Health, Safety & Environment Technical Guidelines

TG - 02

Biological Safety

Produced by

HSE – Facilities & GS Department

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1 Purpose

1.1.1 The purpose of this document is to protect the health and well-being of all Qatar University (**QU**) staff, students, and visitors, and to prevent damage to property, equipment, facilities, and the environment associated with the usage of biohazardous agents as part of the university's activities.

1.1.2 This document provides guidelines on the application of the requirements and principles of the QU Environment Health Safety Management System (**HSEMS**) to activities associated with these QU workplaces.

2 Scope

2.1.1 This HSE Technical Guideline applies to all operations and activities associated with QU activities where biohazardous agents are involved, to enable the effective management of HSE aspects and risks within these workplaces.

2.1.2 Biohazardous agents are infectious microorganisms, or their toxins, which cause or may cause human disease. Although the OSHA "Occupational Exposures to Hazardous Chemicals in Laboratories" (referred to as the "Laboratory Standard") does not apply to biological agents, the University shall apply the same basic requirements - responsibilities, training, laboratory safety plan, reporting of accidents/exposures, etc. - to biological agents.

3 Responsibilities

3.1 Top Management

3.1.1 QU top management shall allocate sufficient resources for the effective implementation of the HSEMS, including the application of this HSE Technical Guideline, and ensure that QU employees, students, contractors and visitors are aware of their responsibilities through appropriate regulation, delegation and communication.

3.1.2 The QU Top Management is also accountable for monitoring and reporting HSE performance and appropriate programs and actions to ensure compliance with the QU HSE Policy.

3.2 Other Accountabilities

3.2.1 The QU HSE and the HSE Committee are accountable to the QU Top Management for the implementation of this HSE Technical Guideline.

3.2.2 Vice President (VPs), , Deans, Directors, Managers, Head Sections/Units and Project Managers are accountable to the QU Top Management for the application of this HSE Technical Guideline in areas under their supervision.

3.2.3 All QU staff are responsible for performing their duties by complying with the requirements of this HSE Technical Guideline as it applies to their activities and workplaces, observing and obeying safety postings and rules, and promptly reporting all incidents and accidents to their supervisors.

4 Guidelines

4.1 Principles of Biological Safety

The term “containment” is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Both good microbiological technique and the use of appropriate safety equipment provide primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents. The use of vaccines may provide an increased level of personal protection. Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials, through a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice, technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

4.1.1 Laboratory Practice and Technique

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infectious materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely.

Each laboratory shall adopt this laboratory biosafety policy to identify the hazard that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A person trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct the laboratory activities.

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 laboratory in-charge and HSE are responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

4.1.2 Safety Equipment (Primary Barrier)

Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of the infectious splashes or aerosols generated by many microbiological procedures. Open-fronted Class I and Class II biological safety cabinets are primary barriers which provide significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of another primary barrier is the safety centrifuge cup; an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this

hazard, containment controls such as BSCs or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure.

Safety equipment also may include items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with the biological safety cabinets and other devices that contain the agents, animals, or materials being worked with. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the primary barrier between the personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

4.1.3 Facility Design (Secondary Barrier)

The design of facility is important in providing a barrier to protect persons working inside and outside the laboratory within the facility, and to protect persons or animals in the community from infectious agents that may be accidentally released from the laboratory. The Laboratory supervisor in-charge in cooperation with the HSE shall be responsible for providing the facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be in direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may be necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks such as laboratory entrances, or separate buildings or modules for isolation of the laboratory.

4.2 Biological Safety Levels (BSLs)

4.2.1 Biosafety Level 1 (BSL-1)

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

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

4.2.1.1 Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory in-charge when experiments or work with cultures and specimens are in progress.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or face shields. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited; yet, mechanical pipetting devices are used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.
- A biohazard sign shall be posted at the entrance to the laboratory whenever infectious agents are present.

4.2.1.2 Safety Equipment (Primary Barriers)

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

4.2.1.3 Laboratory Facilities (Secondary Barriers)

- Laboratories should have doors for access control.
- Each laboratory contains a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Bench tops are water resistant and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
- Laboratory furniture shall be capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

4.2.2 Biosafety Level 2 (BSL-2)

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

4.2.2.1 Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory in-charge when experiments are in progress.
- Persons should wash their hands after handling hazardous materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices should be used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- 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.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.

4.2.2.2 Special Practices

- Access to the laboratory is limited or restricted by the laboratory in-charge 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, which are not allowed in the laboratory or animal rooms. The laboratory in-charge has the final responsibility for assessing each circumstance and for determining who may enter or work in the laboratory or animal room.
- The laboratory supervisor in-charge 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.
- A biohazard sign must be posted on the entrance to the laboratory. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the laboratory in-charge name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- 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).
- When appropriate, consider 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.
- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory supervisor in-charge. The personnel are advised of special hazards and are required to read and follow instructions on all practices and procedures.
- The laboratory in-charge 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.

- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative. Plastic ware should be substituted for glassware whenever possible.
- 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.
- Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.
- 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.
- Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- 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 state, or international regulations before it is sent for repair or maintenance or to be packaged for transport in accordance with applicable state, or international regulations, before removal from the facility.
- Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the laboratory supervisor in-charge. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- Animals not involved in the work being performed are not permitted in the lab.

4.2.2.3 Safety Equipment (Primary Barriers)

- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
- 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.
- 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.
- Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
- 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 any of the personnel.

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

4.2.2.4 Laboratory Facilities (Secondary Barriers)

- Provide lockable doors for facilities that house restricted agents.
- Consider locating new laboratories away from public areas.
- Each laboratory contains a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in the laboratories are inappropriate.
- Bench tops are water resistant and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- The laboratory furniture is capable of supporting anticipated loading and uses. Spaces between the benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in the laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- 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.
- An eyewash station is readily available.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- 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 shall be fitted with fly screens.

4.2.3 Biosafety Level 3 (BSL-3)

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

All procedures involving the manipulation of infectious materials are conducted within the 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). Under 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 through 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 in-charge.

4.2.3.1 Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory in-charge when experiments are in progress.
- Persons wash their hands after handling infectious materials, after removing gloves, and when they leave the laboratory.
- 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.
- Mouth pipetting is prohibited; mechanical pipetting devices should be used.
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method, such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory. Infectious waste from BSL-3 laboratories should be decontaminated before removal for off-site disposal.

4.2.3.2 Special Practices

- The laboratory doors should be kept closed when experiments are in progress.
- 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. The Laboratory in-charge 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.
- The laboratory supervisor in-charge shall ensure 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.
- When infectious materials or infected animals are around in the laboratory or containment module, a hazard warning sign, incorporating the universal biohazard symbol, is posted on all the laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory supervisor in-charge 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.

- The 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.
- The 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.
- A biosafety manual specific to the laboratory is prepared or adopted by the laboratory in-charge and biosafety precautions are incorporated into standard operating procedures. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- The 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.
- The laboratory supervisor in-charge 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.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- 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. Plastic ware should be substituted for glassware whenever possible.
- 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.
- Syringes which re-sheathe the needle, needleless systems, and other safe devices are used when appropriate.
- 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 state regulations.
- 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.
- Laboratory equipment and work surfaces should be decontaminated routinely with an effective disinfectant, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination with infectious materials.
- 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.

- 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.
- Cultures, tissues, specimens of body fluids, or wastes are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- All potentially contaminated waste materials (e.g., gloves, lab coats, etc.) from laboratories are decontaminated before disposal or reuse.
- Spills and accidents that result in overt or potential exposures to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
- Animals and plants not related to the work being conducted are not permitted in the laboratory.

4.2.3.3 Safety Equipment (Primary Barriers)

- 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.
- Gloves must be worn when handling infectious materials, infected animals, and when handling contaminated equipment.
- Frequent changing of gloves should be accompanied by hand washing as recommended. Disposable gloves should not be reused.
- 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.
- When a procedure or process cannot be conducted within a biological safety cabinet, then the appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are to be used.
- Respiratory and face protection are used when in rooms containing infected animals.

4.2.3.4 Laboratory Facilities (Secondary Barriers)

- The laboratory must be separate 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 may be located in the passageway.
- Each laboratory room contains a sink for hand washing. The sink is hands- free or automatically operated and is located near the room exit door.
- 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 the 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.
- Bench tops are water resistant and are resistant to moderate heat and to the organic solvents, acids, alkalis, and those chemicals used to decontaminate the work surfaces and equipment.
- The 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.

- All windows in the laboratory should be closed and sealed.
- 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.
- Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
- A ducted exhaust air ventilation system is provided. This system creates directional airflow which pumps air into the laboratory from the "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 should 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.
- HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.
- 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 annually at least. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.
- 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).
- An eyewash station is readily available inside the laboratory.
- Illumination must be adequate for all activities, avoiding reflections and glare that could impede vision.
- 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.
- 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 the risk assessment, the site conditions, or other applicable state regulations.

4.2.4 Biosafety Level 4 (BSL-4)

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases. 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 such 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.

4.2.4.1 Standard Microbiological Practices

- Access to the laboratory is limited by the laboratory director when experiments are in progress.
- Policies for safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All waste is decontaminated before disposal by an approved method such as autoclaving.

4.2.4.2 Special Practices

- Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. Persons who are immunocompromised or immunosuppressed may be at risk of acquiring infections. Therefore, persons who may be at increased risk of acquiring infection or for whom infection may be unusually hazardous, such as children or pregnant women are not allowed in the laboratory or animal rooms.
- The laboratory supervis*or* 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 supervisor, HSE, or other person responsible for the physical security of the facility. Before entering, people 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.
- 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).
- The laboratory supervisor 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.

- The laboratory personnel receive available immunizations for the agents handed or potentially present in the laboratory.
- Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.
- A biosafety manual is prepared and adopted. The personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- 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. The personnel receive annual updates or additional training as necessary for procedural changes. Personnel enter and leave the laboratory only through the clothing change and shower rooms. They take a decontaminating shower each time they leave the laboratory. The personnel use the airlocks to enter or leave the laboratory only in an emergency.
- Personal clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jumpsuits, shoes, and gloves, is provided and used by all personnel entering the laboratory. When leaving the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing in the inner change room. Soiled clothing is autoclaved before laundering.
- Supplies and materials needed in the facility are brought in by way of the double-door 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.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- 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. Plastic ware should be substituted for glassware whenever possible. PS: This page is repeated already
- 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.
- Syringes that re-sheath the needle, needleless systems, and other safety devices are used when appropriate.
- 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.
- 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 non-breakable, sealed primary

container and then enclosed in a non-breakable, sealed secondary container. This is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

- No materials, except biological materials that are to remain in a viable or intact state, are removed from the Biosafety Level 4 laboratory unless they have been autoclaved or decontaminated before they leave the laboratory. Equipment or material that might be damaged by high temperatures or steam may be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.
- Laboratory equipment is decontaminated routinely after work with infectious materials is completed, and especially after overt spills, splashes, or other contamination with infectious materials. Equipment is decontaminated before it is sent for repair or maintenance.
- 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.
- A system is established for reporting laboratory accidents and exposures and staff 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 inflicted with potential or known laboratory-associated illnesses.
- Materials not related to the experiment being conducted (e.g., plants, animals, and clothing) are not permitted in the facility.

4.2.4.3 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.

4.2.4.4 Laboratory Facility (Secondary Barriers)

There are two models for Biosafety Level 4 laboratories: the Cabinet Laboratory where all handling of the agent is performed in a Class III Biological Safety Cabinet, and the Suit Laboratory where personnel wear a protective suit. Biosafety Level-4 laboratories may be based on either a 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.

Cabinet Laboratory

- The Biosafety Level 4 facility consists of either a separate building or a clearly demarcated and isolated zone within a building. The rooms in the facility are arranged to ensure passage through a minimum of two doors prior to entering the room(s) containing the Class III biological safety cabinet (cabinet room). Outer and inner change rooms separated by a shower are provided for personnel entering and leaving the cabinet room. A double-door autoclave, dunk tank, fumigation chamber, or ventilated anteroom for decontamination is provided at the containment barrier for passage of those materials, supplies, or equipment that are not brought into the cabinet room through the change room.
- 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.
- Walls, floors, and ceilings of the cabinet room and inner change room are constructed to form a sealed internal shell which facilitates fumigation and is resistant to entry and exit of animals and insects. Floors are integrally sealed and covered. The internal surfaces of this shell are resistant to liquids and chemicals to facilitate cleaning and

decontamination of the area. All penetrations in these structures and surfaces are sealed. Openings around doors into the cabinet room and inner change room are minimized and are capable of being sealed to facilitate decontamination. Any drains in the cabinet room floor are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters and protection against vermin.

- Bench tops have seamless or sealed surfaces which are water resistant and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- 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 hand washing sink is provided near the door of the cabinet room(s) and the outer and inner change rooms.
- If there is a central vacuum system, it does not serve areas outside the cabinet room. Inline 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.
- If water fountains are provided, they are automatically or foot-operated and are located in the facility corridors outside the laboratory. The water service to the fountain is isolated from the distribution system supplying water to the laboratory areas and is equipped with a backflow preventer.
- Access doors to the laboratory are self-closing and lockable.
- Any windows are breakage-resistant and sealed.
- Double-door autoclaves are provided for decontaminating materials passing out of both the Class III biological safety cabinet(s) and the cabinet room(s). Autoclaves that open outside of the containment barrier must be sealed to the wall of the containment barrier. The autoclave doors are automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed. 12. Pass-through dunk tanks, fumigation chambers, or equivalent decontamination methods are provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from both the Class III biological safety cabinet(s) and the cabinet room(s).
- 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.
- 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.

- 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 pre-filtration of the supply air.
- 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.
- Appropriate communication systems are provided between the laboratory and the outside (e.g. voice, fax, computer, etc.).

Suit Laboratory

- 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 well ventilated by a life-support system protected by HEPA filtration. The life support system includes redundant breathing air compressors, alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker leaves the area. An automatically starting emergency power source is provided at a minimum for the exhaust system, life support systems, alarms, lighting, entry and exit controls, and BSCs. The air pressure within the suit is positive to the surrounding laboratory. The air pressure within the suit area is lower than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit area, chemical shower, and airlocks, are sealed.
- A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems should be completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.
- A double-door autoclave is provided at the containment barrier for decontaminating waste materials to be removed from the suit area. The autoclave door, which opens to the area external to the suit area, is sealed to the outer wall of the suit area and is automatically controlled so that the outside door can be opened only after the autoclave "sterilization" cycle. A dunk tank, fumigation chamber, or ventilated airlock for decontamination is provided for passage of materials, supplies, or equipment that are not brought into the suit area through the change room. These devices can be also used for the safe removal of materials, supplies, or equipment from the laboratory that cannot be decontaminated in the autoclave.
- Walls, floors, and ceilings of the suit area are constructed to form a sealed internal shell, which facilitates fumigation and is animal and insect repellent. The internal surfaces of this shell are resistant to liquids and chemicals, facilitating cleaning and

decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floor of the suit area contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer vents and other service lines contain HEPA filters.

- Internal facility appurtenances in the suit area, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area.
- Bench tops have seamless surfaces which are water proof and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- 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 the laboratory work should be covered with a non- fabric material that can be easily decontaminated.
- A hands- free or automatically operated hand washing sink is provided in the suit area(s); hand washing sinks in the outer and inner change rooms should be considered based on the risk assessment.
- 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.
- Any windows are breakage-resistants' are sealed.
- 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 into 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.
- 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.
- The supply air to the suit area, decontamination shower, and decontamination airlock are protected by passage through a HEPA filter. The general room exhausts 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 pre-filtration of the supply air.

- The positioning of the supply and exhaust points should be such that dead air space in the suit room should be minimized.
- The treated exhaust air from Class II biological safety cabinets, located in a facility where workers wear an anti-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.
- 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 the operation. Facilities should be re- verified annually against these procedures as modified by operational experience.
- Appropriate communication systems should be provided between the laboratory and the outside.

4.3 Animal Biosafety Levels

4.3.1 Animal Biosafety Level 1 (ABSL-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 all that is of minimal potential hazard to laboratory personnel and the environment.

4.3.1.1 Standard Practices

- Only those persons required for the program or the support purposes are authorized to enter the facility. Before entering, persons shall be advised of the potential biohazards and are instructed on the appropriate safeguards.
- An appropriate medical surveillance program shall be provided.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use shall only be done in the designated areas and are not permitted in the animal or procedure rooms.
- All procedures shall be carefully performed to minimize the creation of aerosols or splatters.
- Work surfaces shall be decontaminated after use or after any spill of viable materials.
- All wastes from the animal room (including animal tissues, carcasses, and contaminated bedding) shall be transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional or local requirements. Incineration is recommended.
- Policies for the safe handling of sharps shall be followed.
- The personnel shall wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- 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).
- An insect and rodent control program shall be implemented.

4.3.1.2 Safety Equipment (Primary Barriers)

- Wearing of laboratory coats, gowns, and/or uniforms in the facility shall be strictly applied. The laboratory coats remain in the animal room. Gowns and uniforms shall not be worn outside the facility.
- Persons having contact with non-human primates shall assess their risk of mucous membrane exposure and wear appropriate eye and face protection.

4.3.1.3 Facilities (Secondary Barriers)

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

4.3.2 Animal Biosafety Level 2 (ABSL-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.

4.3.2.1 Standard Practices

- Access to the animal room shall be 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.
- An appropriate medical surveillance program shall be provided. All personnel shall 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 shall be implemented.
- The personnel shall be advised of special hazards, and shall be required to read and follow instructions on the practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use shall only be done in designated areas and are not permitted in animal or procedure rooms.
- All procedures shall be carefully performed to minimize the creation of aerosols or splatters.

- Equipment and work surfaces in the room shall be routinely decontaminated with an effective disinfectant after work with the infectious agent and especially after overt spills, splashes, or other contamination by infectious materials.
- All infectious samples shall be 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) shall be 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 shall be disinfected prior to moving the material. Autoclaving of the contents prior to disposal is recommended.
- Needles and syringes or other sharp instruments shall be 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.
- Syringes that re-sheathe the needle, needle-less systems, and other safe devices shall be used when appropriate.
- Plastic ware shall be substituted for glassware whenever possible.
- Personnel shall wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- 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.
- An insect and rodent control program shall be implemented.

4.3.2.2 Special Practices

- Animal care laboratory and support personnel shall receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures.
- The personnel shall 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, shall not be allowed in the animal facility unless special procedures can eliminate the extra risk.
- Only animals used for the experiment(s) shall be allowed in the room.
- All equipment must be appropriately decontaminated prior to removal from the room.
- Spills and accidents which result in overt exposures to infectious materials must be immediately reported to the facility in-charge and HSE. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records shall be maintained.

4.3.2.3 Safety Equipment (Primary Barriers):

- Gowns, uniforms, or laboratory coats shall be worn while in the animal room. The laboratory coat shall be removed and left in the animal room. Gowns, uniforms, and laboratory coats are removed before leaving the animal facility. Gloves shall be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
- Personal protective equipment shall be used based on risk assessment determinations. Appropriate face/eye and respiratory protection shall be worn by all personnel entering animal rooms that house nonhuman primates.
- Biological safety cabinets, other physical containment devices, and/or personal protective equipment (e.g., respirators, face shields) shall be 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.

- When needed, animals shall be housed in primary biosafety containment equipment appropriate for the animal species. Filter top cages shall always be handled in properly designed and operating animal bio-containment cabinets recommended for rodents.

4.3.2.4 Facilities (Secondary Barriers)

- The animal facility shall be separated from areas that are open to unrestricted personnel traffic within the building.
- Access to the facility shall be limited by secure locked doors. External doors are self-closing and self-locking. Doors to animal rooms open inward, shall be self-closing, and shall be kept closed when experimental animals are present. Cubicle room inner doors may open outward or be horizontal or vertical sliding.
- The animal facility shall be designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) shall be water resistant.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, shall be arranged to minimize horizontal surface areas.
- Any windows must be resistant to breakage where possible, windows shall be sealed. If the animal facility has windows that are open, they shall be fitted with fly screens.
- If the floor drains are provided, the traps shall always be filled with an appropriate disinfectant.
- The exhaust air shall be discharged to the outside without being recirculated to the other rooms. Ventilation shall be. The direction of airflow in the animal facility shall open inwardly; animal rooms shall maintain negative pressure compared to the adjoining hallways.
- Cages shall be washed manually or in an appropriate cage washer. The mechanical cage washer shall have a final rinse temperature of at least 180F.
- An autoclave shall be available in the animal facility to decontaminate infectious waste.
- A hand washing sink shall be provided in the animal room where infected animals are housed, as well as elsewhere in the facility.
- Illumination shall be adequate for all activities, avoiding reflections and glare that could impede vision.

4.3.3 Animal Biosafety Level 3 (ABSL-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.

4.3.3.1 Standard Practices

- The laboratory or animal facility in-charge limits access to the animal room to the fewest number of individuals possible. The personnel who must enter the room for program or service purposes when work is in progress shall be advised of the potential hazard.
- An appropriate medical surveillance program shall be provided. All personnel shall 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 shall 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.

- The personnel shall be advised against special hazards, and shall be required to read and follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use shall be done only in designated areas and shall not be permitted in animal or procedure rooms.
- All procedures shall be carefully performed to minimize the creation of aerosols or splatters.
- Equipment and work surfaces in the room shall be routinely decontaminated with an effective disinfectant after work with the infectious agent and especially after overt spills, splashes, or other contamination by infectious materials.
- All wastes from the animal room (including animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse animal tissues) shall be transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with the University and State of Qatar requirements.
- Needles and syringes or other sharp instruments shall be 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.
- Syringes that re-sheathe the needle, needle-less systems, and other safety devices shall be used when appropriate.
- Plastic ware shall be substituted for glassware whenever possible.
- Personnel shall wash their hands after handling cultures and animals, after removing gloves, and before leaving the animal facility.
- 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).
- All infectious samples shall be collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s).
- The laboratory and support personnel shall receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. As necessary, personnel receive updates and/or additional training on procedural or policy changes. Records of all training provided shall be maintained.
- An insect and rodent control program shall be implemented.

4.3.3.2 Special Practices

- Cages shall be autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. The equipment must be decontaminated according to any University and State of Qatar regulations before being packaged for transport or removal from the facility for repair or maintenance.
- A spill procedure shall be posted. Only the personnel properly trained and equipped to work with infectious materials shall clean up the spills. Spills and accidents that result in overt exposures to infectious materials must be immediately reported to the facility in-charge and HSE. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records are maintained.
- All wastes from the animal room must be autoclaved prior to disposal.
- Materials not related to the experiment (e.g., plants, animals**)** shall not be permitted in the animal room.

4.3.3.3 Safety Equipment (Primary Barriers)

- Uniforms or scrub suits shall be worn by personnel entering the animal room. Wrap-around or solid-front gowns shall 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 shall be removed and appropriately contained and decontaminated prior to laundering or disposal.
- Personal protective equipment used is based on risk assessment determinations.
- Personal protective equipment shall be used for all activities involving manipulations of infectious material or infected animals.
- Personnel shall wear gloves when handling infected animals. Gloves shall be removed aseptically and autoclaved with other animal room wastes before disposal.
- Appropriate face/eye and respiratory protection (e.g., respirators and face shields) shall be worn by all personnel entering animal rooms.
- Boots, shoe covers, or other protective footwear, and disinfectant foot baths shall be available and used where indicated.
- The risk of infectious aerosols from infected animals or their bedding also can be reduced if animals are housed in 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.
- Biological safety cabinets and other physical containment devices shall be 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 shall be done in a primary barrier; otherwise respirators shall be worn by the personnel in the room.

4.3.3.4 Facilities (Secondary Barriers)

- The animal facility shall be separate from areas that are open to the unrestricted personnel traffic within the building.
- Access to the facility shall be 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 the change room and shower(s). An additional double-door access (air-lock) or double-doored autoclave may be provided for movement of supplies and wastes into and out of the facility, respectively. Doors to animal rooms open inward and shall be self-closing. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.
- The animal facility shall be designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) shall be water resistant. Penetrations in floors, walls and ceiling surfaces shall be sealed and openings around ducts and the spaces between doors and frames shall be capable of being sealed to facilitate decontamination.
- A hands-free or automatically operated hand washing sink shall be provided in each animal room near the exit door. The sink trap shall be filled with an appropriate disinfectant after each use.
- Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, shall be arranged to minimize horizontal surface areas.
- Windows are not recommended. Any windows must be resistant to breakage and must be sealed.
- If floor drains are provided, they shall always be filled with an appropriate disinfectant.
- A ducted exhaust air ventilation system shall be provided. This system creates directional airflow which pumps 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

shall 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. A visual monitoring device that indicates and confirms directional inward airflow shall 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 shall be installed to notify personnel of HVAC system failure.

- 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 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 shall 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 cabinet pressurization.
- Cages shall be washed in a cage washer. The mechanical cage washer has a final rinse temperature of at least 180°F.
- An autoclave shall be 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.
- If vacuum service (i.e., central or local) is provided, each service connection shall 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 shall be installed to permit in-place decontamination and replacement.
- Illumination must be adequate for all activities, avoiding reflections and glares that could impede vision.
- 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.
- Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services, and the provision of effluent decontamination) shall be considered if recommended by the agent summary statement, as determined by risk assessment of the site conditions.

4.3.4 Animal Biosafety Level 4 (ABSL-4)

Animal Biosafety Level 4 involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening diseases, 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.

4.3.4.1 Standard Microbiological Practices

- The laboratory or animal facility in-charge shall limit the access to the animal room to the fewest individuals possible. The personnel who must enter the room for program or service purposes when work is in progress are warned against the potential hazard.
- A medical surveillance program must be instituted for all the 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.

- The personnel shall be warned of special hazards, and are required to read and to follow instructions on practices and procedures.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use shall be done only in designated areas and are not permitted in animal or procedure rooms.
- All procedures are carefully performed to minimize the creation of aerosols or splatters.
- The equipment and work surfaces in the room shall be routinely decontaminated with an appropriate disinfectant after work with the infectious agent, and especially after overt spills, splashes, or other contaminations by infectious materials.
- Only the 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 in-charge and HSE. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- All wastes (including animal tissues, carcasses, and contaminated bedding), and other materials for disposal, and clothing to be laundered, shall be sterilized in a double-door autoclave located in the secondary barrier wall of the facility.
- Needles and syringes or other sharp instruments shall be 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 the laboratory animals and diaphragm bottles.
- Syringes that re-sheathe the needle, needle- less systems, and other safe devices shall be used when appropriate.
- Plastic ware shall substitute glassware whenever possible.
- 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).
- Laboratory personnel shall 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 are maintained on all training provided.
- Cages shall be autoclaved or thoroughly decontaminated before bedding is removed and before they are cleaned and washed. The equipment and work surfaces are routinely decontaminated with appropriate disinfectants after work with infectious materials, and especially after spills, splashes, or other contamination by infectious materials. Equipment must be decontaminated before removal from the facility for repair or maintenance.
- The personnel assigned to work with infected animals shall work in pairs. Use of squeeze cages, working only with anesthetized animals, or other appropriate procedures to reduce possible worker exposure must be implemented.
- Materials not related to the experiment (e.g., plants, animals) shall not be permitted in the facility.

4.3.4.2 Special Practices

- Additional measures shall be implemented to control access (e.g., 24-hour guard and check in/out system). The personnel enter and leave the facility only through the clothing change and shower rooms. The personnel shower each time they leave the facility. The personnel shall not enter or leave the facility through the air locks, except in an emergency.

- In a Class III cabinet operation, personal clothing shall be removed in the outer clothing change room and kept there. Complete laboratory clothing, including undergarments, pants and shirts or jump suits, shoes, and gloves, shall be provided and used by the personnel entering the facility. When exiting, the personnel should remove laboratory clothing in the inner change room before entering the shower area. Soiled clothing is sterilized in an autoclave.
- In an ABSL-4 suit operation, a complete clothing change is required. A personal shower shall be required following removal of the decontaminated suit. Soiled lab clothing shall be autoclaved before laundering.
- Supplies and materials shall be introduced into the facility via a double-door autoclave or fumigation chamber. After the outer door is secure, the personnel inside the facility open the inner door to retrieve the materials. The doors of the autoclave and fumigation chamber shall be 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.

4.3.4.3 Safety Equipment (Primary Barriers)

- The laboratory animals infected with Biosafety Level 4 agents must be housed within a Class III biological safety cabinet in a BSL-4 Cabinet Laboratory. In a BSL-4 Suit Laboratory, all personnel shall be required to wear one-piece anti-pressure suits ventilated with a life support system. Infected animals shall 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).
- 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 before disposal.

4.3.4.4 Facilities (Secondary Barriers)

BSL-4 animal areas may be included as an integral part of BSL-4 Cabinet Laboratories or Suit Laboratories as described in Section 3.3.4.4.4 Laboratory Facility (Secondary Barriers). The facility requirements described in the BSL-4 Laboratory section should be utilized in conjunction with the caging described in the equipment section above.

4.4 Biological Safety Cabinet

Laboratory techniques may produce aerosols, which can contain hazardous research materials, such as infectious agents or chemical carcinogens that can be inhaled by laboratory workers. Biological safety cabinets (BSC) are used as primary barriers to contain hazardous research materials in order to prevent exposure of laboratory personnel and contamination of the general environment. Some biological safety cabinets are designed also to provide a clean work environment to protect cell cultures or sterile apparatus.

4.4.1 Principles of Containment

Containment of hazardous aerosols in biological safety cabinets is achieved by the use of air barriers, physical barriers, and HEPA filtration. Air barriers provide containment by use of directional airflow from the laboratory past the researcher into the cabinet via the work opening. Hazardous aerosols generated during the experimental procedures inside the cabinet are captured and carried by the flow of air and then trapped in HEPA filters. Some biological safety cabinets provide protection of experimental procedures using uniform, unidirectional HEPA filtered air, referred to as laminar air flow that continuously flows over the work area. Turbulence inside the cabinet is minimized by the laminar air flow allowing for immediate removal of contaminants generated by the procedures. The integrity of the containment provided by air barriers can be compromised by the disruption of the air flow patterns in the cabinet. Air barriers are therefore believed to provide only partial containment and should not be used with highly toxic or infectious materials requiring Biosafety Level 4 containment.

Physical barriers are impervious surfaces such as metal sides, glass panels, rubber gloves and gaskets, which physically separate the experimental procedures from the researcher. Biological safety cabinets incorporating all of these physical barriers (class III BSC), and not relying on air barriers, can be used for higher risk agents since compromising containment is less likely than with air barriers.

High efficiency particulate air (HEPA) filters are defined as filters with a filtration efficiency of 99.97% for thermally generated monodisperse dioctylphthalate (DOP) 0.3 mm diameter particles. Because of their high efficiency, HEPA filters are used in biological safety cabinets to remove virtually all particulates, including hazardous microbiological and chemical aerosols, in the air stream passing through the filter. All biological safety cabinets have exhaust filters that remove contaminants as air is discharged from the cabinet. Some types of biological safety cabinets also have supply HEPA filters which provide clean air to the work area. HEPA filters are not effective in capturing chemical vapors.

4.4.2 Classification of Biological Safety Cabinets

There are three classes of biological safety cabinets, designated as Class I, Class II, and Class III. Class I and II cabinets have a protective air barrier across the work opening that separates the laboratory researcher from the work area. Class II cabinets have an additional feature of providing a HEPA filtered, clean work area to protect the experiment from room contamination. There are several variations of Class II cabinets which are described below. Class III biological safety cabinets have a physical barrier between the operator and the work area. Arm length rubber gloves are sealed to glove ports on the cabinet to provide the operator with access to the work area.

4.4.2.1 Class I (Figure 1)

- The Class I cabinet is ventilated for personnel and environmental protection with an inward airflow away from the operator. It is similar in air movement to a chemical laboratory hood.
- The minimum air flow through the work opening is 75 feet per minute (fpm).
- The cabinet exhaust air is HEPA filtered to protect the environment before it is discharged to the outside atmosphere.
- This cabinet is suitable for work with low and moderate risk biological agents, where no product protection is required.

- Provide good operator protection but do not protect the material within the cabinet (the product) from contamination

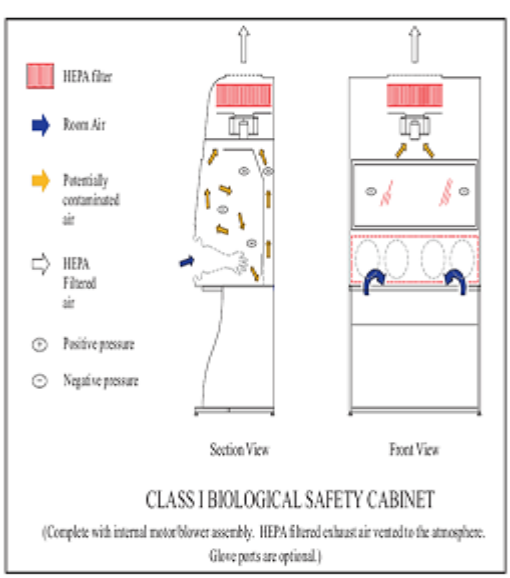

Figure 1a. Class I BSC

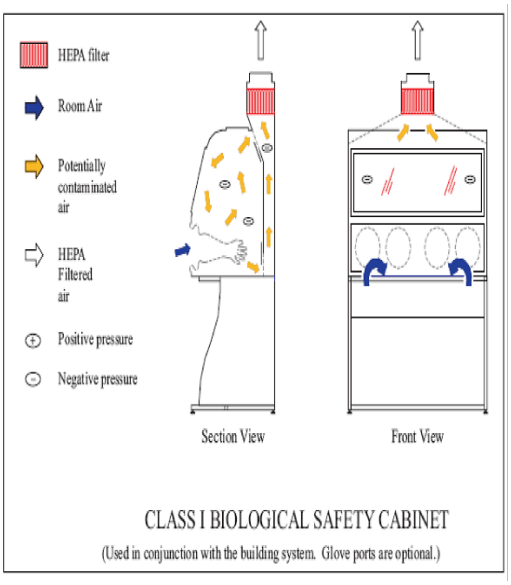

Figure 1b. Class I BSC

4.4.2.2 Class II

- The class II cabinet is ventilated for the personnel, product, and environmental protection having an open front and inward airflow for personnel protection.
- Product protection is provided by HEPA filtered laminar airflow from a diffuser located above the work area. The down flow air splits at the work surface, and exits the work area through grilles located at both the rear and front of the work surface, respectively.
- The cabinet has HEPA filtered exhausted air for environmental protection.
- Designed for work involving microorganisms in containment levels 2, 3 and 4 laboratories and are divided into two types (A and B) on the basis of construction type, airflow velocities and patterns, and exhaust systems(4).
- Within type (A), there are two subtypes, A1 (formerly designated type A) and A2 (formerly designated type B3).
- Within type (B), there are two subtypes, B1 and B2. Class II cabinets are most commonly used in biomedical research laboratories because of their characteristics.

4.4.2.3 Class II, Type A1 Cabinets (Figure 2)

- The cabinet air may be recirculated back into the laboratory or ducted out of the building by means of a "thimble" connection (i.e., a small opening around the cabinet exhaust filter housing) whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system. The thimble must be designed to allow for proper certification of the cabinet (i.e., provide access to permit scan testing of the HEPA filter).
- Maintain a minimum average face velocity of 0.38 m/s (75ft/min).
- May have positive pressure contaminated ducts and plenums.
- Are not suitable for work with low levels of volatile toxic chemicals and volatile radionuclides

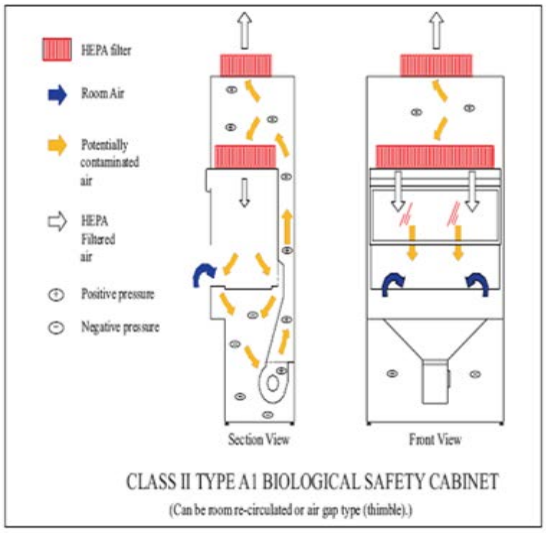

Figure 2. Class II Type A1 BSC

4.4.2.4 Class II, Type A2 Cabinets (Figure 3)

- Cabinet air may be recirculated back into the laboratory or ducted out of the building by means of a "thimble" connection (i.e., a small opening around the cabinet exhaust filter housing) whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system. The thimble must be designed to allow for proper certification of the cabinet (i.e., provide access to permit scan testing of the HEPA filter).
- Maintain a minimum average face velocity of 0.5 m/s (100ft/min).
- Have ducts and plenums under negative pressure.
- Is suitable for work with minute quantities of volatile toxic chemicals and trace amounts of radionuclides.

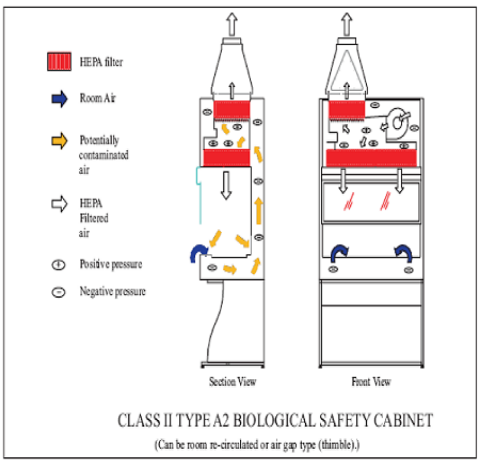

Figure 3. Class II Type A2 BSC

4.4.2.5 Class II, Type B1 Cabinets (Figure 4)

- Hard-ducted through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter; containing negative pressure plena.
- Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- Recirculate 30% of the air within the cabinet.
- Suitable for work with low levels of volatile toxic chemicals and trace amounts of radionuclides.

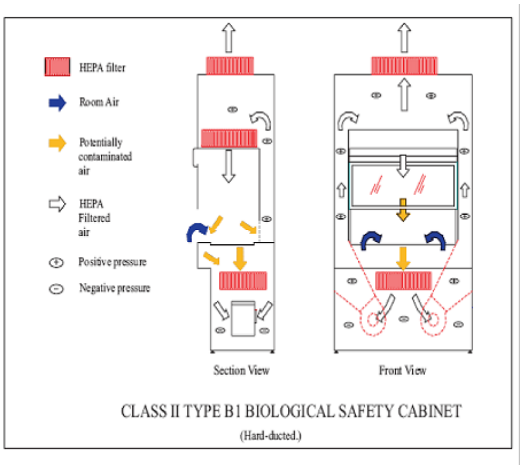

Figure 4. Class II Type B1

4.4.2.6 Class II, Type B2 Cabinets (Figure 5)

- Does not recirculate air within the cabinet.
- Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- Hard-ducted through a dedicated duct exhausted into the atmosphere, 100% of cabinet air, after passage through a HEPA filter; contain negative pressure plena.
- Suitable for work with volatile toxic chemicals and radionuclides.
- The exhaust canopy must allow for proper BSC certification. An alarm should be provided that is audible at the cabinet to indicate loss of exhaust flow from the building exhaust system. The cabinet internal fan should also be interlocked to shut down when the building exhaust system fan fails to prevent pressurization of the cabinet.

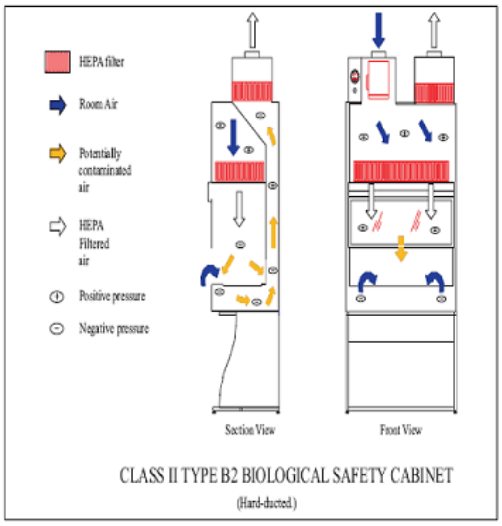

Figure 5. Class II Type B2

4.4.2.7 Class III Cabinets (Figure 6)

- The cabinets are totally enclosed and gas-tight with HEPA filtered supply and exhaust air.
- Work is performed with attached long-sleeve- gloves.
- The cabinet is kept under negative pressure of at least 120 Pa, and airflow is maintained by a dedicated exterior exhaust system.
- Cabinets protect the worker and the product.
- Designed for work with level 4 pathogens and provide an alternative to the positive-pressure suit made for the maximum containment laboratories.
- Cabinet lines consisting of several Class III cabinets (e.g., for centrifuges, animal cages, incubators, refrigerators) and transfer devices joined together are traditionally custom built.
- The exhaust air is double HEPA filtered or treated by HEPA filter and incineration. Removal of materials from the cabinet must be through a dunk tank, double door autoclave or air-lock pass-through for decontamination. Interlock or protocols must be used for the autoclave and pass-through doors to prevent both doors from being opened at the same time.

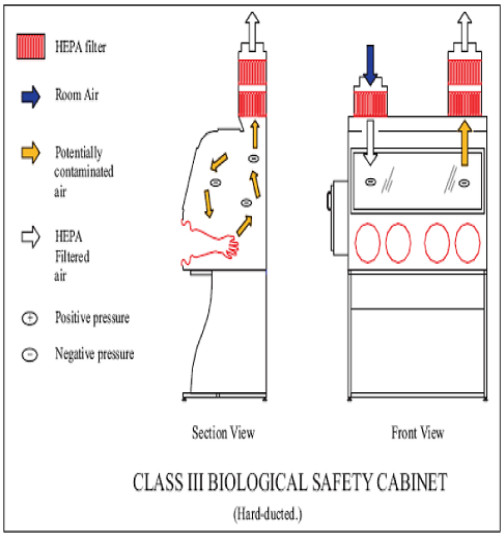

Figure 6. Class III BSC

4.4.3 Installation and Certification of Biological Safety Cabinets

The air curtain at the front of the cabinet is fragile and can easily be disrupted by people walking parallel to it, by the open windows, the air supply registers the laboratory equipment that creates air movement (e.g., vacuum pumps, centrifuges). BSCs should be installed in accordance with the requirements outlined in the NSF Standard 49. They should be located away from high traffic areas, doors and air supply/exhaust grilles that may interrupt airflow patterns. A minimum unobstructed distance of 40 cm should be provided between the exhaust outlet on top of the cabinet and any overhead obstructions. Whenever possible, a 30 cm clearance should be provided on each side of the cabinet to allow for maintenance access. For ducted cabinets, blowers on the exhaust system should be located at the terminal end of the ductwork; failure of exhaust flow should signal an alarm to the user. To prevent pressurization of the cabinet, an interlock system should be installed to prevent the cabinet blower from operating whenever the exhaust flow is insufficient; an anti-backflow device to prevent reverse airflow through the HEPA filter may be required.

Continuous operation of BSCs helps to control dust levels and other airborne particulates in the laboratory. If BSCs are operated only when needed in order to conserve energy, the balancing of laboratory room air must be considered. In some cases, room exhaust is

balanced to include the air exhausted through ducted BSCs, and these cabinets must not be turned off.

The provision of natural gas to BSCs is not recommended. Open flames in the BSC would create turbulence, disrupt airflow patterns and can damage the HEPA filter. When suitable alternatives (e.g., disposable sterile loops, micro-incinerators) are not possible, touch-plate microburners that have a pilot light to provide a flame on demand may be used.
The correct operation of BSCs must be verified before they are used and annually then, and after any repairs or relocation, in accordance with the field tests outlined in annex F of NSF Standard 49. Moving a cabinet can cause damage to the HEPA filter and its seals. These tests include the downward velocity profile, the work access face velocity, the HEPA is the filter leak test and the airflow smoke patterns. Measuring and testing equipment must be calibrated and maintained in accordance with the NSF Standard 49 standard. A copy of the certification report must be provided to the user and kept on file. A label indicating the date of certification, the date of the next certification, to what standard the tests were performed and the name of the certifier should be affixed to the exterior of the cabinet. On-site field testing must be performed by experienced qualified individuals. The NSF accreditation program for BSC certifiers provides a list of individuals who have demonstrated their competence by means of written and practical examinations administered by the NSF. Whenever possible, it is recommended that NSF-accredited field certifiers be used.

4.4.4 Use of the Cabinet

Follow these **start-up procedures** when preparing for work in the BSC:

- Turn off UV lights if in use and ensure that the sash is in the appropriate position.
- Turn on fluorescent light and cabinet blower, if off.
- Check the air intake and exhaust grilles for obstructions.
- If the cabinet is equipped with an alarm, test the alarm and switch it to the "on" position.
- Confirm inward airflow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.
- Disinfect the interior surfaces with a suitable, noncorrosive disinfectant.
- Assemble all materials required for the procedure and load them into the cabinet; do not obstruct the air grilles; the working surface may be lined with absorbent paper with plastic backing; segregate "clean" items from “contaminated" items.
- Wait 5 minutes to purge airborne contaminants from the work area.

Follow these procedures for **working in the cabinet**:

- Don protective clothing and gloves as appropriate.
- Perform operations as far to the rear of the work area as possible.
- Avoid movement of materials or excessive movement of hands and arms through the front access opening during use; when you do enter or exit the cabinet, do so from straight on; allow the cabinet to stabilize before resuming work.

4.5 Biohazards and Potentially Infectious Materials

4.5.1 Biological Risk Assessment

It is the responsibility of the principal investigator or laboratory supervisor in-charge to conduct a risk assessment to determine the proper work practices and containment requirements for work with biohazardous materials. The risk assessment process (*refer to QU HSEMS Section 6.0 Risk Management*) should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to meet with a biohazard incident. This responsibility cannot be shifted to inexperienced or untrained personnel.

The principal investigator or laboratory supervisor in-charge should consult with HSE to ensure that the laboratory complies with the established guidelines and regulations. When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Factors to consider when evaluating a risk include the following:

- Pathogenicity: The more severe the potentially of the acquired disease, the higher the risk.
- Route of transmission: Agents that can be transmitted by the aerosol route which have been known to cause the most laboratory-acquired infections. The greater the aerosol potential, the higher the risk of the infection. Work with *Mycobacterium tuberculosis* is performed at Biosafety Level 3 because disease is acquired via the aerosol route.
- Agent stability: The greater the potential for an agent to survive in the environment, the higher the risk can be. Consider factors such as a desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.
- Infectious dose: Consider the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units. An individual's immune status can also influence the infectious dose.
- Concentration: Consider whether the organisms are in solid tissue, viscous blood, sputum, etc., the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, etc.). In most instances, the risk increases as the concentration of microorganisms increases.
- Origin: This may refer to the geographic location (domestic or foreign), host (infected or uninfected human or animal), or nature of the source (potential zoonotic or associated with a disease outbreak).
- Availability of data from animal studies: If the human data is not available, information on the pathogenicity, infectivity, and route of exposure from animal studies may be valuable. Use caution when translating infectivity data from one species to another.
- Availability of an effective prophylaxis or therapeutic intervention: Effective vaccines, if available, should be offered to the laboratory personnel in advance of their handling of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment (PPE). The availability of post-exposure prophylaxis should also be considered.
- Medical surveillance: Medical surveillance programs may include monitoring the staff health status, participating in post-exposure management, staff counseling prior to offering vaccination, and annual physicals.
- Experience and skill level of at-risk personnel: Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to

working with biohazardous materials. Laboratory workers may have to go through additional training (e.g., HIV training, BSL-3 training, etc.) before they are allowed to work with materials or in a designated facility.

- Infectious agents may be classified into risk groups based on their relative hazard. The table below, presents the "Basis for the Classification of Biohazardous Agents by Risk Group."

Risk Group 1	Agents that are not associated with diseases in healthy adult humans
Risk Group 2	Agents that are associated with human diseases which are rarely serious and for which preventive or therapeutic interventions are often available
Risk Group 3	Agents that are associated with serious or lethal human diseases for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
Risk Group 4	Agents that are likely to cause serious or lethal human diseases for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

4.5.2 Agent List

The following agents have been listed according to the most appropriate Biological Safety Level to be used. The list presented below is based upon the risk groups given in the March 1996 *Guidelines for Research Involving Recombinant DNA Molecules* (National Institute for Health, NIH Guidelines), Appendix B, the agent summary statements in the Center for Disease Control and Prevention, CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th edition (2007), guidance from state and local regulatory agencies, and recommendations of the CDC.

Please note that the Biological Safety Levels are not inherent to an agent but are performance recommendations and should be chosen after a risk assessment is completed. A proper risk assessment takes into account the characteristics of the agent involved, the activities to be performed, and the environment in which the work will be completed. Therefore, certain agents may be used at different Biological Safety Levels depending upon the circumstances. For instance, human clinical samples from HIV-positive patients may be safely handled at BSL-2. Growth of HIV in culture should be performed under BSL-3 containment. Biological Safety Levels may be higher or lower than what is given below for a particular agent depending upon the circumstances of its use.

The Laboratory Safety Subcommittee reviews all the projects involving recombinant DNA, infectious disease agents, and agents of concern to livestock and agriculture and will assist you in the risk assessment process. Once the Laboratory Safety Subcommittee assigns a Biological Safety Level, it must be adhered to unless new information to warrant a change, in most cases from peer-reviewed literature, is provided. The Laboratory Safety Subcommittee will review the literature and make an adjustment, if warranted.

4.5.2.1 Agents - Biological Safety Level 1 (BSL-1)

Agents that are not associated with diseases in healthy adult humans, are of minimal potential hazard to the laboratory personnel, and of minimal potential hazard to the environment may be used at BSL-1. Agents that may be used at BSL-1 include *Lactobacillus* spp., asporogenic

Bacillus subtilis or *Bacillus licheniformis*, *Escherichia coli*-K12 (cloning strains), Baculoviruses, and adeno-associated virus types 1 through 4 in low concentrations (<109 IP/ml).

Those agents not listed under the Biological Safety Levels 2, 3 and 4 are not automatically or implicitly classified as BSL-1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

4.5.2.2 Agents - Biological Safety Level 2 (BSL-2)

Agents to be used at BSL-2 are associated with human diseases which are rarely serious and for which preventive or therapeutic interventions are often available. They are of moderate potential hazard to the laboratory personnel and/or the environment

BSL-2 - Bacterial Agents Including Chlamydia:

- *Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)*
- *Actinobacillus*
- *Actinomyces pyogenes (formerly Corynebacterium pyogenes)*
- *Aeromonas hydrophila*
- *Amycolata autotrophica*
- *Archanobacterium haemolyticum (formerly Corynebacterium haemolyticum)*
- *Arizona hinshawii - all serotypes*
- *Bacillus anthracis*
- *Bartonella henselae, B. quintana, B. vinsonii*
- *Bordetella including B. pertussis*
- *Borrelia recurrentis, B. burgdorferi*
- *Burkholderia (formerly Pseudomonas species) except those listed under BSL-3*
- *Campylobacter coli, C. fetus, C. jejuni*
- *Chlamydia psittaci, C. trachomatis, C. pneumoniae*
- *Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
- *Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
- *Dermatophilus congolensis*
- *Edwardsiella tarda*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7*
- *Haemophilus ducreyi, H. influenzae*
- *Helicobacter pylori*
- *Klebsiella - all species except K. oxytoca (BSL-1)*
- *Legionella including L. pneumophila*
- *Leptospira interrogans - all serotypes*
- *Listeria*
- *Moraxella*
- *Mycobacterium (except those listed under BSL-3) including M. avium complex, M. asiaticum, M. bovis BCG vaccine strain, M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
- *Mycoplasma, except M. mycoides and M. agalactiae which are restricted animal pathogens*
- *Neisseria gonorrhoeae, N. meningitidis*
- *Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*

- *Rhodococcus equi*
- *Salmonella including S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi, A, B, C, S. typhi, S. typhimurium*
- *Shigella including S. boydii, S. dysenteriae, type 1, S. flexneri, S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus including S. pneumoniae, S. pyogenes*
- *Treponema pallidum, T. carateum*
- *Vibrio cholerae, V. parahemolyticus, V. vulnificus*
- *Yersinia enterocolitica*

BSL-2 - Fungal Agents:

- *Blastomyces dermatitidis*
- *Cladosporium bantianum, C. (Xylohypha) trichoides*
- *Cryptococcus neoformans*
- *Dactylaria galopava (Ochroconis gallopavum)*
- *Epidermophyton*
- *Exophiala (Wangiella) dermatitidis*
- *Fonsecaea pedrosoi*
- *Microsporum*
- *Paracoccidioides braziliensis*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Trichophyton*

BSL-2 - Parasitic Agents:

- *Ancylostoma human hookworms including A. duodenal, A. ceylanicum*
- *Ascaris including Ascaris lumbricoides suum*
- *Babesia including B. divergens, B. microti*
- *Brugia filaria worms including B. malayi, B. timori*
- *Coccidia*
- *Cryptosporidium including C. parvum*
- *Cysticercus cellulosae (hydatid cyst, larva of T. solium)*
- *Echinococcus including E. granulosis, E. multilocularis, E. vogeli*
- *Entamoeba histolytica*
- *Enterobius*
- *Fasciola including F. gigantica, F. hepatica*
- *Giardia including G. lamblia*
- *Heterophyes*
- *Hymenolepis including H. diminuta, H. nana*
- *Isospora*
- *Leishmania including L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
- *Loa loa filaria worms*
- *Microsporidium*
- *Naegleria fowleri*
- *Necator human hookworms including N. americanus*
- *Onchoerca filaria worms including, O. volvulus*

- *Plasmodium including simian species, P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
- *Sarcocystis including S. sui hominis*
- *Schistosoma including S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
- *Strongyloides including S. stercoralis*
- *Taenia solium*
- *Toxocara including T. canis*
- *Toxoplasma including T. gondii*
- *Trichinella spiralis*
- *Trypanosoma including T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
- *Wuchereria bancrofti filaria worms*

BSL-2 – Viruses:

- *Adenoviruses, human - all types*
- *Alphaviruses (Togaviruses) - Group A Arboviruses*
- *Eastern equine encephalomyelitis virus*
- *Venezuelan equine encephalomyelitis vaccine strain TC-83*
- *Western equine encephalomyelitis virus*
-
- *Arenaviruses*
- *Lymphocytic choriomeningitis virus (non-neurotropic strains)*
- *Tacaribe virus complex*
- *Other viruses as listed in the BMBL*
-
- *Bunyaviruses*
- *Bunyamwera virus*
- *Rift Valley fever virus vaccine strain MP-12*
- *Other viruses as listed in the BMBL*
- *Calciviruses*
- *Coronaviruses*
- *Flaviviruses (Togaviruses) - Group B Arboviruses*
- *Dengue virus serotypes 1, 2, 3, and 4*
- *Yellow fever virus vaccine strain 17D*
- *Other viruses as listed in the Biosafety in Microbiological and Biomedical Laboratories (BMML)*
- *Hepatitis A, B, C, D, and E viruses*
- *Herpesviruses - except Herpesvirus simiae (Monkey B virus), BSL-4*
- *Cytomegalovirus*
- *Epstein Barr virus*
- *Herpesvirus ateles*
- *Herpesvirus saimiri*
- *Herpes simplex types 1 and 2*
- *Herpes zosterHuman herpesvirus types 6 and 7*
- *Marek's disease virus*
- *Murine cytomegalovirus*
- *Pseudorabies virus*
- *Orthomyxoviruses*
- *Influenza viruses types A, B, and C*

- *Other tick-borne orthomyxoviruses as listed in the BMBL*
- *Papovaviruses*
- *All human papilloma viruses*
- *Bovine papilloma virus*
- *Polyoma virus*
- *Shope papilloma virus*
- *Simian virus 40 (SV40)*
- *Paramyxoviruses*
- *Newcastle disease virus*
- *Measles virus*
- *Mumps virus*
- *Parainfluenza viruses types 1, 2, 3, and 4*
- *Respiratory syncytial virus*
- *Parvoviruses*
- *Human parvovirus (B19)*
- *Picornaviruses*
- *Coxsackie viruses types A and B*
- *Echoviruses - all types*
- *Polioviruses - all types, wild and attenuated*
- *Rhinoviruses - all types*
- *Poxviruses*
- *Vaccinia - all types except Monkeypox virus (BSL-3) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (restricted to the CDC, Atlanta, GA)*
- *Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)*
- *Retroviruses*
- *Avian leukosis virus*
- *Avian sarcoma virus*
- *Bovine leukemia virus*
- *Clinical samples from HIV-positive patients*
- *Feline immunodeficiency virus*
- *Feline leukemia virus*
- *Feline sarcoma virus*
- *Gibbon leukemia virus*
- *Mason-Pfizer monkey virus*
- *Mouse mammary tumor virus*
- *Murine leukemia virus*
- *Murine sarcoma virus*
- *Rat leukemia virus*

NOTE: *Murine Retroviral Vectors*
Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

- *Rhabdoviruses*
- *Rabies virus - all strains*
- *Vesicular stomatitis virus - laboratory adapted strains ONLY including VSV-Indiana, San Juan, and Glasgow*
- *Togaviruses (see Alphaviruses and Flaviviruses)*
- *Rubivirus (rubella)*

4.5.2.3 Agents - Biological Safety Level 3 (BSL-3)

Agents to be used at BSL-3 are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

BSL-3 - Bacterial Agents Including Rickettsia:

- *Bartonella*
- *Brucella including B. abortus, B. canis, B. suis*
- *Burkholderia (Pseudomonas) mallei, B. pseudomallei*
- *Coxiella burnetii*
- *Francisella tularensis*
- *Mycobacterium bovis (except BCG strain, BSL-2), M. tuberculosis*
- *Pasteurella multocida type B -"buffalo" and other virulent strains*
- *Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)*
- *Yersinia pestis*

BSL-3 - Fungal Agents:

- *Coccidioides immitis* (sporulating cultures; contaminated soil)
- *Histoplasma capsulatum*, *H. capsulatum* var.. *duboisii*

BSL-3 - Parasitic Agents:

None

BSL-3 - Viruses and Prions:

- *Alphaviruses (Togaviruses) - Group A Arboviruses*
  - *Semliki Forest virus*
  - *St. Louis encephalitis virus*
  - *Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83 is BSL-2)*
- *Arenaviruses*
  - *Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)*
  - *Flexal*
- *Bunyaviruses*
  - *Hantaviruses including Hantaan virus*
  - *Rift Valley fever virus*
- *Flaviviruses (Togaviruses) - Group B Arboviruses*
  - *Japanese encephalitis virus*
  - *Yellow fever virus*
- *Poxviruses*
  - *Monkeypox virus*
- *Prions*
  - *Transmissible spongiform encephalopathies (TME) agents, Creutzfeldt-Jacob disease and kuru agents (see BMBL for specific containment instruction)*
- *Retroviruses*

- *Human immunodeficiency virus (HIV) types 1 and 2*
- *Human T cell lymphotropic virus (HTLV) types 1 and 2*
- *Simian immunodeficiency virus (SIV)*

- *Rhabdoviruses*
  - *Vesicular stomatitis virus*

4.5.2.4 Agents - Biological Safety Level 4 (BSL-4)

Agents to be used at BSL-4 are likely to cause serious or lethal human diseases for which preventive or therapeutic interventions are not usually available.

BSL-4 - Bacterial Agents:

None

BSL-4 - Fungal Agents:

None

BSL-4 - Parasitic Agents:

None

BSL-4 - Viral Agents:

- *Arenaviruses (Togaviruses) - Group A Arboviruses*
  - *Guanarito virus*
  - *Lassa virus*
  - *Junin virus*
  - *Machupo virus*
  - *Sabia virus*

- *Bunyaviruses (Nairovirus)*
  - *Crimean-Congo hemorrhagic fever virus*

- *Filoviruses*
  - *Ebola virus*
  - *Marburg virus*
- *Filoviruses (Toga viruses) - Group B Arboviruses*
  - *Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses*

- *Herpesviruses (alpha)*
  - *Herpesvirus simiae (Herpes B or Monkey B virus)*

- *Paramyxiviruses*
  - *Equine morbillivirus*

- *Hemorrhagic fever agents*

4.6 Project Registration

Some research projects involve work with potentially hazardous biological agents, known infectious disease agents, or biological materials regulated by the state government. Many granting agencies require that the university monitor the use of biological hazards, infectious disease agents, and recombinant DNA in order for them to raise funds to investigators. Therefore, we have developed a registration system to ensure that all biological materials are handled properly and disposed of appropriately. The Laboratory Safety Subcommittee administers four registration programs for research projects.

4.6.1 Bio-Agent (BA) Registration

Use of the following materials requires that the principal investigator completes and submits the bio-agent registration document for approval by the Laboratory Safety Subcommittee.

Agents to be used at Biosafety Level 2 (BSL-2) or Biosafety Level 3 (BSL-3):

- All human, animal, or plant pathogens that require BSL-2 or BSL-3 containment and handling (see previous section: “Agents List”) must be registered. Please note that BSL-4 agents may not be used at Qatar University.
- Unknown human and animal pathogens must be registered. These are considered BSL-2 until identified.
- Cell lines or cultures that:
  - have been immortalized with a virus (such as EBV or a retrovirus),
  - are known to be tumorigenic in primates (including humans), or
  - are primary human tumor cells. These are considered BSL-2 (or higher in many cases).
- Human blood or other tissues that are known to be HIV positive (or positive for any human disease-causing virus or other agent), when used in research, must be registered.

4.6.2 Recombinant DNA (R-DNA) Registration

All R-DNA projects that involve a living recombinant organism require registration. A subset of R-DNA projects requires review and approval from the Laboratory Safety Subcommittee. The committee oversees all research projects and issues involving R-DNA. Use of the following requires that the principal investigator completes and submits an R-DNA registration document.

- All R-DNA projects, including the growth of recombinant bacteria for probe isolation (plasmid or phage preparations) require registration. Projects must be registered regardless of where the material came from or who originally constructed it.
- The development of transgenic animals and plants requires registration.

R-DNA projects are performed at BSL-1, BSL-2, and BSL-3. The Laboratory Safety Subcommittee will make the final determination.

4.6.3 Acute Toxins (AT) Registration

The use and storage of chemicals with a mammalian LD_{50} of $\leq$ 100 µg/kg must be registered with the Laboratory Safety Subcommittee. For a partial list, see the Toxins Table in Appendix A. If a toxin is not on the list, it still may require registration, depending upon the LD_{50}.

4.6.4 Select Agents

Lists of agents, toxins, and pathogens in Appendix B are classified as Select Agents. Any possession, use, transfer or shipment of these materials is strictly controlled by regulation.

Researchers considering work with any of these materials must first contact the HSE for the approvals, permits, clearances and other necessary paperwork.

4.7 Safe Handling of Laboratory Animals

The personnel involved in the care and use of research animals work in an environment that presents a number of unique hazards from several sources:

- Hazards related to the equipment, materials and practices used in performing routine animal husbandry.
- Hazards related directly or indirectly to animal contact.
- Hazards related to the techniques or materials (e.g., biohazardous substances) that may be used during the course of animal experimentation.

Regardless of the source of hazard, several basic measures should be taken to reduce the risk of personal exposure. These include understanding the hazards likely to be encountered during animal care and use, using properly designed and maintained facilities and equipment to minimize exposures, wearing appropriate personal protective equipment (PPE) and demonstrating the technical proficiency necessary to accomplish experimental manipulations or animal care procedures in safe and humane fashion.

4.7.2 Common Injuries Associated with Animal Husbandry and Care

Possible injuries and accidents associated with working in the laboratory of animal facilities are provided below:

- Burn injuries due to working around cage washers, autoclaves or other sources of hot water or live steam.
- Crush injuries or lacerations from moving caging equipment, operating sanitation equipment or working with intractable large animals.
- Musculoskeletal injuries (strains, sprains or fractures) due to the use of improper technique in lifting or moving heavy equipment or improper restraint and handling of large animals.
- Slip and fall injuries from walking on wet flooring.
- Hearing impairment resulting from working around loud machinery or animals.
- Visual impairment from direct trauma (equipment), splash exposure (detergents, disinfectants, or particulate matter) or exposure to ultraviolet light resulting in corneal damage.
- Skin irritation or contact dermatitis from exposure to chemicals used in cleaning, latex or talc allergy, or in experimental procedures in the animal facility.
- Respiratory exposure to irritating vapors, aerosols or particulates from working with disinfectants and bedding materials.

- Needle stick exposures from attempts to recap hypodermic needles, improper injection technique or delay or improper disposal of used needles.

Personal awareness of hazardous conditions or factors in the environment is critical to avoiding these types of injuries, and individuals shall develop the habit of assessing the environments in which they work. Research staff are encouraged to consult with their supervisors about the hazards which have been identified in particular work areas and the work practices which have proven to be most effective in preventing incidents. Facility supervisors shall also provide a useful orientation about the available resources and use of the facility. MSDS information for all agents used in the animal care operation shall be available for review in each animal facility. Facility supervisor should provide PPE in all of its facilities consisting of masks, gloves, and in many sites, coveralls, booties, bonnets and hearing protection. For most activities conducted in animal rooms or procedure areas involving laboratory animals, research personnel are encouraged to wear a clean lab coat (or equivalent external cover), latex gloves, face (particle) mask and safety glasses. Shoes rather than sandals also should be worn for adequate foot protection.

Refer to *QU HSEMS Section 12.0 Hazard, Near Miss, Incident Reporting and Investigation* for procedures on hazard, near miss and incident reporting and investigation.

4.7.3 Animal Related Hazards

Bites, scratches and other injuries represent a significant portion of the animal-associated hazards encountered by individuals with laboratory animal contact and are readily preventable through proper animal handling technique and the use of proper PPE. Unusual noises, defective equipment, slippery surfaces and conditions conducive to entrapment or distraction of the animal handler shall be eliminated prior to animal handling. Inappropriate animal handling may induce discomfort, pain and distress in the animal provoking a fractious response, introducing undesirable experimental variables and providing the animal the opportunity to inflict injury upon the handler.

The personnel should always wear a long sleeve lab coat or use other sleeve protection when handling rabbits or larger animals to avoid scratch injury, and in some cases special gloves (e.g., stainless steel mesh or heavy leather gauntlets) should be worn to prevent bites. Special attention shall be given to the training of the personnel involved in the handling and restraint of large animals, especially nonhuman primates. In addition to posing a bite and scratch hazard, nonhuman primates can be challenging and difficult to handle safely because of their remarkable strength, dexterity, intelligence and tenacity. Unsuspecting personnel have been injured when nonhuman primates have grabbed and pulled neckties, loose-fitting lab coats or long hair. When it is compatible with the experimental conditions of animal use and/or the clinical condition of the animal, consideration should be given to chemical immobilization of many nonhuman primate species to enable safe animal handling and to reduce the risk of injury for personnel. Specific PPE requirements are in effect for personnel working with monkeys and are posted in the housing areas for these species.

Animal bites continue to be a relatively common occurrence among research personnel and should be regarded seriously even when they have been inflicted by small rodents causing minor tissue damage. The persons who have been bitten should seek prompt medical help with the wound and their tetanus immunization status by their supervisor, and initiate the veterinary evaluation of the animal involved if warranted. Animal bites also prompt a veterinary review of the animal handling circumstances to ensure that proper animal handling techniques were used. A specific, detailed protocol is in effect for bites, scratches or mucous membrane exposures involving some monkey species due the Herpes B virus, an agent which can cause

fatal infection. The list of other specific viral agents that can be involved as wound contaminants includes rabies virus (all mammals), hantavirus (rodents), lymphocytic choriomenigitis virus (rodents) and or virus (sheep and goats). Numerous bacterial agents and at least one fungal agent have also recorded as wound contaminants resulting in serious localized or systemic infections.

4.7.4 Animal Associated Allergy

An estimated 10 to 30 percent of individuals who work with laboratory animals may eventually develop allergy to laboratory animals manifested by reddened, itchy eyes, nasal symptoms and skin rashes. Individuals with pre-existing allergy to other agents have a predisposition to develop an additional sensitivity to animal allergens. Asthma, which is characterized by cough, wheezing, chest tightness and shortness of breath, develops as a further complication in approximately 10 percent of individuals with animal-associated allergy. Also, anaphylaxis, a generalized allergic reaction presenting as diffuse itching, hives and facial and oral swelling can develop and produce life-threatening consequences from laryngeal edema, airway obstruction and shock in certain individuals with massive allergen exposure, often through saliva.

Although rodents, rabbits and cats are most often incriminated in cases of laboratory animal-associated allergy, other mammals and birds also can be involved. Work practices which minimize contact with animal proteins reduce risk for development of allergy. For example, various levels of PPE are available for personnel working with laboratory animals to reduce exposure to allergen.

4.7.5 Zoonoses

Zoonoses are diseases that are transmissible from animals to humans. Laboratory animal species potentially harbor numerous zoonotic agents, including viruses, bacteria, fungi, protozoa and internal and external parasites, but the reported cases of zoonotic transmission to individuals with laboratory animal contact have been infrequent and sporadic. However, because many of the zoonotic disease episodes likely have remained unreported and because those which have been reported involved serious disease and even fatalities, individuals with laboratory animal contact should be aware of these diseases and take appropriate precautionary measures. The likelihood of encountering a zoonoses varies with the species of laboratory animal used, its source and history of veterinary care. Individuals, who become ill and/or feel they have contracted a disease from a laboratory animal, should consult with their supervisor. The major zoonoses are summarized below.

4.7.5.1 Rodents and Rabbits.

The modern conditions of production and care for most laboratory rodents and rabbits have led to the eradication of zoonoses in most of these species. However, these animals can rarely become contaminated through environmental sources, contact with wild rodents or other infected animals or through tumors, cell lines or other biologics used experimentally. In most circumstances, only wild-caught, laboratory maintained rodents would be regarded as a high risk for the transmission of zoonotic diseases. Personnel should be familiar with several zoonoses associated with rodents and rabbits. Two serious systemic viral zoonoses have been associated with the use of laboratory rodents. Lymphocytic choriomeningitis virus causes a flu-like disease with neurological complications, and hantavirus infection produces a disease marked by renal failure and respiratory complications. Other than the bite-associated bacterial

infections from rodents (i.e., rat-bite fever) there are few bacterial zoonoses in these species. The rabbit is a potential source for human bacterial pathogens especially those which cause human diarrheal disease such as salmonellosis. Rodents and rabbits also can be source for human ringworm infection usually recognized as a reddened, annular lesion of the skin of the affected individual. A similar focal dermatitis can be caused by infestation with the rabbit fur mite and, rarely, other mite species of rodents. The dwarf tapeworm infestation of rodents also is capable of infecting man. The complete absence or extremely low incidence of these agents in our laboratory animal populations has obviated our need to adopt intensive health surveillance measures for individuals who work with these species. However, all personnel should use appropriate PPE when working with these species and are encouraged to report unusual illnesses or conditions possibly related to animal contact

4.7.5.2 Dogs and Cats.

Source control and sound programs of veterinary care at the vendors' facilities and at Qatar University ensure that the majority of zoonotic infections are eradicated in these animal species prior to their experimental utilization. In some cases, subclinical infections may go undetected and untreated posing a risk for the personnel who work with these animals. Such infections would include intestinal bacterial infections (salmonellosis, yersiniosis, and campylobacterosis), systemic bacterial infections (brucellosis, cat-scratch fever, leptospirosis and Q-fever) and intestinal parasitic infections (giardiasis and toxoplasmosis). Also, the dog and cat can also harbor the dermatophytes causing human ringworm and other external parasites capable of infesting humans. Proper use of PPE is essential to minimize exposure to these zoonotic hazards. The personnel involved in the use of laboratory cats that have not been specifically bred for research purposes should give consideration to participation in the rabies vaccination program.

4.7.5.3 Non-human Primates

The list of zoonotic diseases in nonhuman primates is long and includes numerous viral (e.g., B virus, hepatitis A and B, measles and SIV), bacterial (e.g., tuberculosis, salmonellosis and shigellosis) and protozoal (e.g., giardiasis and amebiasis) diseases, and there are many documented cases of zoonotic transmission. Consequently, non-human primates must undergo an extensive quarantine period to preclude the presence of many of these zoonoses before experimental work with these animals can be started. Even after release from quarantine, rigorous disease surveillance continues for some agents such as tuberculosis. The personnel also must participate in periodic tuberculin testing if they have any nonhuman primate contact. The persons who work with macaques must undergo special training concerning the prevention and management of potential exposure to B virus, an agent which has caused many fatalities among laboratory personnel. Strict adherence in the use of PPE is expected of all personnel with nonhuman primate contact.

4.7.5.4 Birds and Livestock.

Q fever has proven to be the most important zoonosis associated with livestock in the laboratory animal facility. Although all ruminants and many other animals are potential carriers, infection of laboratory personnel has most often been associated with pregnant sheep that copiously shed the organisms. The disease causes a flu-like illness which can progress to a serious systemic infection with heart involvement. Or, a pox viral disease of sheep and goats, can also infect humans through contaminated wounds producing firm, nodular lesions. Livestock and birds can harbor bacterial zoonoses causing diarrhea in humans. Birds also can shed the agent of psittacosis (*Chlamyia psittaci*), a serious respiratory and systemic disease of

humans. Proper use of PPE is essential to minimize exposure to these potential zoonotic hazards.

4.7.6 Use of Hazardous Agents in Animal Experimentation

Many studies involve the use of hazardous agents in laboratory animals. Often the use of a hazardous substance is incidental to the research, whereas in other circumstances it is an integral component of the study intended to produce a particular experimental effect. Examples of the former include inhalant anesthetic agents (e.g., ether, methoxyflurane, halothane or isoflurane), injectable anesthetic agents (urethane), and adjuvants (particularly Freund's Complete Adjuvant). Examples of the latter include carcinogens, teratogens, mutagens, toxicants, microbial pathogens, radioisotopes, and organisms modified through recombinant DNA techniques. In either case, the use of hazardous agents is noted during the review of the animal protocol and is referred to the HSE or the appropriate Qatar University campus committee to verify or establish the conditions under which the hazardous materials can be used safely. In some cases it may be necessary for the institution to engage outside expert consultants and work with the investigator to develop a more elaborate safety protocol and ensure appropriate personnel training before the animal studies can be initiated.

4.7.7 Special Requirements

- Animal facilities shall have a ventilation system of 10 to 20 air changes per hour.
- Animal holding facilities shall have a 30 to 70 % relative humidity and a temperature of 18 to 26 ^{o}C.
- Laboratory workers who work with items possibly contaminated with disease communicable to humans, including tissues, fluids, fecal materials, and equipment which have come into contact with any of these, shall require immunizations.
- Tetanus shots are required for all works with animals, while those who work with wild animals shall require rabies vaccinations.
- A pre-employment medical examination is mandatory and shall include medical and work histories.
- Any worker who comes into contact with human or primate tissue, blood , and fluids must receive training and be offered shots for Hepatitis-B.
- A decontamination process shall be conducted for individual who work with animals which have diseases that are communicable to humans or other animals. Animals shall be kept in isolation areas.

4.8 Decontamination and Disposal

Decontamination and disposal in laboratories which utilize biohazardous materials are closely interrelated acts in which sterilization and disinfection constitute the first phase of disposal. The goals of decontamination are the protection of personnel and the environment from exposure to biological agents. Blood and body fluids in individual containers that contain greater than 20 ml, microbiological waste, and pathological waste, must be treated before any disposal in order to render the waste nonhazardous.

Sterilization is the process of treating an object or material so as to remove or kill all living organisms. Disinfection is the process of the removal or inactivation of all pathogenic microorganisms. It may not remove all microorganisms and therefore, disinfection is not necessarily sterilization. Whether or not sterility is achieved depends on several factors: the number and nature of the contaminating microorganisms, the presence of bacterial spores, the concentration of the germicide, the length of time of contact between the germicide and the material being disinfected, the type and condition of the material, the amount of soil present, and the temperature.

Sterilizing and disinfecting agents may attack microorganisms in several ways. Some disinfectants coagulate the cell protein so that the cell cannot function. They may injure or destroy the cell membrane altering the normal selective permeability allowing toxins to enter metabolically important components to escape, or prevent the entrance of food. Disinfectants may also react with a specific enzyme to prevent it from reacting with its natural substrate.

Microorganisms exhibit a range of resistance to the inactivating agents. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

4.8.1 Steam Sterilization

Autoclaving, or steam sterilization, is the most dependable procedure for the destruction of all forms of microbial lives. Saturated steam is employed under pressure to achieve a chamber temperature of at least 121 C (250 F) for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121 C. The critical factors in insuring the reliability of this method other than proper temperature and time is the prevention of entrapment of air that is replaced by the steam and adequate exposure time as related to the "soil" load on the contaminated items.
Gravity displacement autoclaves take advantage of the difference in density of the air related to steam. Steam entering the upper-rear of the chamber displaces the air downward and out of the drain line that is located in the lower front of the chamber. A valve in the drain line remains open until a specific pre-set temperature is reached. After this temperature is reached, the valve closes and the steam continues to enter until the pre-set pressure and/or temperature is obtained. The concern with this type of autoclave is that the air in closed or upright containers, or air trapped in closed systems (items with valves, etc.), or densely loaded chamber packages is not readily replaced. If air is not removed from an area, the temperature in that area may remain sub-lethal throughout the decontamination period. Because of this, autoclaves of this type should not be overloaded, densely packed materials should be avoided, systems should be kept open and containers should be turned on their side.

High vacuum autoclaves pump vacuum into the chamber prior to the entrance of the steam. If the vacuum is high (greater than 27 inches Hg.), the air removal concern is alleviated. However, it should be noted that a small load should not be placed in a high-vacuum autoclave because the air remaining in the chamber can be entrained in this load.
Heavily soiled items, especially if the soil is of proteinaceous nature, should be autoclaved for longer periods of time. The reason for this is that soil may protect the microorganism from the lethal effects of the wet heat. Because of this, an exposure time of 60 minutes or greater for soiled items is not unreasonable.

Other practices to improve the effectiveness of autoclave use include removing the plug screen or strainer daily to make sure it is free from dirt, dust, or sediment that may collect in it and cleaning the interior surfaces of residues collected from the steam or materials being sterilized. The use of spore strips (*Bacillus stearothermophilus* spores) placed at locations throughout the autoclave, can serve as a biological indicator of sterility.

Microbiological waste must be steam sterilized in an autoclave, before disposal. Steam under pressure should be provided to maintain a minimum temperature of 250 F for 45 minutes at 15 psi of gauge pressure. The autoclave should be provided with a chart recorder which accurately records time and temperature for each cycle. Monitoring under conditions of full loading for effectiveness should be performed at least once per week through the use of biological indicators. A log of each test should be maintained, which includes the type of indicator used, date, time, and result of the test.

Criteria for autoclaving typical materials

Material	Temperature	Time
Laundry	121 C (250 F)	30 minutes
Trash	121 C (250 F)	1 hour
Glassware	121 C (250 F)	1 hour
Liquids	121 C (250 F, each gallon	1 hour
Animals	121 C (250 F)	8 hours

4.8.2 Dry Heat Sterilization

Dry heat is useful for the sterilization of anhydrous oils, greases, powders, etc., that can be easily permeated by steam. Dry heat sterilization is less efficient than wet heat sterilization and requires longer times and/or higher temperatures. The specific times and temperatures must be determined for each type of material being sterilized. Generous safety factors are usually added to allow for the variables that can influence the efficiency of this method of sterilization. The moisture of the sterilization environment as well as the moisture history of organisms prior to heat exposure begins to affect the efficiency of dry heat sterilization.
Sterilization can usually be accomplished at 160 - 170 C (320 - 338 F) for periods of 2 -4 hours. High temperatures and shorter times may be used for heat resistant materials. The heat transfer properties and the spatial relationships or arrangement of the articles in the load are critical in insuring effective sterilization. If items are heat sensitive then a temperature of 120 C (248 F) must be used, the exposure time necessary for decontamination is usually greater than 24 hours.

The hazards of handling hot solids and liquids are generally well known. The laboratory personnel should be cautioned that steam under pressure can be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable sizes should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too promptly after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers. Items being handled following dry heat sterilization can cause severe burns if protective gloves are not used.

4.8.3 Gas Sterilization

A variety of gases and vapors possess germicidal properties. The most useful of these are formaldehyde and ethylene oxide. When these are employed in closed systems and under controlled conditions of temperature and humidity, sterilization can be achieved. Vapor and gas disinfectants are primarily useful in sterilizing biological safety cabinets and associated effluent air-handling systems and air filters; bulky or stationary equipment that resist penetration by liquid surface disinfectants; instruments and optics that might be damaged by other methods; and rooms and buildings that are associated with air-handling systems.

Ethylene oxide (ETO) gas is lethal for microorganisms including spores, viruses, molds, pathogenic fungi and highly resistant thermophilic bacteria. Some of the principal variables that determine the rate of destruction includes: temperature, concentration, humidity, and exposure time.

Temperature affects the penetration of ETO into microbial cell walls and the wrapping and/or packaging materials. The activity of ethylene oxide will increase approximately 2.7 times for each 10 C (18 F) rise in temperature (between 5-37 C, concentration 884 mg/l). Normally, ethylene oxide sterilization is conducted at temperatures between 49-60 C (120-140 F).
Sterilization times may be reduced when the concentration is increased. For practical sterilization, gas concentrations of 500-1000 mg/l at approximately 49-60 C are recommended. The effect of moisture appears to be related to the moisture content of the exposed bacterial cell. A relative humidity of 30-60% is frequently employed in ethylene oxide chambers during exposure conditions.

All materials that have been sterilized with ethylene oxide must be aerated at least 24 hours before contact with human skin. Mixtures of 3-10% ethylene oxide in air are explosive. Commercially available mixtures of ethylene oxide in Freon or CO2 are not explosive and can be safely utilized.

Formaldehyde is the chemical of choice for space disinfection of safety cabinets, incubators, refrigerators, laboratory rooms, buildings, or other enclosed spaces. Formaldehyde can be generated by vaporizing aqueous solutions of formalin or heating paraformaldehyde. Generally, the generation of formaldehyde gas from powdered or flake paraformaldehyde by heating to a temperature above 150 F is the preferred method. A concentration of 0.3 g per cubic foot of space to be treated is employed, at a temperature above 20 C and relative humidity of 70% or higher, for an exposure of 8 hours or overnight. Aeration to remove excess formaldehyde should follow, with length of time related to area decontaminated.

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas in the event of inadvertent leakage. In preparing to use dilutions and during the application, the personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide and formaldehyde; toxic and hypersensitivity effects are well established for formaldehyde.

4.8.4 Liquid Disinfection

A chemical disinfection is necessary in the laboratory operations because steam under pressure is not feasible for use in large spaces, surfaces, stationary equipment, high temperatures and moistures may damage delicate instruments. There are many disinfectants

available under a wide variety of trade names. In general, these disinfectants can be classified as acids or alkalines, halogens, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, and alcohols. Unfortunately, the more active the disinfectant, the more likely it will possess undesirable characteristics.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and the presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay system employed. Ineffectiveness of a decontaminant may also be due to the failure of the decontaminant to contact the microorganisms rather than the failure of the decontaminant to act. If one places an item in a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Similarly, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contracted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful; a decontaminant should have incorporated surface-active agents and other detergent properties.

4.8.5 Selecting Chemical Decontaminants

No single chemical decontaminant or method will be effective or practical for all the situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

- What is the target microorganism(s)?
- What are the decontaminants, and in what form they are known to, or can be expected to inactivate the target microorganism(s)?
- What degree of inactivation is required?
- In what menstruum is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?
- What is the highest concentration of cells anticipated to be encountered?
- Can the decontaminant be either as an aqueous solution, a vapor, or a gas reasonably expected to contact the microorganisms, and can be effective duration of contact to be maintained?
- What restrictions apply with respect to compatibility of materials? Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations of infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials involves certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact

times in excess of those shown to be effective in the test tube. Similarly, a major portion of the decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which basis to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acids. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Owing to the innate differences in the chemistry of RNA and DNA, the effectiveness of a decontaminant for one cannot be extrapolated for the other. For example, RNA molecules are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2' position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

4.8.6 Properties of Some Common Decontaminants

4.8.6.1 Alcohol

Ethyl or isopropyl alcohol in a concentration of 70 - 85% by volume is often used. Alcohols rapidly lose their cidal activity when diluted below 50% concentration. The cidal action of ethyl alcohol is very rapid and includes all microorganisms except spores. Isopropanol is not very effective against either spores or non-lipid viruses. They are also not effective when organic soil is present. Alcohols become ineffective as soon as they start to evaporate. This property has the advantage of having no residue on treated surfaces, but it often makes repeated applications desirable in order to get adequate exposure.

4.8.6.2 Formaldehyde

In concentration of 8% formalin, this is an effective liquid disinfectant against vegetative bacteria, spores, and viruses. Considerable activity is lost at refrigeration temperatures. Care must be taken when using solutions in the laboratory because of its irritating odor.

4.8.6.3 Phenol

Phenol itself is not often used as a decontaminant because it is extremely toxic. The odor is somewhat unpleasant and a sticky, gummy residue remains on the treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsia, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

4.8.6.4 Quaternary Ammonium Compounds or Quats

After 30 years of testing and use, there is still a considerable controversy over the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The

Quats have the advantages of being nontoxic, odorless, no staining, noncorrosive to metals, stable, and inexpensive.

4.8.6.5 Chlorine

This halogen is a universal decontaminant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from the household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

4.8.6.6 Iodine

The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The range of dilution of Wescodyne recommended by the manufacturer is 1 ounce in 5 gallons of water giving 25 ppm, of available iodine to 3 oz. in 5 gallons giving 75 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 with water or 10% ethyl alcohol (a reasonably good decontaminant itself) which will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

Particular care should be taken when handling concentrated stock solutions of disinfectants. The personnel assigned the task of making up use-concentrations from stock solutions must be properly informed of the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Protective face shields and goggles should be used for eye protection and long-sleeved garments and chemically resistant gloves, aprons and boots should be worn to protect from corrosive and depigmentation effects to the skin. One of the initial sources for hazard information or any given product will be the label on its container.

4.9 Biological Waste Disposal

Disposal of biohazardous waste shall be disposed according to *QU SOP-04 Hazardous Waste Disposal*.

4.10 Biological Laboratory Closeout Procedures

Whenever a Laboratory Supervisor (or a person under their charge performing work with biological materials in their laboratory) leaves the university or is transferred to a different location, proper disposition of hazardous materials, glassware, benches, laboratory equipment, fume hoods, etc. is required. Laboratory closeout is also required for renovations or constructions taking place in the laboratory. This undertaking shall be properly coordinated with Campus Facilities Department prior to the start of laboratory close out.

If proper management of bi-logical materials at close-out requires removal services from an outside contractor, the responsible department will be charged for this service.

4.10.1 Biological and Hazardous Chemical Disposal in Laboratories and Containment Areas

- Ensure that containers of chemicals and biological materials are labeled.
- All containers must be securely closed. Beakers, flasks, evaporating dishes, etc., should be emptied. Hazardous chemical wastes must not be sewered or trashed; they must be collected for disposal.
- Clean chemicals from glassware and assure proper waste disposal guidelines are followed.
- Never pour chemical residues down the sink unless it is safe.
- Check refrigerators, freezers, fume hoods, storage cabinets and bench tops for chemical containers and thoroughly clean these locations.
- If another room or facility (such as a freezer or refrigerator, stock rooms, etc.) is shared with other researchers, remove, transfer or dispose of items used by the departing researcher.
- Contact the Campus Facilities Department for pick-up of biological and hazardous waste at least one week prior to vacating the laboratory.
- For gas cylinders, remove regulators, replace cap and return to supplier. If cylinders are nonreturnable, arrange for pick up by authorized hazardous waste collector. Gas cylinders used in the containment area must be decontaminated prior to return.
- As an alternative to disposal, if the chemical is still usable, transfer the responsibility of the chemical to another Laboratory in-charge who is willing to take charge of the chemical.
- Follow all guidelines in the University Hazardous Waste Disposal Guide for disposal of unwanted chemicals. The authorized hazardous waste collector will pick up all hazardous waste provided:
  - All chemical containers are properly labeled as "hazardous waste" and are accompanied with a completely filled out hazardous waste tag.
  - All containers are securely closed.
- Notify the concerned laboratory department and Campus Facilities Department when laboratories or containment area/rooms have been cleared.

4.11 Transportation of Biological Materials on Campus

All biological materials that are of potential risk to humans and/or animals must be stored and transported in primary and secondary containers. Primary containers can be culture tubes, flasks, vials etc. All containers must meet the following requirements:

- Rigid
- Puncture resistant
- Leak proof
- Impervious to moisture
- Of sufficient strength to prevent tearing or bursting under normal conditions of use and handling
- Sealed to prevent leakage during transport
- Labeled with a biohazard or infectious substance label

All containers should be accompanied by a list of content, name of the person responsible for this material, a contact person and phone number.
If materials are to be transported in liquid nitrogen or with other protection from ambient or higher temperatures, all containers and packaging should be capable withstanding very low temperatures, and both primary and secondary packaging must be able to withstand a pressure differential of at least 95 kPa and temperatures in the range of - 40°C to + 50°C.
If the material is perishable, warnings should appear on accompanying documents, e.g., "Keep cool, between + 2°C and + 4°C."

4.12 Equipment

If the laboratory equipment is to be left for the next occupant, clean or decontaminate it before departing the laboratory.
If the laboratory equipment is to be discarded, be aware that capacitors, transformers, mercury switches, mercury thermometers, radioactive sources and chemicals must be removed before disposal.
Use the following guide for Biological Safety Cabinets (BSC):
Remove all the contents.

- Disconnect tissue culture media vacuum flask.
- Decontaminate all accessible surfaces with an appropriate disinfectant.
- Decontaminate the BSC by a certified contractor, if a BSC is being relocated to a location outside of the building.
- Re-certify the BSC using a certified contractor when a BSC is relocated.
- If the BSC is not being moved and repair work will not open the contaminated inner space, a surface decontamination with an appropriate disinfectant is sufficient.

5 Document Control

This Technical Guideline is a controlled document. The controlled version of this guideline is located on the QU Electronic Documentation Management System.
Any printed copies of this controlled document are reference copies only. It is the responsibility of all of those with printed copies to ensure their copy is kept up to date.
Refer to *QU HSEMS Section 16.0 – Document Control and Record Retention*.

6 Appendices

Appendix A: Toxins Table

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Toxin	Toxicity (LD50 (µg/kg))
Abrin	0.7
Aerolysin	7.0
Botulinin toxin A	0.0012
Botulinin toxin B	0.0012
Botulinin toxin C1	0.0011
Botulinin toxin C2	0.0012
Botulinin toxin D	0.0004
Botulinin toxin E	0.0011
Botulinin toxin F	0.0025
β-bungarotoxin	14.0
Caeruleotoxin	53
Cereolysin	40-80
Cholera toxin	250
***Clostridium difficile* enterotoxin A**	0.5
***Clostridium difficile* cytotoxin B**	220
***Clostridium perfringens* lecithinase**	3
***Clostridium perfringens* kappa toxin**	1500
***Clostridium perfringens* perfringolysin O**	13-16
***Clostridium perfringens* enterotoxin**	81
***Clostridium perfringens* beta toxin**	400
***Clostridium perfringens* delta toxin**	5
***Clostridium perfringens* epsilon toxin**	0.1
Conotoxin	12-30
Crotoxin	82
Diphtheria toxin	0.1
Listeriolysin	3-12
Leucocidin	50
Modeccin	1-10
Nematocyst toxins	33-70
Notexin	25
Pertussis toxin	15
Pneumolysin	1.5
Pseudomonas aeruginosa toxin A	3
Ricin	2.7
Saxitoxin	8
Shiga toxin	0.250
Shigella dysenteriae neurotoxin	1.3
Streptolysin O	8
Staphylococcus enterotoxin B	25
Staphylococcus enterotoxin F	2-10
Streptolysin S	25
Taipoxin	2
Tetanus toxin	0.001
Tetrodotoxin	8
Viscumin	2.4-80
Volkensin	1.4
Yersinia pestis murine toxin	10