

Biosafety in Biomedical Laboratories



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Biohazard

- An agent of biological origin that can cause disease in humans
 - Microorganism
 - Toxin
 - Allergen



Biorisk definition

(adapted from ISO/IEC Guide 51:1999)

- combination of the **probability** of occurrence of harm and the **severity** of that harm where the source of harm is a biological agent or toxin

Biosafety definition

- All measures that prevent **accidental** exposure at biological agents and infections and their release in environment

(WHO Biosafety manual)

Biorisk assessment definition

(adapted from OHSAS 18001:2007)

Process of **evaluating** the biorisk(s) arising from a biohazard(s), taking into account the **adequacy of any existing controls**, and deciding whether or not the biorisk(s) is acceptable

According to Kaplan and Garrick,

risk analysis consists of answering **three** specific questions:

- **what** can happen?
- what is **the chance** that it will happen?
- if it happens, what are **the consequences**?

Biosafety risks are defined as a function of the likelihood of infection by the agent and the likelihood of exposure through an infectious route based on the procedures and work practices and the consequences of disease assuming infection

4 scenarios

1. Risk to individuals in the laboratory
2. Risk to individuals outside the laboratory
3. Risk to animals outside the laboratory (the animal community)
4. Risks to humans and animals resulting from a secondary exposure

Routes of exposure

- droplets or droplet nuclei (upper or lower respiratory tract)
- compromised skin or direct injection into the blood stream
- the mucosal membranes
- the gastrointestinal tract

Risk Assessment

Assessing the risk for acquiring a laboratory associated infection is influenced by the following factors:

- **Pathogenicity**
- **Route of Transmission**
- **Agent Stability**
- **Infectious Dose**
- **Susceptibility**
- **Concentration and Volume**
- **Origin**

■ Pathogenicity

- The greater the pathogenicity of the infectious or suspected infectious agent, the more severe is the potentially acquired disease, and so the higher is the risk.
- For example:
 - Since *Staphylococcus aureus* rarely causes a severe or life-threatening disease in a laboratory situation, it is assigned to **BSL-2**.
 - **Ebola**, **Marburg**, and **Lassa** fever viruses cause diseases with high mortality rates and have no vaccines or treatment, so **BSL-4** is the appropriate level to work with those viruses.
 - Work with human **HIV** and **hepatitis B** virus is done at **BSL-2** because they are not transmitted by the aerosol route, even though potentially lethal disease can result from exposure. **For hepatitis B, there is also an effective vaccine available.**

■ Route of Transmission

- Agents transmitted by the aerosol route have caused the most laboratory infections, versus agents transmitted **parenterally** or by **ingestion**.
- When planning work with an unknown agent with an uncertain mode of transmission, the potential for aerosol transmission must be considered due to the higher risk.

■ Agent Stability

- Desiccation, exposure to sunlight or ultraviolet light, exposure to chemical disinfectants and other factors can affect the agent's stability in the environment.

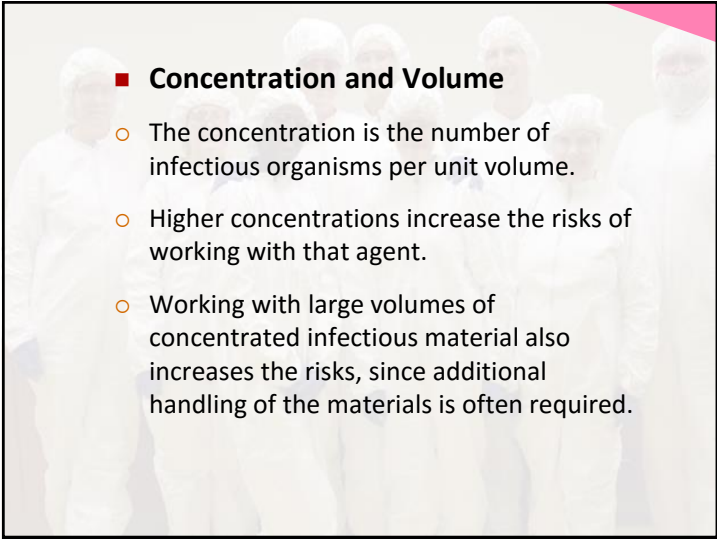
■ Infectious Dose

- Infectious dose can vary from one to hundreds of thousands of units.



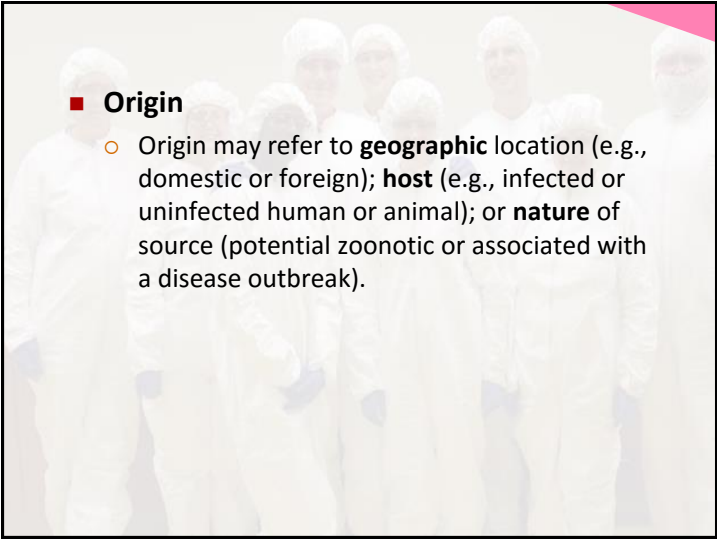
■ Susceptibility

- The infectious dose is affected by the individual's resistance,
- Thus, susceptibility may be greater in **pregnant**, undergone **surgery**, are receiving **immunosuppressant medications** (including steroids), or who have **systemic infections**



■ Concentration and Volume

- The concentration is the number of infectious organisms per unit volume.
- Higher concentrations increase the risks of working with that agent.
- Working with large volumes of concentrated infectious material also increases the risks, since additional handling of the materials is often required.



■ Origin

- Origin may refer to **geographic** location (e.g., domestic or foreign); **host** (e.g., infected or uninfected human or animal); or **nature** of source (potential zoonotic or associated with a disease outbreak).



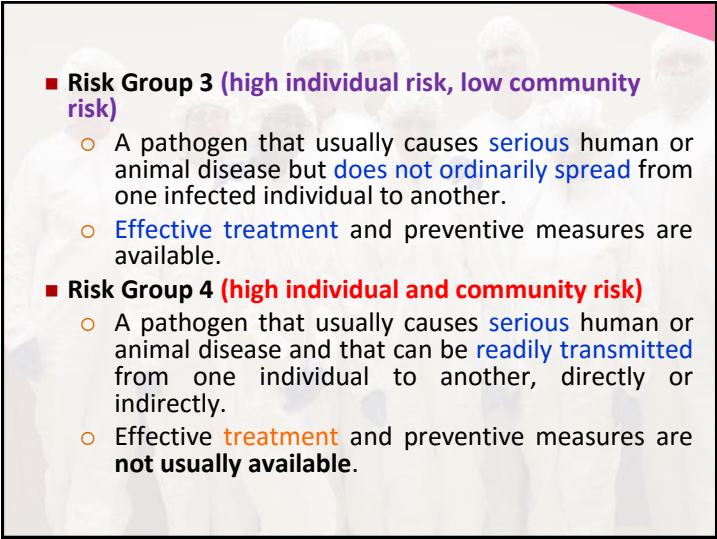
Classification of infective microorganisms by risk groups

■ Risk Group 1 (no or very 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.
- **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**.



Major Safety Concerns

- Safe handling + containment of infectious agents
- Recombinant DNA (rDNA),
- Management of Biohazard Waste
- Blood borne Pathogen Exposure
- Chemical Safety
- Use of Animals in Research
- Radioisotope Use
- Human Subject Protection



Main Principle of Biosafety: Containment

- **Purpose of Containment:** to reduce or eliminate exposure of lab workers and others to hazardous biological agents
 - Bacteria, viruses, fungi, parasites
 - Recombinant DNA
 - Potentially dangerous cell lines



General Principles of Biosafety

- Three elements of containment:
 - Personal protective equipment
 - Facility design
 - Lab practice and techniques
- Extent of containment depends on
 - level of risk of exposure
 - nature of agent

Examples of Safety Equipment

- Biological Safety Cabinets
 - Protect you and cells you are working with!!!
- Centrifuge Safety
- Gloves, lab coats, gowns, face shield, goggles, shoe covers, respirators

Biological Safety Cabinets (BSC)

- **Class I and II** BSCs provide **effective containment system** for safe handling of moderate/high risk organisms (BSL2 and BSL3 agents)
- Class II BSCs also protect the **research material** itself through high efficiency particulate filtration (HEPA) of the air flow down across the work surface

Biosafety Cabinets (BSCs)

- Provide product, personal and environmental protection.
- Various classes of BSCs are available
 - Amount of air exhausted
 - Amount of employee protection

Class I biosafety Cabinet

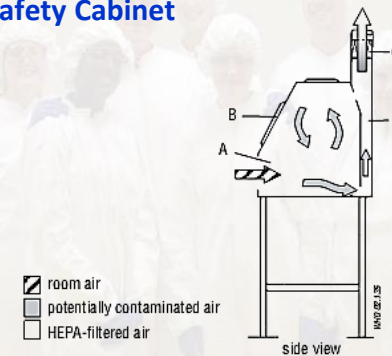


Figure 6. Schematic diagram of a Class I biological safety cabinet.
A, front opening; B, sash; C, exhaust HEPA filter; D, exhaust plenum.

Class IIA biosafety Cabinet

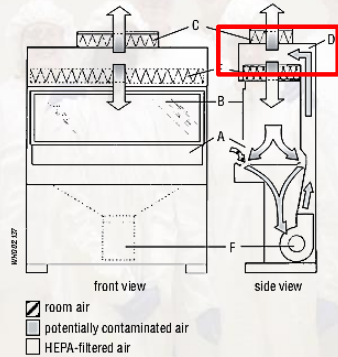


Figure 7. Schematic representation of a Class IIA1 biological safety cabinet.
A, front opening; B, sash; C, exhaust HEPA filter; D, rear plenum; E, supply HEPA filter; F, blower.

Class IIB1 biosafety Cabinet

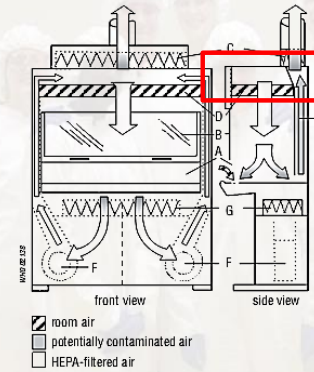


Figure 8. Schematic diagram of a Class IIB1 biological safety cabinet.
A, front opening; B, sash; C, exhaust HEPA filter; D, supply HEPA filter; E, negative-pressure exhaust plenum; F, blower; G, HEPA filter for supply air. Connection of the cabinet exhaust to the building exhaust air system is required.

Class III biosafety Cabinet

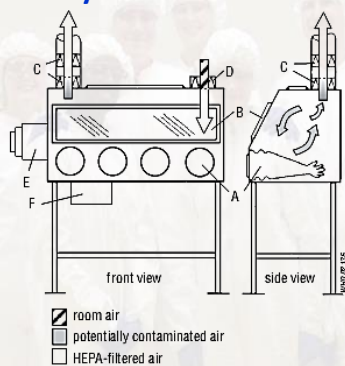


Figure 9. Schematic representation of a Class III biological safety cabinet (glove box).
A, glove ports for arm-length gloves; B, sash; C, double-exhaust HEPA filters; D, supply HEPA filter; E, double-ended autoclave or pass-through box; F, chemical drain tank. Connection of the cabinet exhaust to an independent building exhaust air system is required.

Facility Design and Construction (Secondary Barriers)

- Separation of lab work areas from areas of public access
- Availability of decontamination stations (autoclaves and hand-washing facilities)
- Separate ventilation systems
- Controlled access labs

Biosafety Levels (BSLs)

- A **biosafety level** is the level of **biocontainment** precautions required to isolate dangerous **biological agents** in an enclosed facility
- Each level represents a combination of
 - lab practices,
 - techniques,
 - safety equipment
 - lab facilitiesdesigned to minimize release and exposure.

Biological Safety Level 1



❖ BSL 1

- ❖ BIOSAFETY 1 is 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.
- ❖ Lab is not necessarily separated from general traffic patterns in the building.

❖ BSL 1 (cont)

- ❖ Work is generally conducted on open bench tops using standard microbiological practices.
- ❖ Special containment equipment or facility designs is not required nor generally used.
- ❖ Insect and rodent pest control program should be in effect
- ❖ Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology.

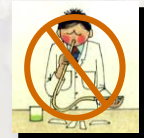
❖ BSL 1(cont)

- ❖ The following standard special practices, safety equipment, and facilities apply to agents assigned to all Biosafety levels.
- ❖ Standard Microbiological Practices
 - ❖ Access to the laboratory are limited or restricted when experiments or work with specimens and cultures are in progress
 - ❖ After handling viable materials and animals hands must be washed after removing gloves and before leaving the laboratory.



❖ Standard Microbiological Practices

- ❖ Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas.
- ❖ Persons who wear contact lenses in laboratories should also wear goggles or a face shield.
- ❖ Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- ❖ Mouth pipetting is prohibited; mechanical pipetting devices are used.



❖ Standard Microbiological Practices

- ❖ All procedures are performed carefully to minimize the creation of **splashes or aerosols**.
- ❖ Work surfaces are **decontaminated** at least once a day and after any spill of viable material.
- ❖ All cultures, stocks, and other regulated wastes are **decontaminated** before disposal by an approved decontamination method.
- ❖ Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory.
- ❖ Materials to be decontaminated at off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

❖ Safety Equipment

- ❖ Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
- ❖ It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
- ❖ Gloves should be worn if the skin on the hands is broken or if a rash exists.

❖ Safety Equipment

- ❖ Protective eyewear should be worn for anticipated splashes of microorganisms or other hazardous materials to the face.



- Lab coat or apron
- Safety glasses or goggles
- Gloves as needed

❖ Laboratory Facilities

- ❖ Each laboratory contains a sink for hand washing.
- ❖ The laboratory is designed so that it can be easily cleaned.
- ❖ Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

❖ Laboratory Facilities (cont)

- ❖ Laboratory furniture is durable. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- ❖ If the laboratory has windows that open, they are fitted with fly screens.



Biosafety Level 1 (BSL 1)

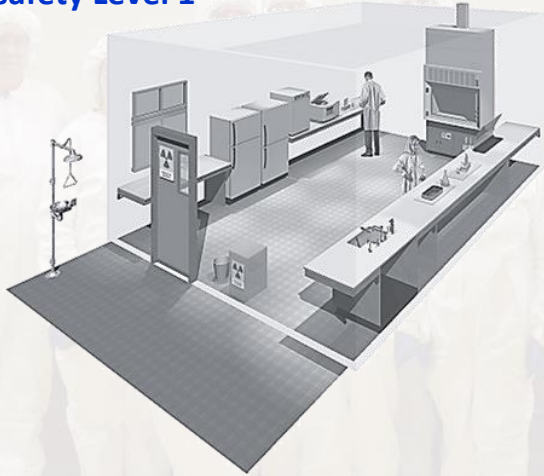
Use BL-1 when working with:

- Well characterized agents **“Avirulent organisms”**
- Agents that are not known to cause disease in health humans
- Agents that are of minimal hazard to lab personnel and the environment

Examples of BL-1 Agents:

- *E. coli* JM109, DH5a
- *Saccharomyces cerevisiae*

Biosafety Level 1



Biological Safety Level 2



❖ BIOSAFETY CONTAINMENT LEVEL 2

- ❖ under normal circumstances, are unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment
- ❖ Laboratory exposures rarely cause infection leading to serious disease;
- ❖ effective treatment and preventive measures are available and the risk of spread is limited.
- ❖ They are a moderate individual risk and limited community risk.
- ❖ **Not spread by aerosol.**

Examples of BL-2 agents:

- Human blood or body fluids
- *E. coli* O157:H7
- *Clostridium botulinum*
- Retroviral vectors
- Hepatitis A,B+C, HIV, some Salmonellae
- Cell culture work and some rDNA

Biosafety Level 2 (BL-2)

- A **biohazard** sign should be present and visible.
- All the surfaces in the laboratory should be readily cleanable and impervious
- Adequate illumination
- Eyewash facility
- Negative air pressure
- Autoclave available
- Biological safety cabinet Class II
- Lab must be separated from public areas

Biosafety Level 2 (BL-2)

Standard Work Practices

- Use mechanical pipetting devices
- Wash hands frequently
- Minimize splashes and aerosols
- Decontaminate work surfaces daily
- Handle wastes properly
- Maintain insect and rodent control program

Biosafety Level 2 (BL-2)

Special Practices

- Place used slides and coverslips in sharps containers, never in any other receptacle.
- Sharps containers are:
 - Red in color
 - Marked with the biohazard symbol
 - Puncture resistant
 - Leak proof
- **DON'T** break, bend, re-sheath or reuse syringes or needles

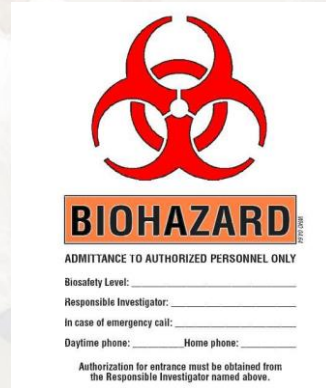


Biosafety Level 2 (BL-2)

Personal Protective Equipment (PPE)

- Lab coat or apron
- Safety glasses or goggles
- Gloves
- Biosafety cabinet
 - Aerosols or splashes
 - Large volumes
 - High concentrations

- Mandatory Warning Sign
- Designate Biosafety Level
- Special Entry Procedures
 - Immunizations
 - PPE
- Contact Information



Biosafety Level 2

Special Practices

Needles & Sharps Precautions (cont.)

- DON'T place needles or sharps in office waste containers



2.4

Biosafety Level 2

Special Practices

Needles and Sharps Precautions (cont.)

- DON'T touch broken glass with hands



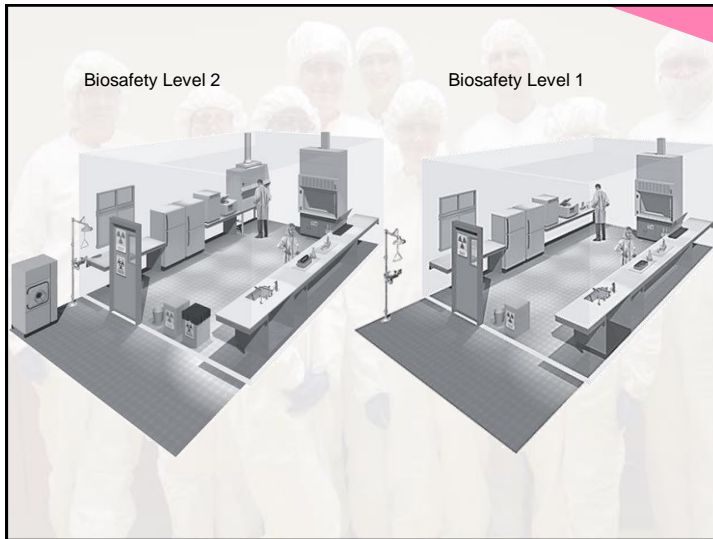
2.4

Biosafety Level 2

Special Practices

- Identify “clean” and “contaminated” areas
 - Use appropriate warning signs
- Decontaminate work surfaces
- Report spills and accidents
- Remove gloves, lab coats before leaving work area
- No animals in laboratories

2.4



❖ BIOSAFETY CONTAINMENT LEVEL 3

- ❖ Risk Group 3 infectious agents are pathogens that
 - ❖ usually cause **serious** human or animal disease,
 - ❖ or which can result in serious economic consequences, but do not ordinarily spread by casual contact from one individual to another (high individual risk, low community risk),
 - ❖ or that can be treated by antimicrobial or antiparasitic agents.
- ❖ Risk Group 3 pathogens include
 - ❖ bacteria such as anthrax, Q Fever, tuberculosis, and
 - ❖ viruses such as hanta viruses, Human immunodeficiency viruses (HIV - all isolates), eastern and western equine encephalitis viruses, **SARS and West Nile viruses**

❖ PROCEDURES REQUIRED TO CONTAIN RISKGROUP 3 ORGANISMS

- ❖ **Laboratory staff** must be **fully trained** in the handling of pathogenic and other hazardous material, in the use of safety equipment, disposal techniques, handling of contaminated waste, and emergency response.
- ❖ **Standard operating procedures** must be **pasted** in visible spot.
- ❖ Equipment must include an **autoclave** and a certified **HEPA** filtered class II biological safety cabinet
- ❖ All activities involving infectious materials to be conducted in biological safety cabinets or other appropriate combinations of personal protective and physical containment devices.

❖ PROCEDURES REQUIRED TO CONTAIN RISKGROUP 3 ORGANISMS

- ❖ Personal protective equipment should include head covers and dedicated footwear, gloves, appropriate respiratory protection
- ❖ Specialized design and construction of laboratories, with controlled access double door entry and **body shower**
- ❖ **Negative air pressure** at all times, **no recirculation**, **HEPA filtration** must be used

2.5

Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

- Separate building or isolated zone
- Double door entry
- Directional inward airflow
- Single-pass air; 10-12 air changes/hour

2.5

Biosafety Level 3

Laboratory Facilities (Secondary Barriers)

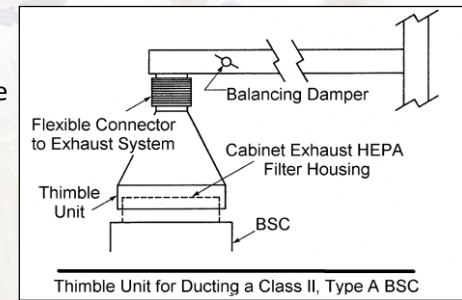
- Enclosures for aerosol generating equipment
- Room penetrations sealed
- Walls, floors and ceilings are water resistant for easy cleaning

2.5

Biosafety Level 3

Safety Equipment (Primary Barriers)

- BSC class II or III to manipulate infectious material



2.5

Biosafety Level 3

Safety Equipment (Primary Barriers)

- Respiratory protection may be indicated



2.5

Biosafety Level 3

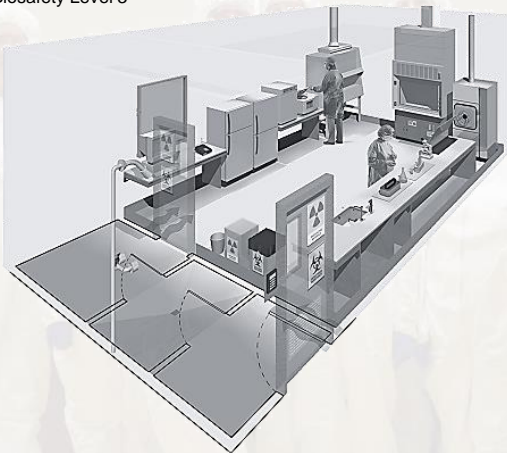
Special Practices

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



2.5

Biosafety Level 3



❖ Biological Safety Level 4



BIOHAZARD

❖ BSL- 4

- ❖ Designed for use with:
 - ❖ Highly toxic/infectious agents
 - ❖ Agents that are at a very high risk for forming infectious aerosols
 - ❖ Life threatening agents
 - ❖ Ex. Ebola, Marburg, Lassa

❖ Special Practices

- ❖ Only those that work in the BSL-4 lab will be allowed entry
- ❖ Immuno-compromised individuals such as children and pregnant women are not allowed to enter
- ❖ A logbook must be signed each time one comes in or out of the lab with the date and time

❖ Special Practices

- ❖ The doors must have a universal hazard label with....
 - Description of the infectious agent (s)
 - Responsible director
 - PPE required such as respirators and any immunization requirements
- ❖ All lab personnel are trained specially for BSL-4 by lab director

❖ Special Practices

- ❖ Lab personnel must be vaccinated for the agents
 - Present in the lab
 - That have potential to be in the lab
- ❖ Baseline serum samples are collected and stored for serological surveillance
 - Makes sure the personnel have sufficient antibodies for the pathogens they are working with
- ❖ Biosafety manual is adopted for that lab

❖ Special Practices

- ❖ Entering the lab
 - Personnel must remove personal clothing in an outer room and leave it
 - All clothing and PPE is provided and expected to be worn
- ❖ Exiting the lab
 - Personnel remove lab clothing in the inner room **after** taking a decontaminating shower
 - The lab clothing is autoclaved then cleaned
 - They exit through the outer room where their personal clothes are

❖ Special Practices

- ❖ Supplies/materials are brought into the lab by a double door autoclave that....
 - is secured so that materials can be removed from the interior door
 - is decontaminated after each use
- ❖ Plastic is substituted for glass when possible
 - Eliminate sharps such as capillaries, scalpels, needles and syringes
 - Broken glass should be handled with a dust pan and broom
 - Non-disposable and disposable sharps collected in a hard container to be transported to an autoclave for decontamination; disposables are thrown out

❖ Special Practices

- ❖ Any viable materials transferred from the class III Biological Safety Cabinet or the lab itself must be put in a primary container and then in a non- breakable secondary container
 - It is then removed by an airlock
 - Pressurizes the chamber so that minimal air is emitted when each door opens one at a time
 - Can also decontaminate the containers

❖ Special Practices

- ❖ Lab equipment is decontaminated after use, after spills or before any repairs
- ❖ Any material not related is not permitted in the laboratory
- ❖ A system is set up for emergencies such as exposure to infectious agents and also employee absenteeism on safety
 - Quarantine
 - Medical care for those with lab acquired illness
 - Records are kept for all accidents

❖ Safety Equipment (primary barriers)

- ❖ Class III biological safety cabinets for handling infectious material or....
- ❖ Class II biological safety cabinet in conjunction with a positive pressure suit ventilated by a life support system
- ❖ BSC must be validated on a 12 month period
- ❖ The PPE required is a...
 - one-piece jumpsuit
 - gloves
 - closed toe shoes
 - All will be removed in the inner room after showering

❖ Laboratory Facility (secondary barrier)

- ❖ BSL-4 labs are housed in separate buildings or isolated within a building with lower BSL labs
- ❖ Outer and inner change rooms
- ❖ Walls, floors and ceilings have sealed internal shells
 - keep pests out
 - Liquid and heat stable for decontaminating purposes
- ❖ Drains have chemical disinfectant traps attached to liquid waste decontamination system
 - All waste must be decontaminated including shower waste
- ❖ HEPA filters in vents for sterile air
 - Filter air exhaust from the class III and II biological safety cabinets and
 - From ventilated positive pressure suits worn in class II safety cabinets

❖ Laboratory Facility (secondary barrier)

- ❖ Windows are break resistant
- ❖ A dunk tank, fumigation chamber or airlock is provided for safe passage of materials outside/inside lab that cannot be decontaminated in the autoclave
- ❖ A non-recirculation ventilation system is provided with directional inward airflow
 - Air flows from the outside of the lab to the inside but not vice versa

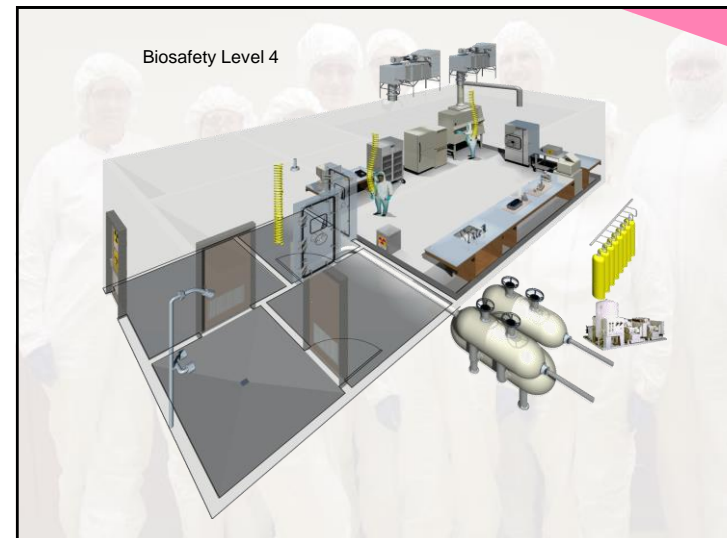


Figure 12. International radiation hazard symbol



Safety Resources

**Biosafety in
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and Biomedical
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5th Edition



U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control and Prevention
National Institutes of Health

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Laboratory biosafety manual
Third edition



World Health Organization
Geneva
2004