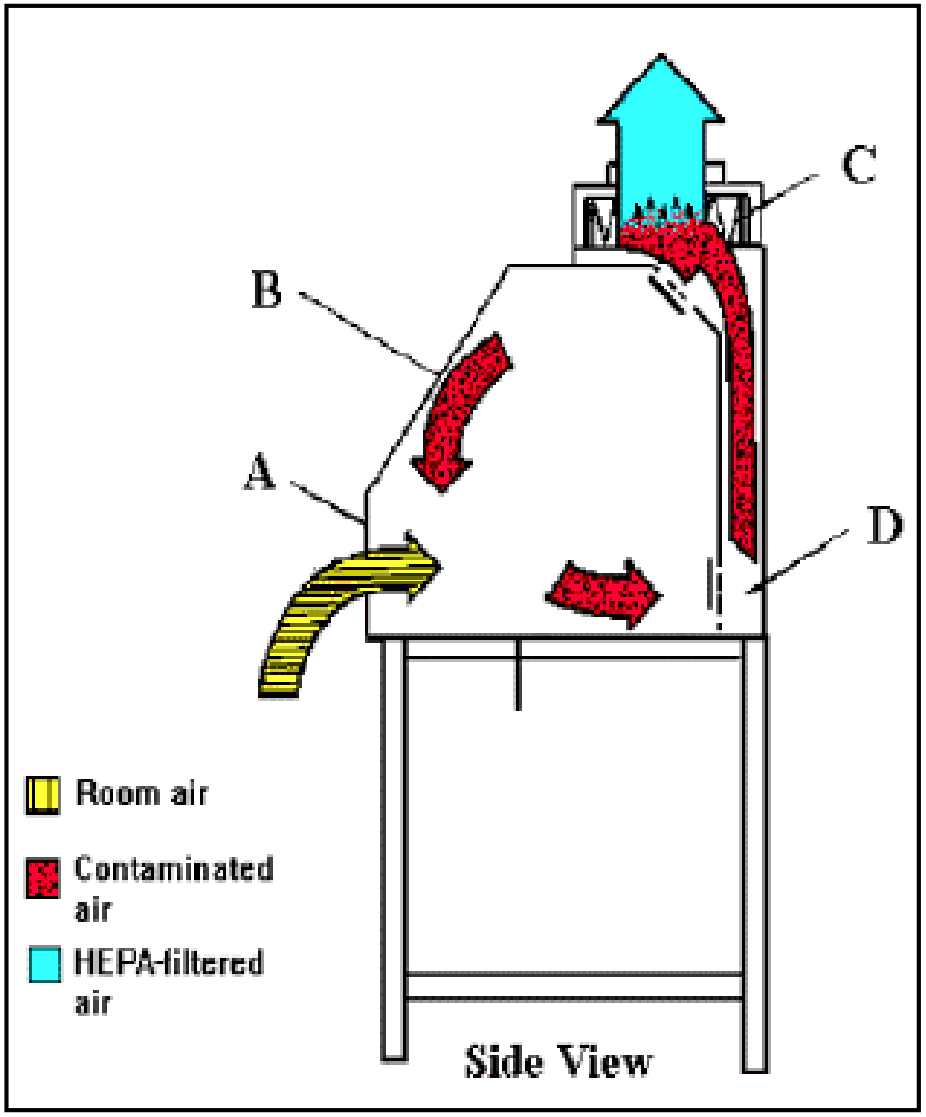
Horizontal Laminar Flow "Clean Bench"



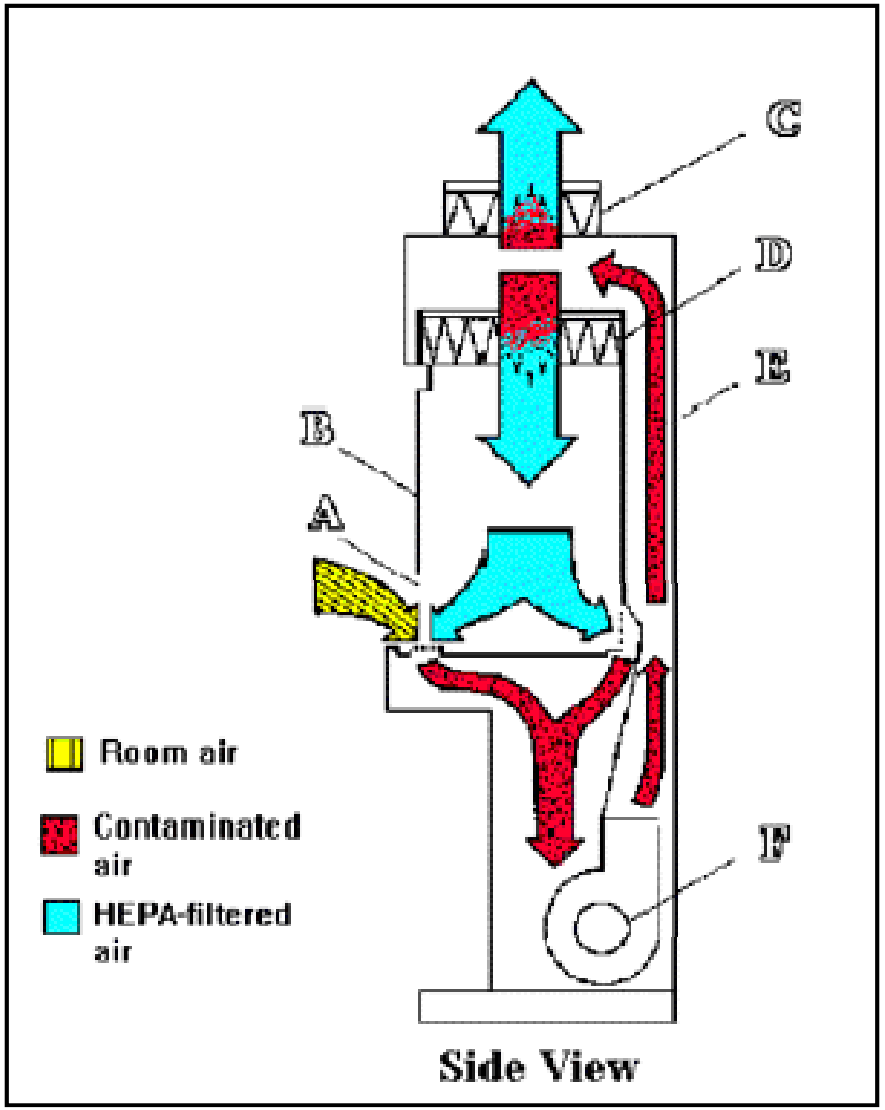
Vertical Laminar Flow "Clean Bench"



Class I BSC

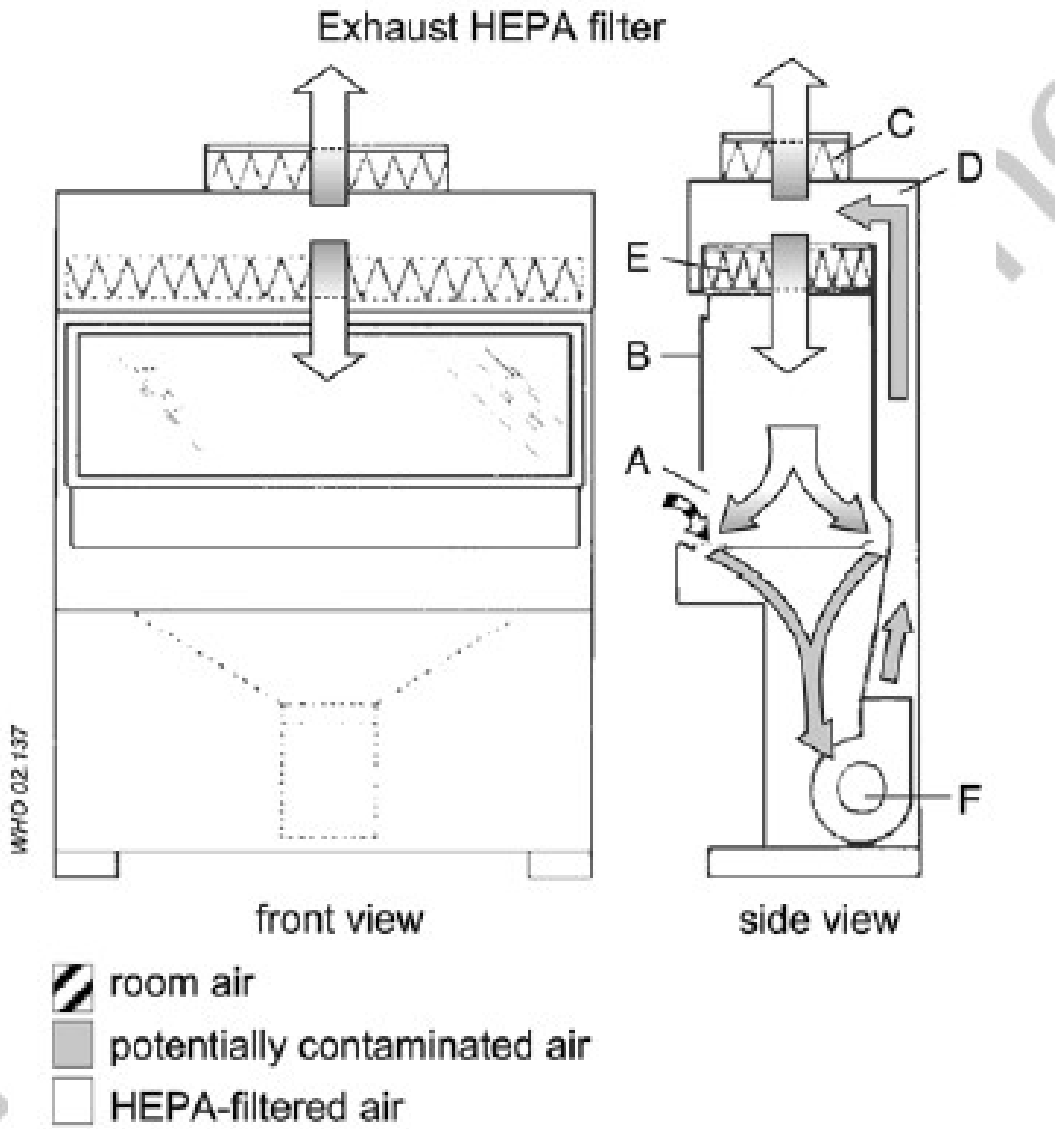


Class II, Type A BSC



A. front open;**B**. sash;**C**. exhaust HEPA; **E**. exhaust plenum;
D. supply HEPA filter;**F.** blower .

Class II, Type AI BSC

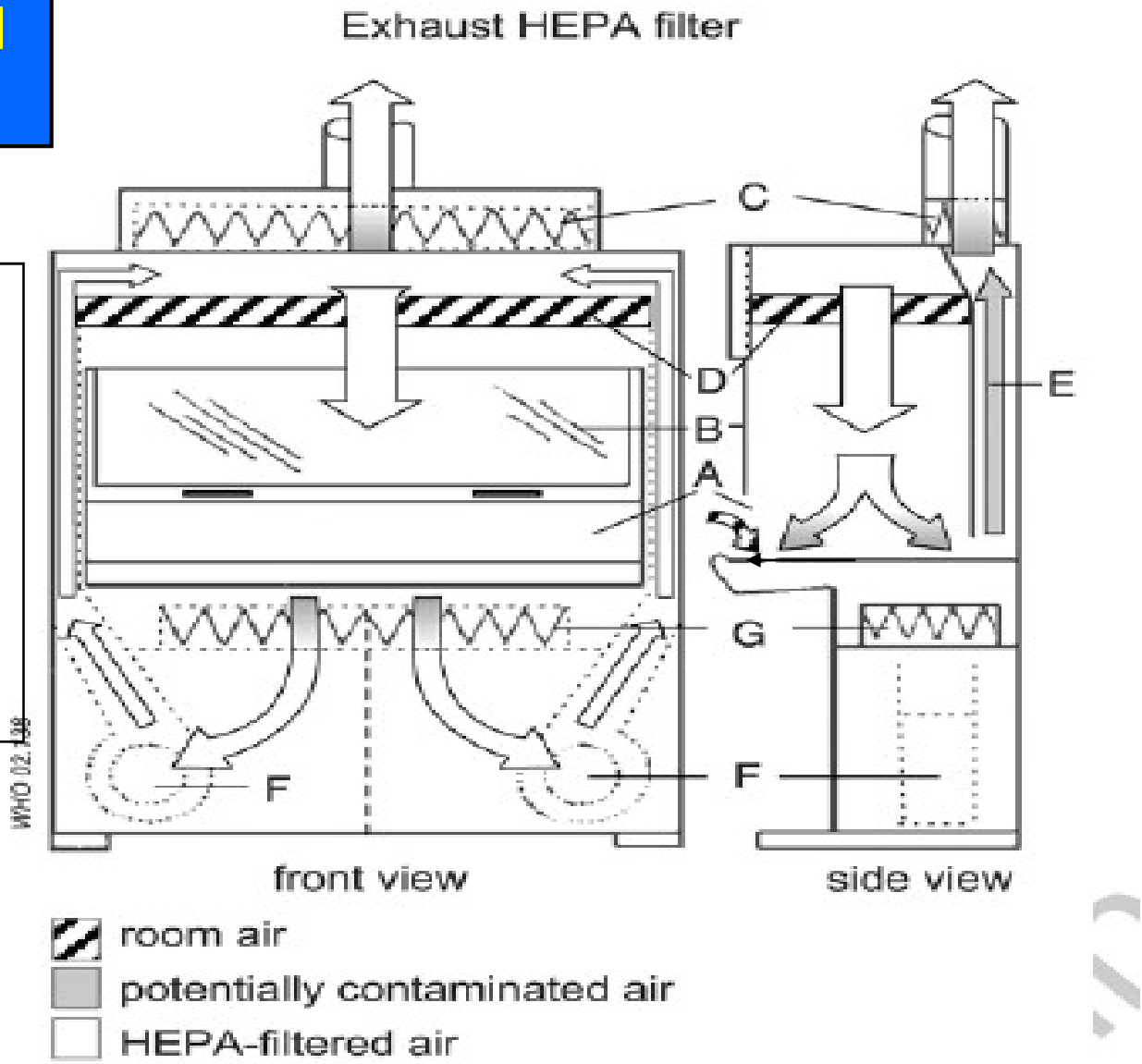


While HEPA filters are effective for trapping particulates and infectious agents, these filters **will not capture volatile chemicals or gases**

Schematic representation of a Class IIA1 biological safety cabinet.
A, front opening; B, sash; C, exhaust HEPA filter; D, rear plenum; E, supply HEPA filter; F, blower.

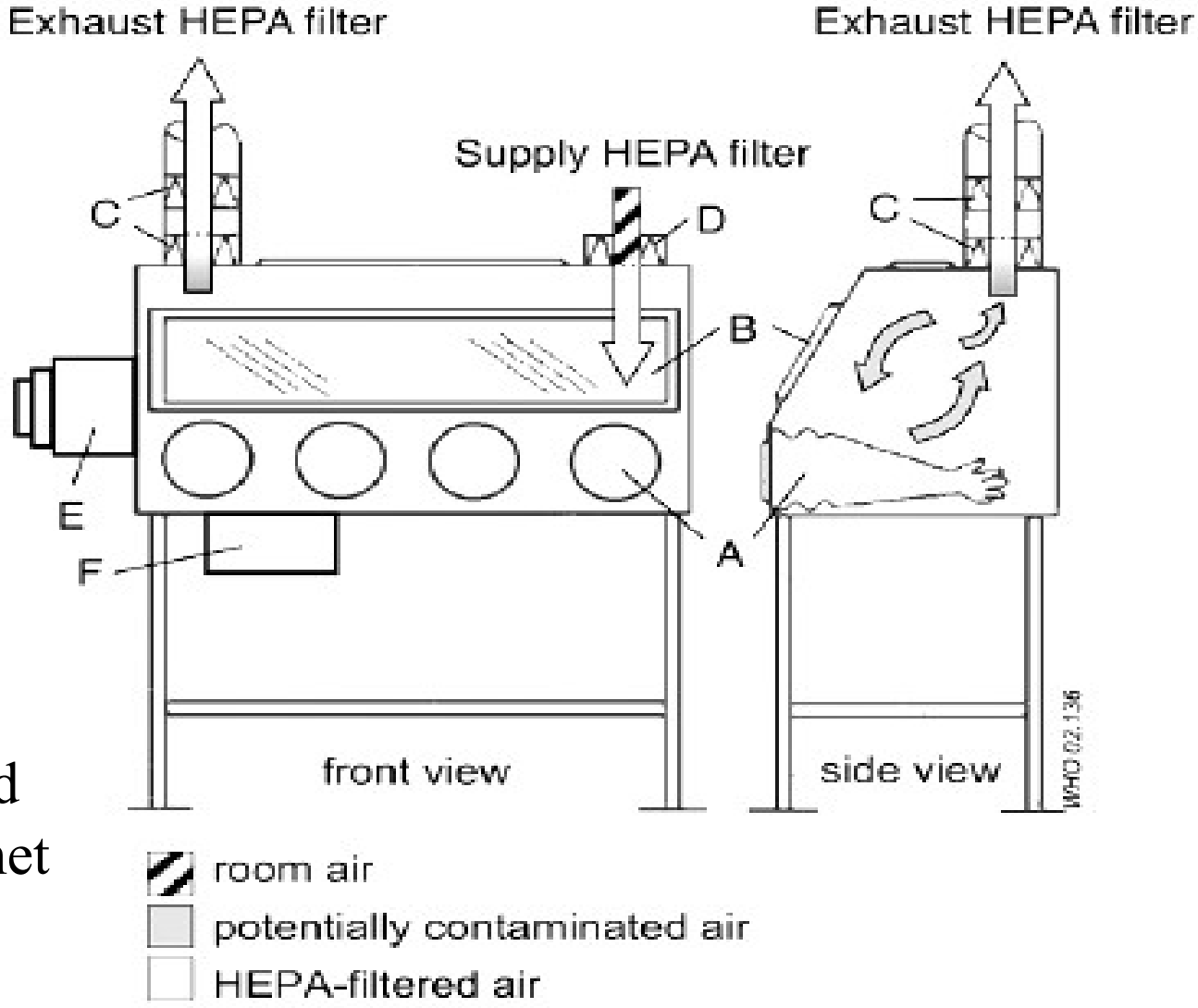
Class II, Type BI BSC

This cabinet may be used with etiologic agents treated **with minute quantities of toxic chemicals and trace amounts of radionuclides** required as an adjunct to microbiological studies if work is done in the directly exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.



Schematic diagram of a Class IIB1 biological safety cabinet.
A, front opening; B, sash: C, exhaust HEPA filter; D, supply HEPA filter; E, negative-pressure exhaust plenum; F, blower; G, HEPA filter for supply air. Connection of the cabinet exhaust to the building exhaust air system is required.

Class III, BSC



Totally enclosed ventilated cabinet of gas-tight construction

Schematic representation of a Class III biological safety cabinet (glove box). A, glove ports for arm-length gloves; B, sash; C, double-exhaust HEPA filters; D, supply HEPA filter; E, double-ended autoclave or pass-through box; F, chemical dunk tank. Connection of the cabinet exhaust to an independent building exhaust air system is required.

Differences between Biological safety cabinets

Table 9. ***Differences between Class I, II and III biological safety cabinets (BSCs)***

BSC	FACE VELOCITY (m/s)	AIRFLOW (%)		EXHAUST SYSTEM
		RECIRCULATED	EXHAUSTED	
Class I[a]	0.36	0	100	Hard duct
Class IIA1	0.38–0.51	70	30	Exhaust to room or thimble connection
Class IIA2 vented to the outside[a]	0.51	70	30	Exhaust to room or thimble connection
Class IIB1[a]	0.51	30	70	Hard duct
Class IIB2[a]	0.51	0	100	Hard duct
Class III[a]	NA	0	100	Hard duct

NA, not applicable.

[a] All biologically contaminated ducts are under negative pressure or are surrounded by negative pressure ducts and plenums.

Selection of Biological safety cabinet

Table 8. ***Selection of a biological safety cabinet (BSC), by type of protection needed***

TYPE OF PROTECTION	BSC SELECTION
Personnel protection, microorganisms in Risk Groups 1–3	Class I, Class II, Class III
Personnel protection, microorganisms in Risk Group 4, glove-box laboratory	Class III
Personnel protection, microorganisms in Risk Group 4, suit laboratory	Class I, Class II
Product protection	Class II, Class III only if laminar flow included
Volatile radionuclide/chemical protection, minute amounts	Class IIB1, Class IIA2 vented to the outside
Volatile radionuclide/chemical protection	Class I, Class IIB2, Class III

Biological Safety Cabinets

Type	Protection			Volatile Chemicals	Application
	Worker	Product	Environment		
1	Yes	No	Yes	Yes*	Enclose equipment or procedures with a potential to generate aerosols tissue homogenization, cage cleaning, etc).
2A	Yes	Yes	Yes	No	Cell culture and infectious material procedures
2B	Yes	Yes	Yes	Yes	Cabinet has total-exhaust, no air is re-circulated. This cabinet provides simultaneous primary biological and **chemical containment.**

III. Biological Safety Cabinet Placement

Air currents. Locate cabinets away from doors and windows, heavily traveled laboratory areas, and fans, air conditioners, and ventilation systems.

Biological safety cabinets should be installed in such a manner that fluctuations of the room supply and exhaust air do not cause the cabinet to operate outside its containment parameters.

Use of Flames: Alternates Electric incinerators, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized.

UV light: radiation should not take the place of wiping down the cabinet interior with a disinfectant or the practice of good aseptic technique

UV light

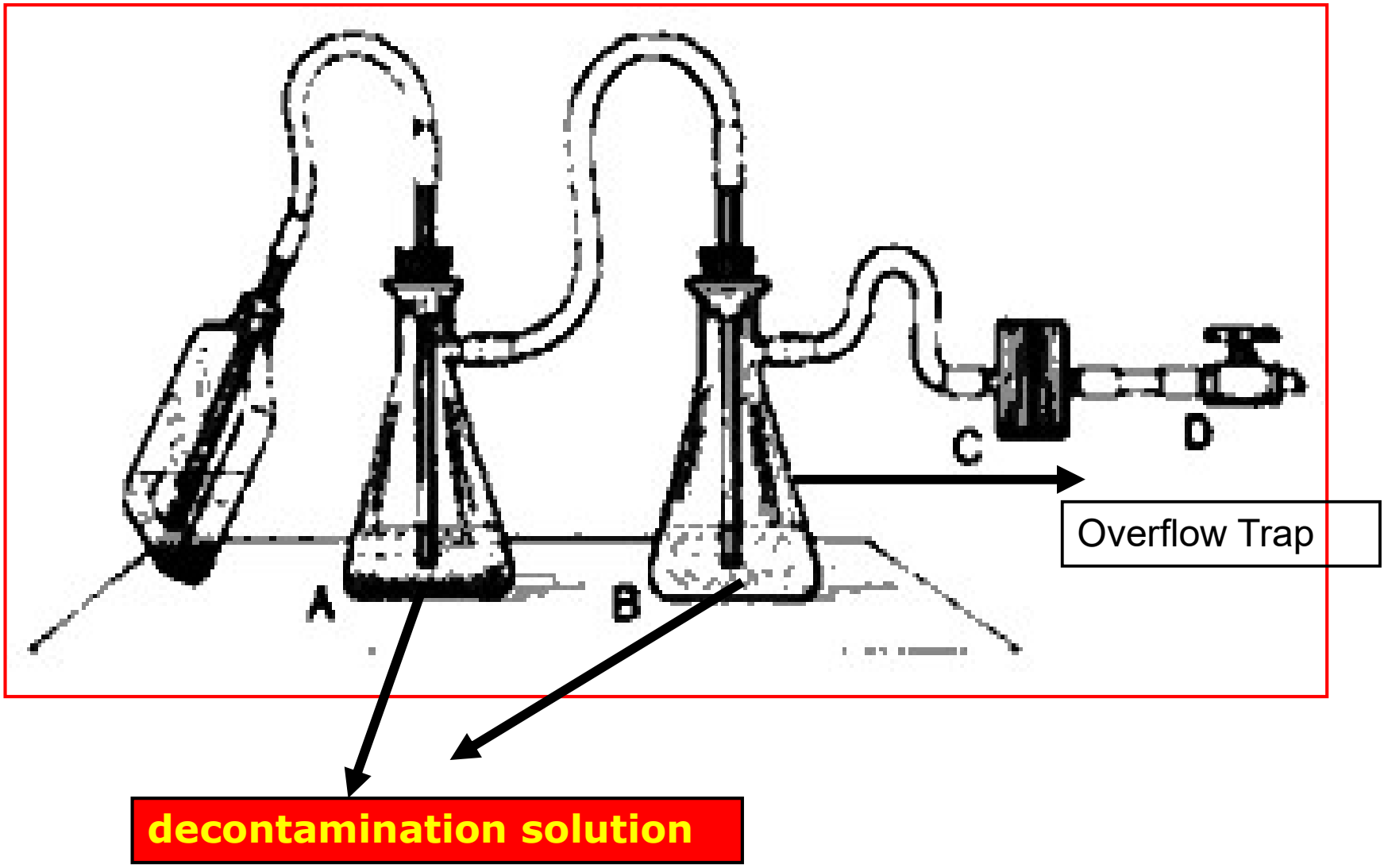
(Germicidal Wavelength-254nm)

The length of time a lamp will be effective depends on the number of hours it is in use.

- Lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted.
- UV light **does not** penetrate cracks or seams so will not disinfect the spill area under the work surface - a favorite hideout for fungal spores.
- Due to mercury content UV lights need to be disposed of as hazardous waste.
- Turn off UV light when the room is occupied - UV exposure can burn corneas and cause skin cancer.
- Be aware that UV lights can cause gas line tubing to deteriorate and present a gas leak hazard.

VII. Vacuum Lines

All vacuum lines should be protected from contamination and fluid intake.



VIII. Safe & Effective Work Practices--***Before work is started:***

Remove all unnecessary equipment and supplies from the cabinet, clutter alters air flow. **Check that air grilles are clear**.

•Turn on blower before using to remove particulates in the cabinet. Wait at least 15 minutes.

•Wipe down surface of cabinet interior with disinfectant.

•Prepare a checklist of materials necessary for the activity. Place supplies and needed equipment in the BSC before beginning work to minimize the number of arm-movement disruptions across the air barrier of the cabinet. Only items required for the immediate work should be placed in the BSC.

•Place absorbent towels and decontaminating solution near the cabinet to facilitate quick clean up of spills.

•**Wipe the exterior of supplies with a disinfectant**, particularly containers removed from a water bath. Segregate items that will remain clean from the ones that may become contaminated.

•**Wash hands and arms**, wear appropriate protective equipment for the work being done and to prevent skin flora from contaminating your work.

•**Adjust stool height** so that your neck and face are above the sash opening.

While working in the cabinet:

•In order to prevent air disturbances that can breach the air barrier, never have more than **one person** at a time use a cabinet - even six-foot cabinets.

•Delay manipulation of materials for approximately one minute after placing the hands/arms inside the cabinet. Do not rest arms on the front grille. Raising arms slightly will lessen disruption of air flow.

•**Work as far back** in the cabinet as practical - at least four inches inside the front grill edge.

•Move arms slowly and limit arm movement in and out of cabinet.

•As a general rule of thumb, keep clean materials at least one foot away from aerosol-generating activities to minimize the potential for cross-contamination.**The work flow** should be from "clean (left) to contaminated or dirty (right) ". Limit the movement of "dirty" items over "clean" ones.

•Note use of Pipetting aids negates pouring of liquids will eliminate the need to flame bottlenecks. Remove media with vacuum and replace with serological pipettes.

Tips to prevent contamination:

•Clean or treat water baths frequently .

•Clean the inside of incubators frequently. HEPA filters on incubator CO2 and air intake lines. Replace regularly.

•Use lab coats designated for working in the biological safety cabinet or tissue culture area, launder frequently. Use disposable sleeve guards if contamination has been a problem.

•Never pour media, remove with vacuum and replace with pipetting aids.

•Do not leave flasks of waste media in cabinet, clean after every use.

•On a regular basis, decontaminate under the air grilles and wherever parts are removable. Media is commonly splattered on the front grille allowing fungus to grow undetected on the under surface of the grille. UV light will not reach this hideout.

•Decontaminate the surface of trays used to transfer culture flasks between the incubator and the biological safety cabinet or microscope.

•Keep pipette aids cleaned, especially the nosepiece, and replace filters regularly.

•Clean and disinfect vacuum tubing.

•Most contamination problems can be traced to incubators, water baths, or using poor aseptic technique. Don't be lulled into believing that the use of UV lights or flaming containers will keep you contamination free.

After work is complete:

- Wipe down the surfaces of all containers and equipment with an appropriate disinfectant and remove from the cabinet.
- Leave blower on for several minutes with no activity so that any airborne contaminants will be purged from the work area.
- Wipe down the cabinet interior with disinfectant.
- Remove gloves and wash hands.

Spill Cleanup

IX. Spill Cleanup

For small spills in a biological safety cabinet:

- Leave the cabinet running.
- Wipe down all interior cabinet surfaces with appropriate disinfectant.
- Wipe down all supplies and equipment in cabinet.

For moderate spills in a biological safety cabinet:

- Leave the cabinet running.
- Cover spill area with absorbent paper.
- Pour disinfectant over towels from edges of spill to center, do not splatter.
- Allow 20-30 minutes of contact time.
- Determine if spill has gone beyond the work surface such as on the grilles or in side seams. **If yes, disassemble as much of cabinet as possible for decontamination.**
- If the cabinet has a catch basin below the work surface that may be involved in the spill, flood the basin with disinfectant. **Do not use 100% alcohol**, as its a flammable hazard. Clean basin after 20 minutes. Follow the manufacture's recommendations for decontamination procedures.
- Autoclave or wipe down all items in cabinet with disinfectant.
- Wipe down all interior surfaces.
- Let cabinet run for at least 10 minutes after cleanup.
- Place gloves and all clean up materials in biohazard bag for pick up or clear autoclavable bag and autoclave for 60 minutes at 121 degrees C (256 F).
- Wash hands.

For major spills in a biological safety cabinet

Contact Professional Help / manufacturer

References

- Australian/New Zealand Standard AS/NZS 2243.3:2002. Safety in laboratories Part 3: Microbiological aspects and containment facilities.(www.standards.com.au and www.standards.com.nz)

- Canada. Minister of Health. Population and Public Health Branch. Center for Emergency Preparedness and Response. 2004. "Laboratory Biosafety Guidelines". 3rd Edition http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index.html2.CDC/NIH. 1999. Biosafety in Microbiological and Biomedical Laboratories" 4th Edition. Government Printing Office http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm (5th edition in press).

- 3.National Institutes of Health. 2002. *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* 59 FR 34496 (July 5, 1994), as amended. The current amended version of the *NIH Guidelines* can be accessed at: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

- 4.European Union. 2000. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.(seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EC Official Journal of the European Communities L262/21. October 17,2000

- 5.World Health Organization. 2004. "Laboratory Biosafety Manual". 3rd Edition. WHO, Geneva. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/index.html