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# HIERARCHICAL BIOSAFETY IMPLEMENTATION IN MEDICAL LABORATORIES: ALIGNING BIOSAFETY LEVELS (BSLs) TO PREVENT INFECTION.

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**ABSTRACT** The Biosafety Level (BSL) framework is a globally accepted, four-tiered biocontainment system developed by the CDC and NIH to support microbiological and diagnostic research while protecting laboratory personnel, the public, and the environment. Risk classification is based on a pathogen's infectivity, disease severity, transmission route, and procedural nature. BSL-1 applies to minimal-risk agents using open bench work and basic P.P.E.BSL-2 involves moderate-risk pathogens in a clinical setting and requires restricted access, class II biosafety cabinets, and autoclaving. BSL-3 covers dangerous airborne pathogens, which require sealed facilities with regulated airflow. BSL-4 is the highest safety level, used for fatal chemicals with no treatment, necessitating airlocks, decontamination showers, Class III cabinets, or positive-pressure suits. This research synthesizes peer-reviewed literature from PubMed and Scopus to evaluate BSL-based infection prevention methods in medical laboratories in line with NABL ISO 15189.

**KEYWORDS :** Biosafety Level [BSL], National Institutes of Health [NIH]

## INTRODUCTION

Biosafety levels are safety precautions to reduce the risk of laboratory personnel exposure to pathogens and to protect the workers, community, and environment. The CDC has a hierarchy of 4 levels of containment (BSL 1-4), each with particular containment techniques, safety equipment, and facility architecture.

Basic safety precautions from lower BSLs apply to most procedures, with enhanced requirements as risk levels increase, ensuring the laboratoryworker and the environment remain protected against biological hazards[1].

### Agent summary statements

Theeffectiveness of BSL shouldfulfilany one of the following four criteria:

1. Details the pathogen's risks (e.g., potential for laboratory-acquired infections (LAIs) and pathogenicity).
2. Recommended Biosafety Level (BSL): Specifies the appropriate containment level (1-4) for handling the agent in laboratories.
3. Outlines necessary practices, safety equipment, and facility safeguards (e.g., use of biological safety cabinets).
4. Pathogen-specific covers epidemiology, modes of transmission, stability, and therapeutic availability, often summarized in [Pathogen Safety Data Sheets] [2].

### Risk Assessment and Biosafety Framework

It is based on standard laboratory practices. However, the absence of an agent summary statement does not indicate that an agent is safe for BSL-1 handling. Laboratory directors must conduct an independent risk assessment before initiating work on any novel agent or procedure. Directors may modify precautions in consultation with colleagues, institutional biosafety committees, biosafety officers, and professional associations.

A modern biosafety theoretical framework is emerging to guide life sciences research beyond traditional laboratory settings. However, disagreement exists within the scientific community regarding the "biosafety" definition[3].

**Three primary perspectives dominate:**

1. Sound laboratory practices for infectious organisms and biological hazards.
2. environmental threats from the release of genetically modified or other organisms affecting biodiversity.
3. potential harm to human health and the environment from biotechnology industry applications. This diversity of perspectives reflects the evolving nature of biosafety considerations in contemporary research.

### Goal of biosafety

- to minimize or eliminate the occurrence and distribution of biological threats to humans and other life forms,
- to protect life forms, ecosystems, and global health and safety [4].

**Stages of historical phases of development of BSLs.**

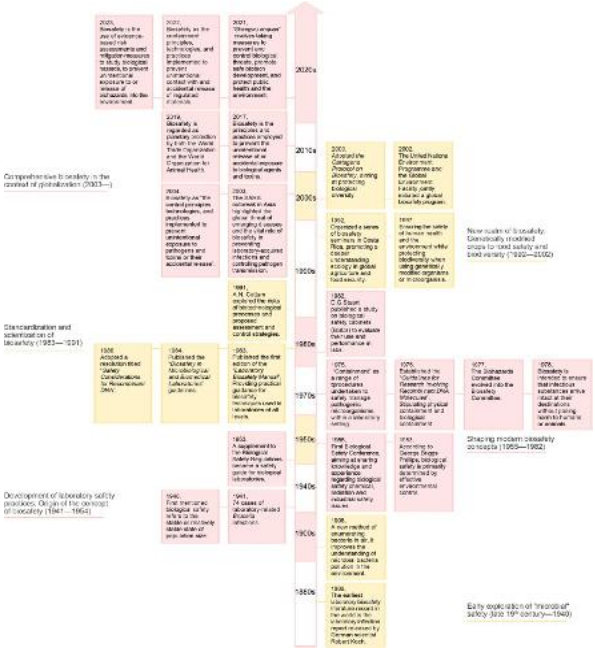

**FIGURE 1: The development of biosafety**

## TYPE

## BIOSAFETY LEVEL 1

The most basic level of biocontainment designed to work with microorganisms that do not naturally cause human disease. While these organisms are generally safe, they can pose a potential health risk to immunocompromised individuals, laboratory personnel, and the surrounding environment under certain circumstances. BSL-1 represents the entry-level biosafety designation and requires only standard microbiological practices.

### Microorganisms Handled in BSL-1 Laboratories

These are classified as GRAS (Generally Regarded as Safe) organisms. Common examples include:

- ***Bacillus licheniformis*** - A non-pathogenic bacterium commonly used in research and industrial applications

- ***Bacillus subtilis*** - A harmless soil bacterium frequently employed in molecular biology research
- ***K-12 E. coli***—A laboratory strain of *Escherichia coli* with limited virulence factors, extensively used in genetic engineering and biotechnology[5].

## PROCEDURE AND PRACTICES

- Wash hands after handling hazardous materials and before leaving the lab.
- No eating, drinking, smoking, cosmetics, contact lenses, or food storage in lab areas.
- Minimize the use of needles, scalpels, and glass pipettes.
- Avoid splashes/aerosols; decontaminate work surfaces after use with BSL-1 agents.
- Wash hands thoroughly before leaving the laboratory.

**Safety Equipment for BSL1**

When working inside a BSL-1 laboratory, there are several available options for protective clothing and personal protective equipment to help reduce exposure risk to staff, the community, and the environment:

1. It is recommended that you wear a standard lab coat as a protective barrier.
2. Protective eyewear must be worn during procedures that may produce aerosols and have splashes of potentially hazardous material.
3. Personnel should wear gloves when handling hazardous or potentially hazardous materials.

**Laboratory Facilities for BSL-1**

1. There is an available sink for hand washing.
2. Doors should be installed to provide separate access to work areas.
3. The laboratory bench must be resistant to water, heat, organic solvents, acids, and bases.
4. Laboratory chairs must be made ofnon-porous materials and easily decontaminated[6].

**FIGURE 2:BIOSAFETY LABORATORY LEVEL 1 [NCB 1]**
**IMAGE SOURCE:** *LABORATORY BIOSAFETY MANUAL, 3RD EDITION*, WHO; 2005

## BIOSAFETY LEVEL 2

Laboratories that are working with moderate-risk agents in the community and that can be associated with humans utilize BSL2 practices, equipment, and facility design. Examples of microorganisms that have been assigned to this containment level are the hepatitis B virus, HIV, *Salmonella, andToxoplasma spp*. When using good microbiological techniques, there is a low potential for producing splashes or aerosols from the agents used; therefore, agents can be safely used in activities that are conducted on the open bench.
Procedures and Practices

- Restrict entry; keep doors closed during research.
- Biohazard Signs: Place at all access points for contaminated equipment.
- Wash hands after handling biological material, removing gloves, and before leaving.
- Eating, drinking, or any consumption is prohibited in the lab.
- Use only when necessary; handle with care and dispose safely.
- Use goggles if splashes or sprays are possible outside cabinets.
- Use secondary containment and carts; avoid public space contact.[7].

**Safety Equipment for BSL2**

It contains all safety equipment and precautions of BSL-1 laboratories, with additional requirements for biological hazard protection.

Engineering Controls A biological safety cabinet or equivalent containment device must be provided for procedures creating infectious aerosols, including pipetting, centrifugation, grinding, blending, shaking, sonicating, and handling open containers of infectious material.

Personal Protective Equipment must be worn when handling hazardous materials and removed before leaving the laboratory. Eye and face protectionmust be worn to protect against anticipated splashes, sprays, and other potential exposures.

**BSL-2 Laboratory Design & Equipment Requirements**

1. Self-closing doors that restrict entry to authorized personnel only.
2. Lab design must facilitate easy cleaning and disinfection of all surfaces.
3. Outdoor windows must be equipped with screens or sealed to minimize airflow into the laboratory.
4. Class II BSCs must be positioned to not obstruct air supply/exhaust flow and placed away from external doors and high-traffic areas.
5. All BSCs require annual certification. Class II BSC exhaust can be safely recirculated within the lab.
6. An autoclave, incinerator, or equivalent waste destruction method must be readily available.
7. All vacuum lines must include liquid disinfectant traps for protection and priming[8].

**FIGURE 3: BIOSAFETY LABORATORY LEVEL 2**
**IMAGE SOURCE:** *LABORATORY BIOSAFETY MANUAL, 3RD EDITION*, WHO; 2005

## BIOSAFETY LEVEL 3

BSL3 builds upon the safety measures and practices outlined in BSL1 and BSL2. All the engineering controls, safety devices,or any special facilities will also be included in BSL-3. The agents considered biohazards will be either indigenous (native to the region) or foreign (not native but found in the region) and may have the potential to cause severe or fatal illness (e.g., SARS coronavirus, *Mycobacterium*, and*Chlamydia psittaci*) through respiratory transmission. Hazards will exist in similar fashion in BSL3 to BSL1 and BSL2; however, the major difference in BSL3 labs is the mode of transmission associated with hazardous agents. Additional measures are required for directional airflow (negative pressure) in BSL3 labs to ensure that air travels from non-laboratory environments to laboratory environments[9].

**Procedures & Practices**

- Long hair must be secured to prevent contact with hands, specimens, containers, or equipment.

- Eating, drinking, smoking, applying cosmetics, handling contact lenses, and storing food are prohibited in laboratories.
- Food consumption must occur in designated areas outside the laboratory.
- Proper removal of PPE is critical of prevent self-contamination and cross-contamination to non-designated areas.

Removal procedures must prevent the transfer of infectious material outside the laboratory [10]

**Safety Equipment for BSL 3**

1. Facility, regardless of type used. Some types of BSL3 PPE can be reused but must be disinfected before washing.
2. Eye, face, and respiratoryoperations within aBSL3 facility that involve dealing with pathogenic materials must occur within a Class II/Class III BSC.
3. Personal protective equipment (PPE) for BSL3 facilities includes laboratory attire with solid fronts, tiebacks, and/or wrap-around gowns; scrubs; or coveralls and must not leave the area. Protection must be worn in areas occupied by infected animals.

**Laboratory Facilities for BSL3**

- Only trained, authorized staff; entry via two self-closing, lockable doors with an antechamber.
- Smooth, sealed, cleanable walls/ceilings; seamless, slip-resistant, waterproof floors; minimal horizontal surfaces; full decontamination before renovation/closure.
- Inward directional airflow; alarms/indicators at entry; exhaust not recirculated; HEPA filters with damper ports, tested annually; vacuum lines with HEPA disinfectant traps.
- Class II BSC exhaust recirculated only if certified annually; negative air supply; HEPA filters tested/replaced yearly; primary aerosol protection.
- Extra safety: Anterooms, shower-out facilities, final HEPA exhaust, effluent decontamination.
- All operations/designs documented before use and verified annually [11].

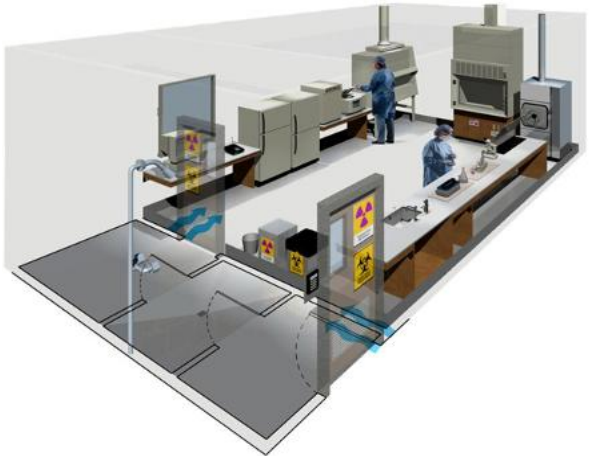

**FIGURE 4:BIOSAFETY LEVEL 3**
**IMAGE SOURCE:** HEALVITA GROUP GmbH. BIOSAFETY LABORATORIES 3 AND 4 [BSL-3 / BSL-4]Cleanroom Design.

**BIOSAFETY LEVEL 4**

BSL-4 is the highest containment level for pathogens with severe disease potential and no available vaccines or treatments.

**Two Configurations**

1. Cabinet Laboratory: Class A BSC only
2. Protective Suit Laboratory: Class B + approved positive-pressure suit

**Key Requirements**

- All work must occur in a Class B BSC.
- Materials decontaminated via autoclave or chemical disinfectant (within Class B BSC)
- Cabinet design: continuous bonded edges, smooth surfaces, no sharp edges (to prevent glove puncture) [12].

**Procedures and Practices**

- Restricted to authorized, essential personnel only
- Must change into laboratory clothing before entry
- Entry/exit allowed only through designated changing rooms (except emergencies)
- Personnel must receive proper SOPs and BSL-4-specific training
- Demonstrate proficiency in containment procedures
- Complete annual refresher training and updates when procedures change
- All materials must pass through a double-door autoclave, fumigation chamber, or airlock with proper decontamination procedures and interlock systems.

**Suit laboratory practices.**

- Work performed in positive-pressure one-piece suits with air supply.
- Infectious materials handled inside HEPA-filtered primary barriers (e.g., BSCs).
- Personnel must wear protective clothing before donning suits.
- Disposable gloves worn over suit gloves; remove before leaving the change area.
- Outer gloves must be regularly decontaminated during work.

**Laboratory Facilities**

1. Separate building or restricted zone
2. Dedicated HVAC, negative pressure, nonrecirculating
3. Double HEPA filters on all air, fans interlocked
4. Sealed, unbreakable windows
5. Pass-through systems: autoclaves, fumigation chambers, dunk tanks, airlocks
6. External autoclaves with airtight bio-seals andtreated exhaust gases
7. Non-autoclavable items → fumigation/dunk tanks[13].

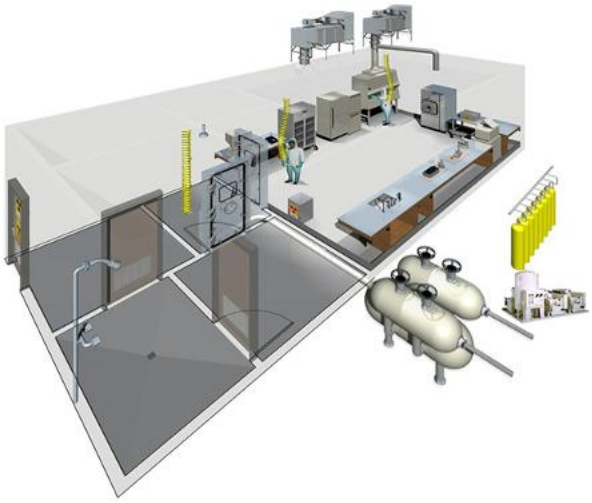

**FIGURE 5: BIOSAFETY LABORATORY LEVEL 4**
**IMAGE SOURCE:** 'Laboratory Biosafety Manual,' 3rd edition, WHO; 2005.

**TABLE: Biosafety level comparison matrix.**

| Parameter | BSL-1 (Minimal Risk) | BSL-2 (Moderate Risk) | BSL-3 (High Risk) | BSL-4 (Extreme Risk) |
|---|---|---|---|---|
| **Agent Type** | Non-pathogenic to humans; GRAS organisms (e.g., *E. coli K-12, B. subtilis*) | Moderate human pathogens (community acquired) or unknown origin blood/body fluids | Human pathogens with respiratory transmission; Indigenous or exotic agents | Exotic agents with no known vaccine or treatment; severe to fatal disease (e.g., Ebola, Marburg) |
| **Disease Severity** | None or minimal risk to healthy adults | Mild to moderate disease (e.g., diarrhea, hepatitis, skin infections) | Serious or potentially lethal disease via respiratory route (e.g., TB, SARS) | Severe to fatal disease with high mortality (15-90%) |
| **Transmission Route** | Not applicable | Percutaneous inoculation (needle stick); Mucosal contact; Low aerosol risk | Respiratory/Aerosol transmission; Lab-acquired infections documented | Highly transmissible via respiratory aerosol; aerosol-generating procedures |
| **Vaccine/Treatment** | Not applicable | Vaccines available for many (e.g., HBV); Effective treatments (antivirals, ABX) available | Limited vaccines (e.g., BCG has variable efficacy); Treatments available (TB Antibiotics) | No vaccines available; Experimental vaccines in development only; No curative treatment |
| **PPE - Coat** | Recommended (optional) | **REQUIRED**: Buttoned lab coat | **REQUIRED**: Wrap-around gown or coveralls | **REQUIRED**: Positive-pressure protective suit with supplied air |

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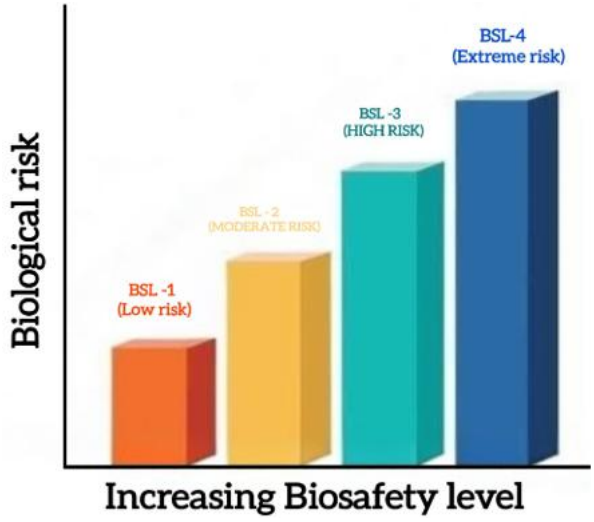
|---|---|---|---|---|
| **PPE - Gloves** | Optional (when contact risk) | **REQUIRED**: Single layer | **REQUIRED**: Double gloves (nitrile or latex) | **REQUIRED**: Multiple layer gloves (disposable + suit gloves) |
| **PPE - Eye/Resp.** | Eye protection optional (splash risk); Resp. not needed | Eye protection **REQUIRED** (goggles/face shield); Resp. not needed | Eye protection **REQUIRED**; Resp. **REQUIRED** (respirator or supplied air) | Eye/Resp. **MANDATORY**: Integrated suit visor and positive-pressure supplied-air system |
| **Containment** | Open bench work; standard lab equipment | Class II BSC for aerosol procedures; | Class II or Class III BSC for ALL procedures; | Class III BSC exclusively; seamless edges; |

| Parameter | BSL-1 (Minimal Risk) | BSL-2 (Moderate Risk) | BSL-3 (High Risk) | BSL-4 (Extreme Risk) |
|---|---|---|---|---|
| | | HEPA-filtered exhaust | HEPA-filtered exhaust | double HEPA filters |
| **Ventilation** | Standard lab HVAC | Not required (standard if installed) | **MANDATORY**: Negative pressure; directional inward airflow | **MANDATORY**: Negative pressure; dedicated system; multiple redundancy; backup |
| **Access Control** | Standard door; no special restrictions | Self-closing door; restricted to trained personnel; biohazard sign | Double self-closing doors with anteroom; clothing change room; restricted access | Separate building or isolated zone; multiple entry/exit with showers; biometric access |
| **Waste/Decontam.** | Standard cleaning and waste management | Biological waste protocols; red bin; autoclaved or chemically treated | Autoclave + chemical treatment for liquid waste; before incineration | Double-door interlocked autoclave; fumigation chamber; dunk tank; chemical shower |

**Sources:** CDC BMBL [6th Edition], WHO Laboratory Biosafety Manual [4th Edition], NABL Guidelines.

**Challenges And Limitations**

BSCs'significant budgetary and operational challenges for clinical laboratories. The high purchase costs and ongoing maintenance costs. Additionally, limited laboratory space complicates proper BSC placement, potentially affecting airflow efficiency. Improper use of BSCs, such as leaving cabinet doors open or not following proper operational procedures, compromises containment integrity and increases the risk of infection. Inadequate decontamination of BSCs can lead to cross-contamination of specimens. However, recent technological advancements and improved BSC design have greatly enhanced infection control practices in clinical laboratories. These improvements have led to better containment of infectious agents and improved protection of laboratory personnel.




## CONCLUSION

Biosafety, protection against biological dangers, is crucial for laboratories. Adherence to biosafety standards and SOPs is critical for laboratory personnel exposed to hazardous microorganisms and infectious illnesses. Safety is key: proper safeguards lower hazards to individuals while allowing critical medical and scientific work to be done. Laboratory workers and staffcan be safe from biological threats through compliance with established biosafety criteria.

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