

VI. **BIOLOGICAL SAFETY**

Microbiological and biomedical laboratories are special, often unique, work environments that may pose special infectious disease risks to persons in or near them. Personnel have contracted infections in the laboratory throughout the history of microbiological and biomedical research. A number of cases have been attributed to carelessness or poor technique in the handling of infectious materials.

The most important element of safety is strict adherence to standard microbiological practices and techniques. When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established four biosafety levels which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by infectious agents or recombinant DNA research. The Principle Investigator is responsible for making a determination of the required levels of physical and biological containment required for the work to be performed.

In addition, vertebrate animal biosafety level criteria are provided. Ideally, facilities for laboratory animals used for studies of infectious or noninfectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities that provide patient care. The recommendations provided describe four combinations (designated Animal Biosafety Levels 1-4) of practices, safety equipment, and facilities for experiments on animals infected with agents which produce, or may produce, human infection.

Each laboratory should develop standard operating procedures which identify the hazards that will or may be encountered and which specify practices and procedures designed to minimize or eliminate risks.

A. **General Laboratory Practice**

1. Engineering controls such as biological safety cabinets should be examined and maintained or replaced on a regular schedule to ensure their effectiveness. It is imperative that Class I and II biological safety cabinets are tested and certified in place at the time of installation within the laboratory, at any time the cabinet is moved, and at least annually thereafter.
2. Employees should wash their hands immediately or as soon as possible after removal of gloves or other personal protective equipment and after hand contact with blood or other potentially infectious materials.
3. All personal protective equipment should be removed immediately upon leaving the work area or as soon as possible, if overtly contaminated, and placed in an appropriately designated area or container for storage, washing, decontamination, or disposal.

4. Immediately after use, contaminated sharps should be disposed of in closable, puncture resistant, disposable containers which are leak proof on the sides and bottom and color coded or labeled with the biohazard symbol.
5. Sharps containers should be easily accessible to personnel and located in the area of use.
6. Used needles and other sharps should not be sheared, bent, broken, recapped, or resheathed by hand.
7. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in areas where work with biohazardous materials is performed.
8. Food and drink must not be stored in refrigerators, freezers, or cabinets where biohazardous materials are stored.
9. All procedures involving biohazardous materials should be performed in such a manner as to minimize splashing, spraying, and aerosolization of these substances.
10. Mouth pipetting/suctioning is prohibited.
11. Broken glassware which may be contaminated should not be picked up directly with the hands. It should be cleaned up using mechanical means such as a brush and dust pan, a vacuum cleaner, tongs, cotton swabs or forceps.
12. All infectious waste must be disposed of in accordance with the procedures found in Section VIII., "Biomedical Waste."
13. House vacuum systems should be protected from aspirations of infectious fluids. For Biosafety Level 2 laboratory work, an in-line flask containing a suitable decontamination solution should be used, serving as a fluid overflow collection vessel, connected to the vacuum system. For Biosafety Level 3 laboratory work, two flasks containing a decontamination solution prior to the HEPA filter should be used.

Source: CDC/NIH *Primary Containment for Biohazards: Selection, Installation, and use of Biological Safety Cabinets*, U.S. Department of Health and Human Services Public Health Services, Centers for Disease Control and Prevention and National Institutes of Health, September 1995

B. Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. 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 not 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.

1. Standard Microbiological Practices
 - a. 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.
 - b. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
 - c. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. 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.
 - d. Mouth pipetting is prohibited; mechanical pipetting devices are used.
 - e. Policies for the safe handling of sharps are instituted.
 - f. All procedures are performed carefully to minimize the creation of splashes or aerosols.
 - g. Work surfaces are decontaminated at least once a day and after any spill of viable material.
 - h. 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.
 - i. 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.

- j. An insect and rodent control program is in effect.
- 2. Special Practices: None
 - 3. Safety Equipment (Primary Barriers)
 - a. Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
 - b. It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
 - c. 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.
 - d. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.
 - 4. Laboratory Facilities (Secondary Barriers)
 - a. Laboratories should have doors for access control.
 - b. Each laboratory contains a sink for handwashing.
 - c. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
 - d. 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.
 - e. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
 - f. If the laboratory has windows that open, they are fitted with fly screens.

C. Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs 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.

1. Standard Microbiological Practices

- a. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
- b. Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
- c. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- d. Mouth pipetting is prohibited; mechanical pipetting devices are used.
- e. Policies for the safe handling of sharps are instituted.
- f. All procedures are performed carefully to minimize the creation of splashes or aerosols.
- g. 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.
- h. 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 facility. Materials to be decontaminated off-site from the laboratory are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
- i. An insect and rodent control program is in effect.

2. Special Practices

- a. 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.

- b. 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 or animal rooms.
- c. 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.
- d. 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).
- e. 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.
- f. 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.
- g. 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.
- h. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - (1) 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.

- (2) 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.
 - (3) Syringes which re-sheath the needle, needleless systems, and other safety devices are used when appropriate.
 - (4) 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.
- i. Cultures, tissues, or 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.
 - j. 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.
 - k. Spills and accidents which 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.

- I. Animals not involved in the work being performed are not permitted in the lab.
3. Safety Equipment (Primary Barriers)
 - a. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
 - (1) 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.
 - (2) 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.
 - b. Face protection (goggles, mask, faceshield or other splatter guards) 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.
 - c. 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.
 - d. Gloves are worn when hands may contact potentially infectious material, 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.

4. Laboratory Facilities (Secondary Barriers)
 - a. Provide lockable doors for facilities that house restricted agents.
 - b. Consider locating new laboratories away from public areas.
 - c. Each laboratory contains a sink for handwashing.
 - d. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
 - e. 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.
 - f. 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.
 - g. 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.
 - h. An eyewash station is readily available.
 - i. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
 - j. 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.

D. Biosafety Level 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.

1. Standard Microbiological Practices
 - a. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
 - b. Persons wash their hands after handling infectious materials and animals, after removing gloves, and when they leave the laboratory.
 - c. 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.
 - d. Mouth pipetting is prohibited; mechanical pipetting devices are used.
 - e. Policies for the safe handling of sharps are instituted.
 - f. All procedures are performed carefully to minimize the creation of aerosols.
 - g. Work surfaces are decontaminated at least once a day and after any spill of viable material.
 - h. 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.

- i. An insect and rodent control program is in effect.

2. Special Practices

- a. Laboratory doors are kept closed when experiments are in progress.
- b. 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.
- c. 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.
- d. 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.
- e. 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.
- f. 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.

- g. 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 to follow instructions on practices and procedures.
- h. 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.
- i. 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.
- j. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels
 - (1) 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.
 - (2) 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.
 - (3) Syringes which re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

- (4) 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.
- k. 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.
- l. Laboratory equipment and work surfaces should be decontaminated routinely with an appropriate disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
 - (1) 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.
 - (2) 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.
- m. Cultures, tissues, or specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- n. All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
- o. Spills and accidents which 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.
- p. Animals and plants not related to the work being conducted are not permitted in the laboratory.

3. Safety Equipment (Primary Barriers)

- a. 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.
 - b. Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
 - c. Frequent changing of gloves accompanied by hand washing is recommended. Disposable gloves are not reused.
 - d. 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.
 - e. 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.
 - f. Respiratory and face protection are used when in rooms containing infected animals.
4. Laboratory Facilities (Secondary Barriers)
- a. 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. A clothes change room (shower optional) may be included in the passage way.
 - b. Each laboratory contains a sink for handwashing. The sink is hands free or automatically operated and is located near the laboratory exit door.
 - c. 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.

Openings such as around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.

- d. Bench tops are impervious to water and resistant to moderate heat and organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.
- e. 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.
- f. All windows in the laboratory are closed and sealed.
- g. 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.
- h. Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily traveled laboratory areas.
- i. 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.
- j. The High Efficiency Particulate Air (HEPA)-filtered exhaust air from Class II biological safety cabinets 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).

- k. 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.
- l. 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).
- m. An eyewash station is readily available inside the laboratory
- n. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- o. 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.
- p. 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.

E. Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents which 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.

1. Standard Microbiological Practices
 - a. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
 - b. Policies for safe handling of sharps are instituted.
 - c. All procedures are performed carefully to minimize the creation of aerosols.
 - d. Work surfaces are decontaminated at least once a day and after any spill of viable material.
 - e. All waste is decontaminated before disposal by an approved method such as autoclaving.
 - f. An insect and rodent control program is in effect.
2. Special Practices
 - a. 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.
 - b. 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,

biohazards 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.

- c. 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).
- d. 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.
- e. Laboratory personnel receive available immunizations for the agents handled or potentially present in the laboratory.
- f. Baseline serum samples for all 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 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.
- g. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and to follow instructions on practices and procedures.
- h. Laboratory 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.

- i. Personnel enter and leave the facility only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the facility. Personnel use the airlocks to enter or leave the laboratory only in an emergency.
- j. 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 facility. 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.
- k. 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.
- l. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - (1) 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.
 - (2) 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.
 - (3) Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.

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- (4) 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.
 - m. 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.
 - n. No materials, except for 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 facility. Equipment or material which 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.
 - o. Laboratory equipment is decontaminated routinely after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials. Contaminated equipment is also decontaminated before it is sent for repair or maintenance.
 - p. 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.
 - q. 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.
 - r. Materials not related to the experiment being conducted (e.g., plants, animals, and clothing) are not permitted in the facility.
3. 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.

4. 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

- (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-doored autoclave, dunk tank, fumigation chamber, or ventilated anteroom is provided at the containment barrier for passage of those materials, supplies, or equipment which 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. 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. Any drains in the cabinet room are connected directly to the liquid waste decontamination system. Sewer vents and other ventilation lines contain HEPA filters and protection against vermin.
- (4) 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.

- (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 automatic 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) A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is 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 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 material, 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 services 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 a 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.

F. Animal Biosafety Level 1

Animal Biosafety Level 1 (ABSL-1) is suitable for work involving well characterized agents that are not known to cause disease in healthy adult humans, and that are of minimal potential hazard to laboratory personnel and the environment.

1. Standard Practices

- a. The animal facility director establishes policies, procedures, and protocols for emergency situations. Each project is subject to pre-approval by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biohazard Committee (IBC). Any special practices are approved at this time.
- b. Only those persons required for program or support purposes are authorized to enter the facility. Before entering, persons are advised of the potential biohazards and are instructed on the appropriate safeguards.
- c. An appropriate medical surveillance program is in place.
- d. A safety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and follow instructions on practices and procedures.

- e. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
 - f. All procedures are carefully performed to minimize the creation of aerosols or splatters.
 - g. Work surfaces are decontaminated after use or after any spill of viable materials.
 - h. All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.
 - i. Policies for the safe handling of sharps are instituted.
 - j. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
 - k. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators.)
 - l. An insect and rodent control program is in effect.
2. Special Practices: None.
3. Safety Equipment (Primary Barriers)
- a. The wearing of laboratory coats, gowns, and/or uniforms in the facility is recommended. Laboratory coats remain in the animal room. Gowns and uniforms are not worn outside the facility.
 - b. Persons having contact with non-human primates should assess their risk of mucous membrane exposure and wear appropriate eye and face protection.
4. Animal Facilities (Secondary Barriers)
- a. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.

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- b. External facility doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
 - c. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
 - d. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
 - e. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
 - f. Windows are not recommended. Any windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens.
 - g. If floor drains are provided, the traps are always filled with water and/or an appropriate disinfectant.
 - h. Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*, latest edition. No recirculation of exhaust air should occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.
 - i. The facility has a hand washing sink.
 - j. Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
 - k. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

G. **Animal Biosafety Level 2**

Animal Biosafety Level 2 involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

1. Standard Practices

- a. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use committee (IACUC) and the Institutional Biohazard Committee (IBC).
- b. Access to the animal room is limited to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- c. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented.
- d. 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.
- e. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should only be done in designated areas and are not permitted in animal or procedure rooms.
- f. All procedures are carefully performed to minimize the creation of aerosols or splatters.
- g. Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contamination by infectious materials.
- h. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. The outer surface of the containers is disinfected prior to moving the material. Autoclaving of the contents prior to incineration is recommended.
- i. Policies for the safe handling of sharps are instituted.

- (1) Needles and syringes or other sharp instruments are restricted for use in the animal facility only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - (2) Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
 - (3) Plasticware should be substituted for glassware whenever possible.
 - j. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
 - k. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements (e.g., the need for immunizations and respirators) for entering the animal room.
 - l. An insect and rodent control program is in effect.
2. Special Practices
 - a. Animal care 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. Records of all training provided are maintained. In general, persons who may be at increased risk of acquiring infection, or for whom infection might be unusually hazardous, are not allowed in the animal facility unless special procedures can eliminate the extra risk.
 - b. Only animals used for the experiment(s) are allowed in the room.
 - c. All equipment must be appropriately decontaminated prior to removal from the room.
 - d. Spills and accidents which result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
 3. Safety Equipment (Primary Barriers)
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- a. Gowns, uniforms, or laboratory coats are worn while in the animal room. The laboratory coat is removed and left in the animal room. Gown, uniforms, and laboratory coats are removed before leaving the animal facility. Gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
 - b. Personal protective equipment is used based on risk assessment determinations. Appropriate face/eye and respiratory protection is worn by all personnel entering animal rooms that house nonhuman primates.
 - c. Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) are used whenever conducting procedures with a high potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals.
 - d. When needed, animals are housed in primary biosafety containment equipment appropriate for the animal species. Filter top cages are always handled in properly designed and operating animal biocontainment cabinets recommended for rodents.
4. Animal Facilities (Secondary Barriers)
- a. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
 - b. Access to the facility is limited by secure locked doors. External doors are self-closing and self-locking. Doors to animal rooms open inward, are self-closing, and are kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
 - c. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) are water resistant.
 - d. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
 - e. Any windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens.
 - f. If floor drains are provided, the traps are always filled with an

appropriate disinfectant.

- g. Exhaust air is discharged to the outside without being recirculated to other rooms. Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals*, latest edition. The direction of airflow in the animal facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.
- h. Cages are washed manually or in an appropriate cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- i. An autoclave is available to decontaminate infectious waste.
- j. A handwashing sink is available in the room where infected animals are housed.
- k. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

H. **Animal Biosafety Level 3**

Animal Biosafety Level 3 involves practices suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.

1. Standard Practices

- a. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
- b. The laboratory or animal facility director limits access to the animal room to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- c. An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or potentially present (e.g., hepatitis B vaccine, TB skin testing). When appropriate, a serum surveillance system should be implemented. In general, persons who may be at

increased risk of acquiring infection, or for whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.

- d. 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.
- e. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
- f. All procedures are carefully performed to minimize the creation of aerosols.
- g. Equipment and work surfaces in the room are routinely decontaminated with an effective disinfectant after work with the infectious agent, and especially after overt spills, splashes or other contamination by infectious materials.
- h. All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse animal tissues) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended. The outer surface of the containers is disinfected prior to moving the material.
- i. Policies for the safe handling of sharps are instituted.
 - (1) Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - (2) Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - (3) Plasticware should be substituted for glassware whenever possible.
- j. Personnel wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.

- k. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, list the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).
- l. All infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s).
- m. An insect and rodent control program is in effect.

2. Special Practices

- a. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment must be decontaminated according to any local, state, or federal regulations before being packaged for transport or removal from the facility for repair or maintenance.
- b. A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious material must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- c. All wastes from the animal room must be autoclaved prior to incineration or other appropriate terminal treatment.
- d. Materials not related to the experiment (e.g., plants, animals) are not permitted in the animal room.

3. Safety Equipment (Primary Barriers)

- a. Uniforms or scrub suits are worn by personnel entering the animal room. Wrap-around or solid-front gowns should be worn over this clothing. Front-button laboratory coats are unsuitable. The gown must be removed and left in the animal room. Before leaving the animal facility, scrub suits and uniforms are removed and appropriately contained and decontaminated prior to laundering or disposal.
- b. Personal protective equipment is used based on risk assessment determinations.

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- (1) Personal protective equipment is used for all activities involving manipulations of infectious materials or infected animals.
 - (2) Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room wastes before disposal.
 - (3) Appropriate face/eye and respiratory protection (e.g., respirators and face shields) is worn by all personnel entering animal rooms.
 - (4) Boots, shoe covers, or other protective footwear, and disinfectant foot baths are available and used when indicated.
- c. The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in partial containment caging systems, such as open cages placed in inward flow ventilated enclosures (e.g., laminar flow cabinets), solid wall and bottom cages covered with filter bonnets, or other equivalent primary containment systems.
 - d. Biological safety cabinets and other physical containment devices are used whenever conducting procedures with a potential for creating aerosols. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, or intranasal inoculation of animals. At BSL-3, all work should be done in a primary barrier; otherwise respirators should be worn by personnel in the room.
4. Animal Facilities (Secondary Barriers)
 - a. The animal facility is separated from areas that are open to unrestricted personnel traffic within the building.
 - b. Access to the facility is limited by a self-closing and self-locking door. This exterior entry door may be controlled by a key lock, card key, or proximity reader. Entry into the animal room is via a double-door entry which includes a change room and shower(s). An additional double-door access (air-lock) or double-doored autoclave may be provided for movement of supplies and waste into and out of the facility, respectively. Doors to animal rooms open inward and are self-closing. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
 - c. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls,

floors, and ceilings) are water resistant. Penetrations in floors, walls and ceiling surfaces are sealed and openings around ducts and the spaces between doors and frames are capable of being sealed to facilitate decontamination.

- d. A hands-free or automatically operated hand washing sink is provided in each animal room near the exit door. The sink trap is filled with an appropriate disinfectant after each use.
- e. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas.
- f. Windows are not recommended. Any windows must be resistant to breakage and must be sealed.
- g. If floor drains are provided, they are always filled with an appropriate disinfectant.
- h. Ventilation should be provided in accordance with criteria from the *Guide for Care and Use of Laboratory Animals*, latest edition. 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 may not be required, but should be considered based on site requirements, and specific agent manipulations and use conditions. The exhaust must be dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Personnel must verify that the direction of the airflow (into the animal areas) is proper. It is recommended that a visual monitoring device that indicates and confirms directional inward airflow be provided at the animal room entry. Consideration should be given to installing an HVAC control system to prevent sustained positive pressurization of the animal spaces. Audible alarms should be considered to notify personnel of HVAC system failure.
- i. The HEPA filtered exhaust air from a Class II biological safety cabinet can be recirculated into the animal room 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 throughout 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 system 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.

- j. Cages are washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F.
- k. An autoclave is available which is convenient to the animal rooms where the biohazard is contained. The autoclave is utilized to decontaminate infectious waste before moving it to other areas of the facility.
- l. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter, placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
- m. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- n. The complete 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.
- o. 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 of the site conditions, or other applicable federal, state, or local regulations.

I. Animal Biosafety Level 4

Animal Biosafety Level 4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission. ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. Procedures must be developed locally to address specific operations of the Class III cabinet line or the suit laboratory.

1. Standard Practices

- a. Aside from the standard policies, procedures, and protocols for emergency situations established by the facility director, appropriate special policies and procedures should be developed

as needed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).

- b. The laboratory or animal facility director limits access to the animal room to the fewest individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- c. A medical surveillance program must be instituted for all persons entering an ABSL-4 facility. This program must include appropriate immunizations, serum collection, and availability of post-exposure counseling and potential prophylaxis. In general, persons who may be at increased risk of acquiring infection, or for whom infection might have serious consequences, are not allowed in the animal facility unless special procedures can eliminate the extra risk. Assessment should be made by the occupational health physician.
- d. A site-specific biosafety manual is prepared or adopted. Personnel are advised of special hazards, and are required to read and to follow instructions on practices and procedures.
- e. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use should be done only in designated areas and are not permitted in animal or procedure rooms.
- f. All procedures are carefully performed to minimize the creation of aerosols.
- g. Equipment and work surfaces in the room are routinely decontaminated with an appropriate disinfectant after work with the infectious agent, and especially after overt spills, splashes or other contamination by infectious material.
- h. A spill procedure is developed and posted. Only personnel properly trained and equipped to work with infectious materials are to clean up spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- i. All wastes (including animal tissues, carcasses, and contaminated bedding), other materials for disposal, and clothing to be laundered, are sterilized in a double-door autoclave located in the secondary barrier wall of the facility. Disposable wastes are

incinerated.

- j. Policies for the safe handling of sharps are instituted.
 - (1) Needles and syringes or other sharp instruments are restricted in the animal facility for use only when there is no alternative, such as for parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
 - (2) Syringes that re-sheath the needle, needle-less systems, and other safe devices should be used when appropriate.
 - (3) Plasticware should be substituted for glassware whenever possible.
- k. A biohazard sign must be posted on the entrance to the animal room whenever infectious agents are present. The hazard warning sign identifies the infectious agent(s) in use, lists the name and telephone number of the responsible person(s), and indicates the special requirements for entering the animal room (e.g., the need for immunizations and respirators).
- l. Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the annual updates, or additional training as necessary for procedural or policy changes. Records are maintained on all training provided.
- m. Cages are autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with infectious materials, and especially overt spills, splashes, or other contamination by infectious materials. Equipment must be decontaminated according to any local, state, or federal regulations before removal from the facility for repair or maintenance.
- n. Personnel assigned to work with infected animals should work in pairs. Based on the risk assessment, use of squeeze cages, working only with anesthetized animals, or other appropriate procedures to reduce possible worker exposure must be instituted.
- o. Materials not related to the experiment (e.g., plants, animals) are not permitted in the facility.

2. Special Practices

- a. Additional measures are effected to control access (e.g., 24-hour guard and check in/out system). Personnel enter and leave the facility only through the clothing change and shower rooms. Personnel shower each time they leave the facility. Personnel should not enter or leave the facility through the airlocks, except in an emergency.
 - b. In a Class III cabinet operation, 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, are provided and used by all personnel entering the facility. When exiting, personnel remove laboratory clothing in the inner change room before entering the shower area. Soiled clothing is sterilized in an autoclave.
 - c. In an ABSL-4 suit operation, a complete clothing change is required. A personal shower is required following removal of the decontaminated suit. Soiled lab clothing is autoclaved before laundering.
 - d. Supplies and materials are introduced into the facility via a double-door autoclave or fumigation chamber. After the outer door is secure, personnel inside the facility open the inner door to retrieve the materials. The doors of the autoclave and fumigation chamber are interlocked in a manner that prevents opening of the outer door **unless** the autoclave has been operated through a "sterilization cycle" or the fumigation chamber has been decontaminated.
 - e. A system is established for the reporting of accidents, incidents, exposures, and employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such a reporting/surveillance system is the availability of a facility for the quarantine, isolation, and medical care of persons with potential or known laboratory-associated illnesses.
 - f. The serum samples collected are analyzed at intervals. The results are communicated to the participants.
3. Safety Equipment (Primary Barriers)
- a. Laboratory animals, infected with agents assigned to Biosafety Level 4, are housed a Class III biological safety cabinet in a BSL-4 Cabinet Laboratory. In a BSL-4 Suit Laboratory, all personnel are required to wear one-piece positive pressure suits ventilated with a life support system. Infected animals should be housed in a partial containment system (such as open cages placed in

ventilated enclosures, solid wall and bottom cages covered with filter bonnets and opened in laminar flow hoods, or other equivalent primary containment systems).

- b. The use of disposable material that does not require cleaning, including animal caging, should be considered. Disposable materials must be autoclaved on exit from the facility and then incinerated.

4. Animal Facility (Secondary Barriers)

BSL-4 animal areas may be included as an integral part of BSL-4 Cabinet Laboratories or Suit Laboratories. The facility requirements described in the BSL-4 Laboratory section should be utilized in conjunction with caging described in the equipment section above.

Source: CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Department of Health and Human Services Public Health Services, Centers for Disease Control and Prevention and National Institutes of Health, HHS Publication No. (CDC) 93-8395, 4th. Edition, May, 1999.

J. ***OU Laboratory Bloodborne Pathogen Exposure Control Plan (Procedures for Working with Human Blood or Other Potentially Infectious Material)***

Laboratories with employees who have reasonably anticipated eye, skin, mucous membrane or parenteral contact with human blood or other potentially infectious materials must follow this *OU Laboratory Bloodborne Pathogen Exposure Control Plan* in compliance with the OSHA Bloodborne Pathogen Standard.

1. Other potentially infectious material includes the following:
 - a. human body fluids:
 - (1) semen,
 - (2) vaginal secretions,
 - (3) pericardial fluid,
 - (4) cerebrospinal fluid,
 - (5) synovial fluid,
 - (6) pleural fluid,
 - (7) pericardial fluid,
 - (8) peritoneal fluid,
 - (9) amniotic fluid,
 - (10) saliva in dental procedures,
 - (11) any body fluid that is visibly contaminated with blood, and
 - (12) all body fluids in situations where it is difficult or impossible to differentiate between body fluids.
 - b. other:
 - (1) any unfixed tissue or organ (other than intact skin) from a

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- (2) human, living or dead, human immunodeficiency virus (HIV)-containing cell or tissue cultures,
 - (3) human organ cultures,
 - (4) HIV or hepatitis B virus (HBV) containing culture medium or other solutions,
 - (5) blood, organs, or other tissues from experimental animals infected with HIV, HBV or other bloodborne pathogens infectious to man, and
 - (6) human cell lines and human cell strains.
 - (a) Only established human cell lines and human cell strains which are characterized (tested by antigenic screening for viral or agent markers, co-cultivation with indicator cells allowing contaminants to grow, or molecular technology such as polymerase chain reaction or nucleic acid hybridization) to be free of bloodborne pathogens (including HIV, HBV, Epstein-Barr virus, Herpes virus and papilloma members of the Papovavirus group, etc.) and documented as such may be excluded from the requirements of the OSHA Bloodborne Pathogen Standard.
 - (b) Cell lines/strains that are procured from commercial vendors or other sources with documented testing to be free of human bloodborne pathogens and which have been protected from contamination may be excluded from the requirements of the OSHA Bloodborne Pathogen Standard.
2. Universal precautions should be observed which dictates that all human blood and other potentially infectious materials should be treated as infectious for HBV, HIV, and other bloodborne pathogens.
 3. Engineering and work practice controls should be utilized first to minimize employee exposure. Where occupational exposure remains after the institution of engineering controls, personal protective equipment (PPE) should also be used as follows:
 - a. Gloves should be worn when it can be reasonably anticipated that the employee may have hand contact with blood or other potentially infectious materials and when handling or touching contaminated items.
 - b. Masks in combination with eye protection devices such as goggles or face shields should be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious material may be generated and eye, nose, or mouth contamination can be

reasonably anticipated.

- c. Gowns, aprons, lab coats, surgical caps or hoods, and/or shoe covers should be worn when gross contamination can be reasonably anticipated. The type and characteristics of this protective clothing will depend upon the task and degree of exposure anticipated.
4. Handwashing facilities should be readily accessible to employees. Personnel in work areas that do not have handwashing facilities readily accessible should be provided with an appropriate hand cleanser in conjunction with clean cloth or paper towels or antiseptic towelettes. Employees should wash their hands with soap and running water as soon as feasible after using antiseptic hand cleansers or towelettes.
 5. All garments should be removed as soon as possible if penetrated by blood or other potentially infectious material.
 - a. Removed PPE should be placed in a designated area or container for storage, washing, decontamination, or disposal. Contaminated PPE should be placed in a designated container labeled with the biohazard symbol.
 - b. PPE should be cleaned, laundered and/or disposed in a proper manner.
 - (1) Contaminated disposable PPE should be placed in a biohazard bag until it can be sterilized/autoclaved.
 - (2) Contaminated launderable PPE should be placed in a container labeled with the biohazard symbol until sent to a laundry which will handle it in accordance with OSHA requirements.
 6. Employees should wash their hands immediately or as soon as feasible after removal of gloves or other PPE.
 7. Employees should wash their hands or other skin with soap and water or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.
 8. Appropriate commercially available and effective safer medical devices designed to eliminate or minimize occupational exposure should be used whenever feasible.
 9. Contaminated needles or other contaminated sharps should not be bent, recapped, or removed. If needles must be recapped, a mechanical means or a one-handed technique should be used.
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10. Immediately or as soon as possible after use, contaminated sharps should be placed in appropriate sharps containers, even if the sharps are reusable and will be reprocessed.
 - a. Sharps containers should be:
 - (1) puncture resistant,
 - (2) labeled with the biohazard symbol or color-coded,
 - (3) leak-proof on the sides and bottom, and
 - (4) not be allowed to overfill (a good guideline is to dispose when approximately two-thirds full).
 - b. Other guidelines for selection of sharps containers should consider issues such as lids that lock tight for safe disposal, a container that is specifically constructed for the method of sterilization that will be used (if sharps containers are not specifically constructed to be autoclaved, the resulting mass of melted plastic is extremely hazardous due to the needles that often protrude), and a clear top that would allow inspection.
 11. Eating, drinking, smoking, applying cosmetics or lip balm and handling contact lenses is prohibited in areas where there is a reasonable likelihood of occupational exposure to human blood or other potentially infectious materials.
 12. Food or drink should not be kept in areas where blood or other potentially infectious materials are present or stored.
 13. Procedures which minimize spraying, splashing, spattering, and generation of droplets of infectious material shall be used whenever possible.
 14. No mouth pipetting should occur.
 15. Biohazard labels should be affixed to all containers of regulated waste, refrigerators, freezers and other containers that hold or are contaminated with blood or other potentially infectious material. Red bags or containers may be substituted for labels.
 16. Specimens of blood or other potentially infectious materials should be placed in a container which prevents leakage during collection, storage, transport, or shipping. This container should be red or labeled with the biohazard symbol and closed prior to being stored, transported, or shipped. If contamination outside this primary container occurs or is likely to occur, it should be placed in a second red or similarly labeled container which prevents leakage during handling processing, storage, transport, or shipping.

17. Equipment which has been in contact with blood or other potentially infected material should be examined prior to servicing or shipping and should be decontaminated as necessary.
 - a. Where complete decontamination cannot occur prior to servicing, a readily observable biohazard label should be attached to the equipment stating which portions of the equipment remain contaminated, and
 - b. the employee requesting the service or repair is responsible for ensuring that information is conveyed to all affected employees, service representatives, and/or the manufacturer prior to handling, servicing, or shipping so that appropriate precautions can be taken.
18. Contaminated work surfaces should be decontaminated after completion of procedures, immediately or as soon as feasible after any spill of blood or other potentially infectious material, and at the end of the work shift if the surface has become contaminated since the last cleaning.
19. Broken glassware which may be contaminated should not be picked up directly with the hands but by mechanical means such as a brush and dustpan, tongs or forceps.
20. All employees with occupational exposure should receive bloodborne pathogen training at the time of assignment to tasks where occupational exposure may take place, when changes affect employees' occupational exposure and at least annually thereafter.
21. The hepatitis B vaccine should be made available to all employees who have reasonable anticipation of occupational exposure to blood or other potentially infectious materials at no cost to the employee (cost to be borne by the department).
22. If an employee sustains an exposure incident (such as a stick with a contaminated needle/scalpel/dental wire or a splash of potentially infectious material in the eye, mouth, mucous membrane, or non-intact skin), the exposed person should immediately:
 - a. clean the wound with soap; flush mucous membranes with water or normal saline solution;
 - b. notify his/her supervisor;
 - c. proceed for treatment at Goddard Health Center or the nearest emergency room within 1-2 hours of the exposure; and
 - d. if possible, for laboratory exposures, bring a sample of the source

material to the treatment facility for testing.

23. Following an exposure incident, a confidential examination and follow-up should be made available to the employee to address such infectious diseases as HBV, HCV, and HIV. This should include confidential post-exposure prophylaxis and counseling in accordance with current CDC protocol.
24. The healthcare professional providing treatment must forward a written opinion (as outlined in the OSHA regulation) to the employee and the employee's supervisor, and maintain a copy on file.

Sources: OSHA *Bloodborne Pathogen Standard* (29 CFR 1910.1030)

K. Recombinant DNA

The National Institutes of Health (NIH) publishes *Guidelines for Research Involving Recombinant DNA Molecules* in the Federal Register which should be followed when constructing and handling recombinant DNA molecules or organisms and viruses containing recombinant DNA molecules. The most current version of this document is available upon request from the EHSO. Under these guidelines, the Principal Investigator must:

1. make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;
2. select appropriate microbiological practices and laboratory techniques to be used for the research;
3. submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the Institutional Biosafety Committee (IBC) for review and approval or disapproval;
4. remain in communication with the IBC throughout the conduct of the project;
5. make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
6. instruct and train laboratory staff in:
 - a. the practices and techniques required to ensure safety, and
 - b. the procedures for dealing with accidents;
7. inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection);

8. supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
9. investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (EHSO), Animal Facility Director (where applicable), IBC, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);
10. correct work errors and conditions that may result in the release of recombinant DNA materials; and
11. ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).
12. comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines*.

Source: NIH *Guidelines for Research Involving Recombinant DNA Molecules*, April, 2002.

L. OU Recombinant DNA-Biohazard Committee

1. The purpose of the OU Recombinant DNA-Biohazard Committee is:
 - a. to assist in complying with all Federal, State, and local guidelines regarding recombinant DNA activities.
 - b. to train OU Norman Campus faculty, students, and staff in proper handling and disposal of all recombinant DNA-related reagents and biological materials.
 - c. to be proactive regarding all OU Norman Campus recombinant DNA activities.
2. All research, including funded Grant Applications and non-funded research, which address the following subjects, must seek Recombinant DNA-Biohazard Committee approval:
 - a. cloning,
 - b. recombinant DNA,
 - c. plasmid,
 - d. ligation or ligate,

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- e. transformation or transform,
 - f. vector
 - g. bacteriophage, and/or
 - h. virus.
3. All research that involves the above materials or subjects must take the following actions.
- a. The Principal Investigator must contact the Recombinant DNA-Biohazard Committee Chair, Dr. Bruce Roe (325-4912), to discuss the planned activities, prior to the final grant submission, so that it can be determined if any recombinant DNA or any potential biological hazardous work is proposed.
 - b. After a preliminary determination that recombinant DNA activities are being planned, the Principal Investigator typically submits a draft of the proposal to Dr. Roe for review to verify that the proposed work is in compliance with all Federal, State, and local guidelines and falls within the category “exempt from the guidelines”.
 - (1) If it is determined that the proposed work meets these guidelines, a letter confirming this will be forwarded to the OU Office of Research Administration.
 - (2) Should the work proposed fall in any existing Recombinant DNA category other than “exempt from the guidelines”, a special meeting of the Recombinant DNA-Biohazard Committee will be held with the Principal Investigator to discuss the proposed research, determine whether it complies with all Federal, State, and local guidelines, and formally notify the PI and the Office of Research Administration of the results of this meeting.

M. Animal Safety

1. Zoonotic Diseases

A zoonotic disease is defined as one that is communicable from animals to humans. Some species posing risks (in decreasing order of hazard) are provided in Table VI-1. Strategies for staying healthy and reducing the risk of exposure are as follows.

- a. Wash your hands frequently - the most common method of contracting a zoonotic infection is by placing infectious material in

your mouth, nose, or eyes.

- b. Wear personal protective clothing and equipment (see Table VI-1) and do not take unlaundered protective clothing home.
- c. Follow recommended safe work practices (see Tables VI-2 and VI-3).
- d. Notify your supervisor immediately if an exposure incident occurs, even if it seems minor, then seek medical attention. First-aid kits are readily available in all buildings housing animals, then report to a medical facility such as Employee Health.
- e. Tell your personal physician that you work with animals. Many zoonotic diseases have flu-like symptoms: your physician needs this information for making an accurate diagnosis.

2. Injury

Environmental factors, as well as factors intrinsic to the animal, can lead to greater risk for injury. Animals respond to sounds and smells, sometimes undetectable to humans, which can frighten the animal. Animals may have a flight zone or a particular sign of distress that animal handlers should be aware of to reduce risk. Inappropriate handling can induce discomfort, pain and distress, provoking an animal to inflict injury on its handler. Animals, especially non-human primates, may grab or get caught in loose clothing, long hair, etc., or may spit or throw feces. Guidelines for preventing injury include:

- a. Know the animal's flight zone and signs of distress.
- b. Use proper handling techniques.
- c. Minimize the use of sharps and glass and ensure proper disposal or same.
- d. Determine the potential risk and wear appropriate protective equipment for the hazard, which may include leather gloves, latex/nitrile gloves, face shield, etc.
- e. If you must lift heavy objects, contact the EHSO for safe lifting procedures and training.
- f. Minimize the amount of time a floor is allowed to remain wet, and use slip-resistant footwear, mats and signage whenever wet floors cannot be avoided.
- g. If a bite or a scratch is sustained, it is important that medical care be sought, even if the injury seems trivial, due to the potential for

disease transmission, which can include *Cercopithecine herpesvirus 1* (B-virus), Rabies, hantavirus infection, cat-scratch fever, tularemia, rat-bite fever, and *Staphylococcus spp.* infection. Procedures to follow in the event of a bite or scratch are:

- (1) While wearing gloves, carefully express the wound and apply gentle pressure around the wound to encourage bleeding.
- (2) Rinse the wound under warm running water for 15 minutes and continue massaging the site.
- (3) If the injury involves a macaque or any animal known to be infected with a zoonotic disease, you may wash the wound and surrounding area with povidone-iodine solution for 5 minutes. Care should be taken when using iodine, as prolonged skin exposure to iodine can increase wound healing time and can cause tissue damage.
- (4) For all other wounds, or if you are concerned about using iodine, wash the wound and surrounding area with soap and water for 5 minutes.
- (5) Continue to rinse periodically. If normal saline is available, rinse with normal saline.
- (6) Pat the injury dry using sterile gauze pads.
- (7) Cover the wound with a pad and secure it with gauze and tape.
- (8) Seek medical attention.

3. Animal Allergies/Asthma

Animals and animal products such as dander, hair, scales, fur, saliva, and body wastes contain powerful allergens that can cause both respiratory and skin disorders. Sources of exposure to animal allergens vary with animal species. For example, allergens have been found in the urine of rats; the urine, saliva, and pelts of guinea pigs; rabbit pelts, cat saliva and dander; dog dander; and horse serum and dander.

Inhalation is one way for animal allergens to enter the body. After a period of time (often several months, but occasionally many years), one may inhale sufficient quantities of allergens to become sensitized - that is, develop symptoms when exposed again, even to tiny amounts of the allergen. Other routes of exposures may come from animal bites or scratches.

In sensitized persons, reactions often occur soon after exposure to the animal or animal product, but they may be delayed for 2 to 8 hours or more. Symptoms vary from mild reactions such as sneezing and runny nose to more serious reactions such as cough, chest tightness, wheezing, or shortness of breath.

The National Institute for Occupational Safety and Health (NIOSH) recommends several measures to reduce exposures to animal allergens in the workplace and prevent animal-induced asthma and allergies, including the following:

- a. Provide training to educate workers about animal allergies and steps for risk reduction.
- b. Perform animal manipulations within ventilated hoods or safety cabinets when possible.
- c. Avoid wearing street clothes while working with animals. Leave work clothes at the workplace to avoid potential exposure problems for family members.
- d. Keep cages and animal areas clean. Take particular care to control exposures during cleaning (minimize dust and aerosols, etc.).
- e. Reduce skin contact with animal products such as dander, serum, and urine by using gloves, lab coats, and approved particulate respirators with faceshields where appropriate.
- f. Provide health monitoring and appropriate counseling and medical followup for workers who have become sensitized or have developed allergy symptoms.

4. Institutional Animal Care and Use Committee (IACUC) Requirements

The OU IACUC is responsible for the overview of animal research at OU, and has determined that annual training in appropriate safety areas and animal handling procedures is mandatory for all OU personnel working with animals. A semiannual program evaluation and facilities inspections provide a review of regulations and guidelines, thus enabling the IACUC to determine the program's overall effectiveness.

Anyone who has a concern about their care, tending, and/or use of research animals should report the matter directly to either the Animal Resources Director, the IACUC chair, or any IACUC committee member. These concerns, and any action that results, will be presented to the IACUC chair and then to the entire IACUC committee.

Sources: *Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials*, National Research Council, National Academy Press, 1989.
Biosafety in Microbiological and Biomedical Laboratories, fourth edition, Centers for Disease Control and

Prevention/National Institutes of Health, U. S. Government Printing Office, 1999.

NIOSH ALERT: Preventing Asthma in Animal Handlers, DHHS (NIOSH) Publication No. 97-116, January 1998.

Occupational Health and Safety in the Care and Use of Research Animals, National Research Council, National Academy Press, 1997.

**TABLE VI-1
DISEASES, PROTECTIVE EQUIPMENT, AND MEDICAL MONITORING/VACCINATIONS FOR CERTAIN ANIMAL SPECIES**

Species	Potential Zoonotic Disease	Minimum Personal Protective Equipment*	Medical Monitoring/Vaccinations
Non-human Primates	<i>Circopithecine herpesvirus 1: CHV 1 (Herpesvirus simiae)</i> Hepatitis A Shigellosis (<i>Shigella</i> spp.) Campylobacteriosis (<i>Campylobacter</i> spp.)	Disposable surgical face mask Disposable gloves Disposable shoe covers Disposable hair bonnet Disposable gown Face shield**	Baseline and annual physical Serum banking (recommended) Tetanus immunization TB test (every 6 months) Hepatitis B immunization (recommended for those working with apes)
Dogs	Campylobacteriosis Leptospirosis (<i>Leptospira interrogans</i>) Salmonellosis (<i>Salmonella</i> spp.) Rabies virus	Gloves appropriate for the hazard (leather to protect against bites, latex/nitrile to protect against biological material) Laboratory coat Face protection (mask, goggles, face shield) when potential for splash of hazardous material exists	Baseline and annual physical Serum banking (recommended) Tetanus immunization Rabies vaccination
Cats	Toxoplasmosis (<i>Toxoplasma gondii</i>) Cat-scratch fever (<i>Bartonella henselae</i>) Salmonellosis Rabies Microsporosis/Ringworm (<i>Epidermophyton floccosum</i> , <i>Microsporium</i> spp., <i>Trichophyton</i> spp.)	Gloves appropriate for the hazard (leather to protect against bites, latex/nitrile to protect against biological material) Laboratory coat Face protection (mask, goggles, face shield) when potential for splash of hazardous material exists	Baseline and annual physical Serum banking (recommended) Tetanus immunization Rabies vaccination Toxoplasmosis antibodies titer for premenopausal personnel
Sheep	Q fever (<i>Coxiella burnetii</i>)	Gloves appropriate for the hazard (leather to protect against bites, latex/nitrile to protect against biological material) Laboratory coat Face protection (mask, goggles, face shield) when potential for splash of hazardous material exists	Baseline and annual physical Serum banking (recommended) Tetanus immunization Q-Fever assessment
Rabbits	Leptospirosis Microsporosis/Ringworm	Gloves appropriate for the hazard (leather to protect against bites, latex/nitrile to protect against biological material) Laboratory coat Face protection (mask, goggles, face shield) when potential for splash of hazardous material exists	Baseline and annual physical Serum banking (recommended) Tetanus immunization

**TABLE VI-1
DISEASES, PROTECTIVE EQUIPMENT, AND MEDICAL MONITORING/VACCINATIONS FOR CERTAIN ANIMAL SPECIES**

Species	Potential Zoonotic Disease	Minimum Personal Protective Equipment*	Medical Monitoring/Vaccinations
Laboratory raised rodents	Leptospirosis Salmonellosis Microsporosis Ringworm Allergies	Gloves appropriate for the hazard (leather to protect against bites, latex/nitrile to protect against biological material) Laboratory coat Face protection (mask, goggles, face shield) when potential for splash of hazardous material exists	Baseline and annual physical Serum banking (recommended) Tetanus immunization

*For routine work where the animal is not known to be infected with an organism for research purposes. Where infection is known, the appropriate Animal Biosafety Level precautions shall be used (see Table VI-3).

**Persons performing cage cleaning, moving, or handling of a conscious animal must wear additional protective equipment.

**TABLE VI-2
RECOMMENDED SAFE WORK PRACTICES**

Practices to Reduce the Number of Employees at Risk of Exposure	<p>Restrict access to the work area. Provide warnings of hazards (such as biohazards or chemical hazards) and advice about special requirements (such as personal protective equipment or immunization requirements).</p>
Practices to Reduce Exposures by Direct and Indirect Contact	<p>Keep hands away from mouth, nose, eyes, and skin. Wash hands when contaminated and when work activity is completed; especially after handling animals and before leaving the work area. Decontaminate work surfaces before and after work and after spills of a hazardous agent. Use appropriate methods to decontaminate equipment, surfaces, and wastes. Substitute less-hazardous materials for hazardous materials whenever possible. Wear personal protective equipment while performing work and remove it before leaving the work area.</p>
Practices to Reduce Percutaneous Exposures	<p>Eliminate the use of sharp objects whenever possible. Use needles with self-storing sheaths or those designed to protect the user. Select products with puncture-resistant features whenever possible. Use puncture-resistant sharps containers for disposal of sharps. Handle animals with care and proper restraint to prevent scratches and bites.</p>
Practices to Reduce Exposure by Ingestion	<p>No mouth pipetting allowed. Do not smoke, eat, drink, or apply cosmetics in areas used for the care and use of research animals. Keep hands and contaminated items away from mouth. Protect mouth from splash and splatter hazards.</p>
Practices to Reduce Exposure by Inhalation	<p>Use chemical fume hoods, biological safety cabinets, and other containment equipment to control inhalation hazards. Handle fluids carefully to avoid spills and splashes and the generation of aerosols. Use in-line HEPA filters to protect the vacuum system.</p>

**TABLE VI-3
ADDITIONAL PRECAUTIONS FOR ANIMAL BIOSAFETY LEVELS 2 AND 3**

Biosafety Level 2	<p>Written standard operating procedures must be developed. Specific training must be provided to laboratory and animal handlers regarding the infectious agent and special procedures to be used. This training must be documented. Only animals used for the experiment should be allowed in the room. Personal protective equipment may need to be disposable as biomedical waste or require decontamination before reuse. Floor drain traps should be filled with an appropriate disinfectant Waste should be evaluated to determine whether there is a need to autoclave prior to disposal. Transport of animals should only occur in a manner that does not cause transmission of infectious materials. Additional immunization or medical monitoring may be warranted depending on the agent.</p>
Biosafety Level 3	<p>All Biosafety Level 2 procedures. Personal protective equipment must include double gloves, eye and face protection, wrap-around or solid-front gowns (front-button laboratory coats are unsuitable) and respiratory protection, and must be disposed as biomedical waste or decontaminated before removal from the room. All wastes must be decontaminated/autoclaved prior to disposal Cages are autoclaved or thoroughly decontaminated before being cleaned or washed. A spill procedure is developed and posted. Materials related to the experiment are not permitted in the animal room.</p>