



Changes in Biosafety Guidelines for BSL2 Laboratories

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Objective

- **Participants will be able to identify changes in Biosafety Guidelines for BSL2 Laboratories**



Types of Changes Between BMBL Editions

- Organizational - harmonize sections
- Additions
- Deletions
- Clarifications - must/should



New Sections in BMBL5

Section I Introduction

Section I Introduction

Section II Biological Risk Assessment

Section II Principles of Biosafety

(5th Edition)
(4th Edition)

Section III Principles of Biosafety

Section III Laboratory Biosafety Level Criteria

Section IV Laboratory Biosafety Level Criteria

Section IV Vertebrate Animal Biosafety Level Criteria



New Sections in BMBL5

Section V Vertebrate Animal Biosafety Level Criteria for Vivarium Research

Section V Risk Assessment

(5th Edition)
(4th Edition)

Section VI Principles of Laboratory Biosecurity

Section VI Recommended Biosafety Levels for Infectious Agents and Infected Animals

Section VII Occupational Health and Immunoprophylaxis
(moved from Appendix B)

Section VIII-G Toxin Agent



New Appendices in BMBL5

Appendix A Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets

Appendix A Primary Containment: Biological Safety Cabinets

Appendix B Decontamination and Disinfection

Appendix B Immunoprophylaxis (moved to Section VII)

(5th Edition)
(4th Edition)

Appendix C Transportation of Infectious Substances

Appendix C Transportation and Transfer of Biological Agents

Appendix D Agricultural Pathogen Biosafety

Appendix D Restricted Animal Pathogens



New Appendices in BMBL5

Appendix E	Arthropod Containment Guidelines	
Appendix E	Resources for Information	
Appendix F	Select Agents and Toxins	(5th Edition) (4th Edition)
Appendix F	Laboratory Security and Emergency Response for Microbiological and Biomedical Laboratories (moved to Section VI)	
Appendix G	Integrated Pest Management	
Appendix G	Integrated Pest Management	



New Appendices in BMBL5



Appendix H	Working with Human, NHP and Other Mammalian Cells and Tissues	
Appendix H	Working with Human and Other Primate Cells and Tissues	
Appendix I	Guidelines for Work with Toxins of Biological Origin	
Appendix I	Guidelines for Work with Toxins of Biological Origin	
Appendix J	NIH Oversight of Research Involving Recombinant Biosafety Issues	
Appendix K	Resources for Information	(5th Edition) (4th Edition)
Appendix L	Acronyms	



Subsections for Biosafety Level Criteria

1. *Standard Microbiological Practices*

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2. *Special Practices*

2. *Special Practices*

3. *Safety Equipment (Primary Barriers and Personal Protective Equipment)*

3. *Safety Equipment (Primary Barriers)*

4. *Laboratory Facilities (Secondary Barriers)*

4. *Laboratory Facilities (Secondary Barriers)*

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(4th Edition)



A. Standard Microbiological Practices

1. Access control
2. Handwashing
3. Eating, drinking etc. prohibitions
4. Mouth pipetting
5. Sharps policies
6. Aerosol control
7. Decontamination of work surfaces
8. Decontamination of infectious materials
9. Signage
10. Pest management
11. Training



B. Special Practices

1. Hazard communication
2. Medical surveillance
3. Serum samples
4. Biosafety manual
5. Proficiency
6. Infectious material control
7. Lab equipment decontamination
8. Exposure response
9. Irrelevant plants/animals
10. Primary containment of aerosols



C. Safety Equipment

(Primary Barriers and Personal Protective Equipment)

1. Biosafety cabinets
2. Lab coats/protective clothing
3. Eye/face protection
4. Gloves
5. Animal room PPE



D. Laboratory Facilities

(Secondary Barriers)

1. Doors/locks
2. Handwashing sinks
3. Easy clean design
4. Lab furniture
5. Lab windows
6. BSC locations
7. Vacuum lines
8. Eyewash
9. Ventilation requirements
10. BSC exhaust
11. Decontamination of lab wastes



Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

...Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.

(5th Edition)
(4th Edition)

...The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

...Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.



Standard Microbiological Practices



3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in Laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

(5th Edition)
(4th Edition)

...Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.



Standard Microbiological Practices



4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

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(4th Edition)

...Mouth pipetting is prohibited; mechanical pipetting devices are used.



Standard Microbiological Practices



5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

(moved from Special Practices, #8 c)

Policies for the safe handling of sharps are instituted...

(5th Edition)

(4th Edition)

.... Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

...Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.



Standard Microbiological Practices



5. (continued)

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

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(4th Edition)

(continued from Special Practices)

a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. . . . Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal;



Standard Microbiological Practices



5. (continued)

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

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(continued from Special Practices)

... rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

... Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

.... Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations. (disposal of wastes is moved to #8)



Standard Microbiological Practices



5. (continued)

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

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(4th Edition)

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.Plasticware should be substituted for glassware whenever possible.



Standard Microbiological Practices



6. Perform all procedures to minimize the creation of splashes and/or aerosols.

...All procedures are performed carefully to minimize the creation of splashes or aerosols.

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(4th Edition)

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

...Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.



Standard Microbiological Practices



8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

(5th Edition) (4th Edition)

... All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.



Standard Microbiological Practices



9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

(5th Edition)
(4th Edition)

(moved from Special Practices)

A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent (s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.



Standard Microbiological Practices



10. An effective integrated pest management program is required. See Appendix G.

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... An insect and rodent control program is in effect (see Appendix G).



Standard Microbiological Practices



11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur.

(5th Edition)
(4th Edition)

(moved from Special Practices)

...The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.



Standard Microbiological Practices



11. (continued)

...Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

(5th Edition)
(4th Edition)

(moved from Special Practices)

...In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immuno-compromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.



Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

(5th Edition)
(4th Edition)

Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress.

The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.



Special Practices

2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

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....Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

...Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.



Special Practices

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

...When appropriate, considering the agent (s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

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(4th Edition)

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.



Special Practices

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

No equivalent statement at BSL2

(5th Edition)
(4th Edition)

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

.... Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.



Special Practices

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

(5th Edition)
(4th Edition)

...Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials.



Special Practices

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

(5th Edition)
(4th Edition)

...Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.



Special Practices

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

,,, Animals not involved in the work being performed are not permitted in the lab.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

...Described in next section

(5th Edition)
(4th Edition)



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

(5th Edition)
(4th Edition)

...Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

1. (continued)

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

(5th Edition)
(4th Edition)

(continued)

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

1. (continued)

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

(5th Edition)
(4th Edition)

(continued)

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

(5th Edition)

(4th Edition)

... Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

(5th Edition)
(4th Edition)

... Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

4. Gloves must be worn to protect hands from exposure to hazardous materials.
Glove selection should be based on an appropriate risk assessment.
Alternatives to latex gloves should be available. Gloves must not be worn
outside the laboratory..

(5th Edition)
(4th Edition)

...Gloves are worn when hands may contact potentially infectious materials,
contaminated surfaces or equipment.

.....Alternatives to powdered latex gloves should be available.

..... and they should not be worn outside the lab.



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

4. (continued)

....In addition, BSL-2 laboratory workers should:

- a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
- b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

(5th Edition)
(4th Edition)

(continued)

....Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. .



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

4. (continued)

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

(5th Edition)
(4th Edition)

(continued)

....Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.),



Safety Equipment

(Primary Barriers and Personal Protective Equipment)

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

No equivalent statement at BSL2

(5th Edition)

(4th Edition)



Laboratory Facilities

(Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

(Note special access control requirements for select agent labs)

...Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).

(5th Edition)
(4th Edition)

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

...Consider locating new laboratories away from public areas.
(deleted from 5th ed.)

...Each laboratory contains a sink for handwashing.



Laboratory Facilities

(Secondary Barriers)

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

(5th Edition)
(4th Edition)

....The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.



Laboratory Facilities

(Secondary Barriers)



4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

(5th Edition) **(4th Edition)**

...Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. ...Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment. ...Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.



Laboratory Facilities

(Secondary Barriers)



5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

(5th Edition)
(4th Edition)

.... If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Note that open windows would not meet security requirements of select agent regulation.



Laboratory Facilities

(Secondary Barriers)



6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

(5th Edition)
(4th Edition)

....Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.



Laboratory Facilities

(Secondary Barriers)



7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

.... No equivalent statement at BSL2.

(5th Edition)
(4th Edition)

8. An eyewash station must be readily available.

...An eyewash station is readily available.



Laboratory Facilities

(Secondary Barriers)



9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

(5th Edition)
(4th Edition)

...There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.



Laboratory Facilities

(Secondary Barriers)



10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

.... No equivalent statement at BSL2.

(5th Edition)
(4th Edition)

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

.... No equivalent statement at BSL2.



Laboratory Facilities

(Secondary Barriers)



.... No equivalent statement at BSL2.

(5th Edition)
(4th Edition)

...Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.



For More Information

APHIS/CDC Select Agent Program web
site: <http://www.selectagents.gov>

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