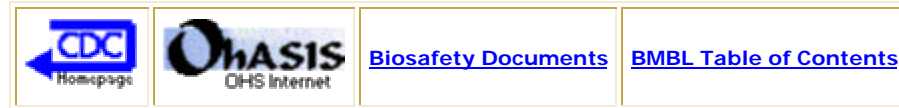


BMBL Section III

Laboratory Biosafety Level Criteria



Laboratory Biosafety Level Criteria

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Tables 1 of this section and Section IV (see pages 52 and 75, BMBL). The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1 (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work with cultures and specimens are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use 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.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. 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 to be placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated outside of the immediate laboratory are packaged in accordance with applicable local, state, and federal regulations before removal from the facility.
9. A biohazard sign can be posted at the entrance to the laboratory whenever infectious agents are present. The sign may include the name of the agent(s) in use and the name and phone number of the investigator.
10. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices None

C. Safety Equipment (Primary Barriers)

1. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
2. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
3. Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
4. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Each laboratory contains a sink for handwashing.
3. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. 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 surface and equipment.
5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
6. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

Biosafety Level 2 (BSL-2)

Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. 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.

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

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

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

7. 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.

8. 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, leakproof 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.

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

B. Special Practices

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

2. 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.

3. 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.

4. 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).

5. 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.

6. 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.

7. 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.

8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

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. Plasticware should be substituted for glassware whenever possible.

b. 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. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; 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.

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

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. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or

federal regulations.

9. 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.

10. 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. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.

11. 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.

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

C. Safety Equipment (Primary Barriers)

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

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.

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.

2. 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.

3. 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.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. 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. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

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

2. Consider locating new laboratories away from public areas.

3. Each laboratory contains a sink for handwashing.

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

5. 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.

6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

7. 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.

8. An eyewash station is readily available.

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

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

Biosafety Level 3 (BSL-3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

It is recognized, however, that some existing facilities may not have all the facility features recommended for Biosafety Level 3 (i.e., double-door access zone and sealed penetrations). In this circumstance, an acceptable level of safety for the conduct of routine procedures, (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, susceptibility testing, etc.), may be achieved in a Biosafety Level 2 facility, **providing** 1) the exhaust air from the laboratory room is discharged to the outdoors, 2) the ventilation to the laboratory is balanced to provide directional airflow into the room, 3) access to the laboratory is restricted when work is in progress, and 4) the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment and facilities apply to agents assigned to Biosafety Level 3:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics

are not permitted in the laboratory. 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 for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of aerosols.
7. Work surfaces are decontaminated at least once a day and after any spill of viable material.
8. 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, leakproof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.
9. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Laboratory doors are kept closed when experiments are in progress.
2. The laboratory director controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. 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 immunocompromised or immunosuppressed may be at risk of acquiring infections. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. No minors should be allowed in the laboratory.
3. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures, enter the laboratory or animal rooms.
4. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for

immunizations, respirators, or other personal protective measures.

5. Laboratory personnel receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing), and periodic testing as recommended for the agent being handled.

6. Baseline serum samples are collected as appropriate and stored for all laboratory and other at-risk personnel. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory.

7. A biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

8. 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 changes.

9. The laboratory director is responsible for ensuring that, before working with organisms at Biosafety Level 3, all personnel demonstrate proficiency in standard microbiological practices and techniques, and in the practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in safe microbiological practices and techniques.

10. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

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. Plasticware should be substituted for glassware whenever possible.

b. 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. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; 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.

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

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. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated before disposal, and disposed of according to any local, state, or federal regulations.

11. All open manipulations involving infectious materials are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench. Clean-up is facilitated by using plastic-backed paper toweling on non-perforated work surfaces within biological safety cabinets.

12. Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.

a. Spills of infectious materials are decontaminated, contained and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious material. Spill procedures are developed and posted.

b. Contaminated equipment must be decontaminated before removal from the facility for repair or maintenance or packaging for transport, in accordance with applicable local, state, or federal regulations.

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

14. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.

15. Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

16. Animals and plants not related to the work being conducted are not permitted in the laboratory.

C. Safety Equipment (Primary Barriers)

1. Protective laboratory clothing such as solid-front or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when overtly contaminated.
2. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
3. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
4. All manipulations of infectious materials, necropsy of infected animals, harvesting of tissues or fluids from infected animals or embryonate eggs, etc., are conducted in a Class II or Class III biological safety cabinet (see Appendix A).
5. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.
6. Respiratory and face protection are used when in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridors. Doors are lockable (see Appendix F). A clothes change room may be included in the passageway.
2. Each laboratory room contains a sink for handwashing. The sink is hands-free or automatically operated and is located near the room exit door.
3. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled are constructed for easy cleaning and decontamination. Seams, if present, must be sealed. Walls, ceilings, and floors should be smooth, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be monolithic and slip-resistant. Consideration should be given to the use of coved floor coverings. Penetrations in floors, walls, and ceiling surfaces are sealed or capable of being sealed to facilitate decontamination. Openings such as around ducts and the spaces between doors and frames are capable of being

sealed to facilitate decontamination.

4. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.

5. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

6. All windows in the laboratory are closed and sealed.

7. A method for decontaminating all laboratory wastes is available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination method). Consideration should be given to means of decontaminating equipment. If waste is transported out of the laboratory, it should be properly sealed and not transported in public corridors.

8. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.

9. A ducted exhaust air ventilation system is provided. This system creates directional airflow which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Laboratory personnel must verify that the direction of the airflow (into the laboratory) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the laboratory entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the laboratory. Audible alarms should be considered to notify personnel of HVAC system failure.

10. HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that

prevents positive pressurization of the cabinets (see Appendix A).

11. Continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.

12. Vacuum lines are protected with liquid disinfectant traps and HEPA filters, or their equivalent. Filters must be replaced as needed. An alternative is to use portable vacuum pumps (also properly protected with traps and filters).

13. An eyewash station is readily available inside the laboratory.

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

15. The Biosafety Level 3 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience.

16. Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

Biosafety Level 4 (BSL-4)

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. They are supervised by competent scientists who are trained and experienced in working with these agents. Access to the laboratory is strictly controlled by the laboratory director. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted.

Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices equipment, and facilities apply to agents assigned to Biosafety Level 4:

A. Standard Microbiological Practices

1. Access to the laboratory is limited by the laboratory director when experiments are in progress.
2. Policies for safe handling of sharps are instituted.
3. All procedures are performed carefully to minimize the creation of aerosols.
4. Work surfaces are decontaminated at least once a day and after any spill of viable material.
5. All waste is decontaminated before disposal by an approved method such as autoclaving.
6. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women, are not allowed in the laboratory or animal rooms.

The *supervisor* has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the laboratory director, biohazard control officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook, signed by all personnel, indicates the date and time of each entry and exit. Practical and effective

protocols for emergency situations are established.

2. When infectious materials or infected animals are present in the laboratory or animal rooms, hazard warning signs, incorporating the universal biohazard symbol, are posted on all access doors. The sign identifies the agent, lists the name of the laboratory director or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

3. The *laboratory director* is responsible for ensuring that, before working with organisms at Biosafety Level 4, all personnel demonstrate a high proficiency in standard microbiological practices and techniques, and in the special practices and operations specific to the laboratory facility. This might include prior experience in handling human pathogens or cell cultures, or a specific training program provided by the laboratory director or other competent scientist proficient in these unique safe microbiological practices and techniques.

4. Laboratory personnel receive available immunizations for the agents handed or potentially present in the laboratory.

5. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.

6. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

7. 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 changes.

8. Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. Personnel use the airlocks to enter or leave the laboratory only in an emergency.

9. Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory. When leaving the laboratory and before proceeding

into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.

10. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock, which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

11. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

a. Needles and syringes or other sharp instruments are restricted in the laboratory for use only when there is no alternative, such as for parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

b. 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. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; 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.

c. Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate.

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. Containers of contaminated needles, sharp equipment, and broken glass must be decontaminated before disposal, according to any local, state, or federal regulations.

12. Biological materials to be removed from the Class III cabinet or from the Biosafety Level 4 laboratory in a viable or intact state are transferred to a nonbreakable, sealed primary container and then enclosed in a nonbreakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

13. No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

14. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.

15. Spills of infectious materials are contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with concentrated infectious material. A spill procedure is developed and posted within the laboratory.

16. A system is established for reporting laboratory accidents and exposures and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for the quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

17. Materials not related to the experiment being conducted (e.g., plants, animals, and clothing) are not permitted in the facility.

C. Safety Equipment (Primary Barriers)

All procedures within the facility are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.

D. Laboratory Facility (Secondary Barriers)

There are two models for Biosafety Level 4 laboratories: (A) the Cabinet Laboratory where all handling of the agent is performed in a Class III Biological Safety Cabinet, and (B) the Suit Laboratory where personnel wear a protective suit. Biosafety Level-4 laboratories may be based on either model or a combination of both models in the same facility. If a combination is used, each type must meet all the requirements identified for that type.

(A) Cabinet Laboratory (See Appendix A)

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior

to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.

2. Daily inspections of all containment parameters (e.g., directional airflow) and life support systems are completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and coved. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.

4. Bench tops have seamless or sealed surfaces which 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.

5. Laboratory furniture is of simple open construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

6. A hands-free or automatically operated handwashing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.

7. If there is a central vacuum system, it does not serve areas outside the cabinet room. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the cabinet room are protected by devices that prevent backflow.

8. If water fountains are provided, they are automatically or foot-operated and

are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.

9. Access doors to the laboratory are self-closing and lockable.

10. Any windows are breakage-resistant and sealed.

11. Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

12. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).

13. Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and clean-side toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

14. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate any system malfunction. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the cabinet room is provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and the HVAC control system is designed to prevent sustained positive pressurization of the laboratory. The Class III cabinet should be directly connected to the exhaust system. If the Class III cabinet is connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinet.

15. The supply air to and exhaust air from the cabinet room, inner change room, and anteroom pass through HEPA filter(s). The air is discharged away from occupied spaces and air intakes. The HEPA filter(s) are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified

annually. The HEPA filter housings are designed to allow for *in situ* decontamination of the filter prior to removal, or removal of the filter in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

16. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.

17. Appropriate communication systems are provided between the laboratory and the outside (e.g., voice, fax, computer).

(B) Suit Laboratory

1. The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through the changing and decontamination areas prior to entering the room(s) where work is done with BSL-4 agents (suit area). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the suit area. A specially designed suit area is maintained in the facility to provide personnel protection equivalent to that provided by Class III biological safety cabinets. Personnel who enter this area wear a one-piece positive pressure suit that is ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing air compressors, alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support systems, alarms, lighting, entry and exit controls, and BSCs.. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.

2. A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

3. A double-doored autoclave is provided at the containment barrier for

decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of materials, supplies, or equipment that are not brought into the suit area through the change room. These devices can be also used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.

4. Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect prohibitive (see Appendix G). The internal surfaces of this shell are resistant to liquids and chemicals, facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.

5. Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.

6. Bench tops have seamless surfaces which 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.

7. Laboratory furniture is of simple open construction capable of supporting anticipated loading and uses. Non-porous materials are preferable. Spaces between benches, cabinets, and equipment are accessible for cleaning and decontamination. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.

8. A hands-free or automatically operated handwashing sink is provided in the suit area(s); handwashing sinks in the outer and inner change rooms should be considered based on the risk assessment.

9. If there is a central vacuum system, it does not serve areas outside the suit area. In-line HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the suit area are protected by devices that prevent backflow.

10. Access doors to the laboratory are self-closing and lockable. Inner and outer doors to the chemical shower and inner and outer doors to airlocks are interlocked to prevent both doors from being opened simultaneously.

11. Any windows are breakage-resistant and are sealed.

12. Liquid effluents from sinks, floor drains (if used), autoclave chambers and other sources within the containment barrier are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment. The process used for decontamination of liquid wastes must be validated physically and biologically.

13. A dedicated non-recirculating ventilation system is provided. The supply and exhaust components of the system are balanced to ensure directional airflow from the area of least hazard to the area(s) of greatest potential hazard. Redundant supply fans are recommended. Redundant exhaust fans are required. The differential pressure/directional airflow between adjacent areas is monitored and alarmed to indicate malfunction of the system. An appropriate visual pressure monitoring device that indicates and confirms the pressure differential of the suit area must be provided and located at the entry to the clean change room. The airflow in the supply and exhaust components is monitored and an HVAC control system is installed to prevent positive pressurization of the laboratory.

14. The supply air to the suit area, decontamination shower, and decontamination airlock is protected by passage through a HEPA filter. The general room exhaust air from the suit area, decontamination shower and decontamination airlock is treated by a passage through two HEPA filters in series prior to discharge to the outside. The air is discharged away from occupied spaces and air intakes. The HEPA filters are located as near as practicable to the source in order to minimize the length of potentially contaminated ductwork. All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for *in situ* decontamination of the filter prior to removal. Alternatively, the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration. The design of the HEPA filter housing should facilitate validation of the filter installation. The use of pre-certified HEPA filters can be an advantage. The service life of the exhaust HEPA filters can be extended through adequate prefiltration of the supply air.

15. The positioning of the supply and exhaust points should be such that dead air space in the suit room is minimized.

16. The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear a positive pressure suit, may be discharged into the room environment or to the outside through the facility air exhaust system.

If the treated exhaust is discharged to the outside through the facility exhaust system, it is connected to this system in a manner that avoids any interference with the air balance of the cabinets or the facility exhaust system.

17. The Biosafety Level 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to operation. Facilities should be re-verified annually against these procedures as modified by operational experience.

18. Appropriate communication systems should be provided between the laboratory and the outside.

This page last reviewed November 30, 2000

[Centers for Disease Control and Prevention](#)

Office of the Director (OD)

[Office of Health and Safety \(OHS\)](#)