Institutional Biological Safety Manual (2010)

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Overview

Over the years, there have been many documented cases of lab personnel acquiring diseases due to their work with infectious agents. Only approximately 20% of these cases have been attributed to a specific incident, with the rest assumed to be related to work practices in the lab, primarily the creation of aerosols. Whenever work with infectious agents is performed, all appropriate steps must be taken to protect personnel and the environment.

Program Administration

Institutional Biosafety Committee (IBC)

Research with biohazardous materials (rDNA, infectious agents, biological toxins, human fluids) is overseen by the Institutional Biosafety Committee (IBC). The IBC reviews research protocols to determine if the proper safety procedures are in place and to recommend biocontainment levels for submitted research. Researchers should not initiate biohazardous research without contacting the IBC. The IBC is administered by the Office of Research Support with the support of Environmental Health and Safety

Institutional Biosafety Officer (BSO)

The Institutional Biosafety Officer is responsible for ensuring that laboratories are working safely with biohazardous materials. This includes the periodic inspection of laboratories, developing emergency plans for handling spills and accidents and investigating laboratory accidents involving biohazardous materials. The BSO also provides technical consultation for researchers on conducting risk assessments, safe practices, and security.

Environmental Health and Safety (EHS)

Environmental Health and Safety provides services necessary for biological research. These services include biomedical waste disposal, autoclave quality assurance, biosafety cabinet certification, biosafety inspections, training and emergency response.

Principal Investigators (PI)

Principal Investigators are responsible to ensuring that their laboratories meet the institutional and contractual requirements for the safe use of biohazardous materials. This includes:

- Conducting a risk assessment to determine appropriate safety measures
- Complying with permit and shipping requirements for biohazardous materials
- Developing Standard Operating Procedures
- Developing a Laboratory Safety Plan that includes emergency procedures
- Ensuring laboratory personnel understand the risks associated with their research
- Ensuring personnel are properly trained
- Ensuring personnel are aware of occupational health requirements associated with their research

- Ensuring waste is properly disposed
- Following this manual, the *NIH Guidelines* and *Biosafety in Microbiological and Biomedical Laboratories* (BMBL)
- Providing appropriate Personal Protective Equipment to personnel and ensuring that it is used.
- Reporting any accidents or research related illness to EHS

Laboratory Personnel

- Know the hazards associated with the biological materials and procedures used in the laboratory
- Follow approved lab procedures and safety guidelines
- Know the emergency procedures
- Complete all required training before conducting any lab activity
- Report any accidents or research related illness to the PI or EHS
- Utilize all required Personal Protective Equipment (PPE)

Training Requirements

Principal Investigators and their personnel who work with biohazardous materials should at a minimum take :

OH 102: (Site Specific Hazard Communication)
OH 201: Laboratory Safety
OH 207: Biological Safety

In addition, if research involves the collection or handling of human or non-human primate tissues or fluids:

OH 218: Bloodborne Pathogens

If the research involves recombinant DNA:

RC 301: NIH Guidelines

If the research is conducted in the BSL-3 lab:

OH 206: BSL-3 Lab Training (Required annually)

Principals of Biosafety

Biohazardous Materials

Biohazardous materials are defined as biological or bio-synthetic agents, biologically derived materials and toxins that present a risk or potential risk to the health of humans, animals, or plants either directly through exposure or infection or indirectly through damage to the environment. Categories of biological materials include the following:

- Human, animal and plant pathogens (bacteria, parasites, fungi, viruses) BSL-1 and above
- Biological toxins and infectious proteins (prions)
- All human and non human primate (NHP) blood, blood products, tissues and body fluids
- Human and NHP cells and cell lines (including established cell lines)
- Animals and animal tissues that are intentionally infected with pathogens or suspected to contain pathogens.

Risk Assessment

Risk assessment is the process of analysis of the agent hazards and the hazards associated with the laboratory procedures. Principal Investigators and their staff must be aware of the characteristics of the agents they are working with as well as the procedures they are conducting that increase the potential for exposure. The Principal Investigator is primarily responsible for the risk assessment for their research. However, the BSO, IBC, IACUC and lab animal veterinarians share in the responsibility.

PIs and their staff should be aware of the agents they are working with. The agents may be exotic, modified to be more or less pathogenic, resistant to antibiotics, or carry novel genes that can be oncogenic or toxic. Information on wild-type agents may be found in the BMBL, *Control of Communicable Diseases Manual* or the Canadian Material Safety Data Sheets for Infectious Substances:

http://www.phac-aspc.gc.ca/msds-ftss/

PIs and their staff should also assess the procedures they are conducting with the agents. The procedures may increase the potential for exposure by creating aerosols or using sharps. The risk assessment should also look at the staff and their experience working with the agent or procedure. The risk assessment should identify what safeguards should be used to protect workers such as assigning a biosafety level, biosafety cabinets, safety centrifuges or personal protective equipment.

The Principal Investigator should document the risk assessment and review it in consultation with the Biosafety Officer or other subject matter expert.

Risk Group

The *NIH Guidelines* (2002) uses a risk classification system of infectious organisms by Risk Group. These agents are listed in the *NIH Guidelines*. While the list of agents is large, it is not comprehensive. Risk Groups can be used in the risk assessment process.

- Risk Group 1 (RG1) Agents that are not associated with disease in healthy adult humans. Includes a list of animal viral etiologic agents in common use.

- Risk Group 2 (RG2) Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
- Risk Group 3 (RG3) Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
- Risk Group 4 (RG4) Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

Routes of Exposure

There are four main routes of exposure in which a person can come in contact with infectious agents. These routes are contact with the skin or mucus membranes, ingestion, inhalation, and inoculation. Each of these routes of exposure is discussed below.

Direct Contact with Skin or Mucus Membranes

Spilled material can come into direct contact with the skin as can droplets produced by pipetting, removal of screw caps, and vortex mixing of unsealed tubes.

- The control of a contact exposure is accomplished through the wearing of appropriate protective clothing such as a face shield, gloves, safety glasses, a mask, and laboratory coats. Other ways to control contact exposure include using absorbent paper on the work bench, performing all procedures carefully, and frequently wiping work surfaces with a disinfectant.
- Keep all non-essential items away from the area where work is being performed to protect personal items from contamination. All contaminated wastes must be handled and stored properly to prevent contact exposure of lab personnel as well as housekeeping staff and waste handlers.

Ingestion

Ingestion may occur either directly or indirectly. Exposure may occur from mouth pipetting or splashing from a container into the mouth or by contaminating the hands and then touching the mouth or items such as a coffee cup, food, or lip balm, that go into the mouth.

The control of an ingestion exposure is accomplished through the use of mechanical pipetting devices *whenever* pipetting and by practicing good personal hygiene, such as washing hands frequently throughout the day and *not* eating or drinking in the work area. Food items also cannot be stored in refrigerators that contain hazardous materials or in the lab where work with infectious agents is being performed.

Inhalation

It is generally known that aerosols are the primary means by which infectious diseases are spread and contracted. An aerosol can be either a liquid or a dry particle. An aerosol with a diameter

of five microns or less can easily be inhaled and carried to the alveoli of the lungs. These aerosols can remain airborne for a long period of time and can spread wide distances, especially after entering the ventilation system. Particles with a diameter larger than five microns tend to settle rapidly and can contaminate the skin or other surfaces.

There are many commonly performed procedures in the lab that can create aerosols. Examples include centrifuging, heating inoculating loops, using a blender, blowing out the last drop in a pipette, and changing animal bedding.

The control of inhalation exposure is accomplished by a combination of using the appropriate safety equipment such as biological safety cabinets and by performing procedures carefully to minimize the creation of aerosols. Refer to the following Section 3 of this chapter for additional information regarding Laboratory Equipment.

Inoculation

Inoculation in a lab usually occurs with a needle and syringe. Exercise extreme caution whenever using a needle. Restrict needle use; whenever an alternative to a needle is possible, it should be used. Inoculation can also occur through animal bites and other sharps such as Pasteur pipettes and razor blades.

The control of an inoculation hazard is accomplished by the safe use, handling, and storage of needles and other sharps. After using a needle, do not recap, bend, break, remove it from the syringe, or manipulate it in any way. Many people have been accidentally stuck with a needle during the process of recapping it. The needle and other sharps should simply be placed into a sharps container to prevent any injuries. Call EHS at 471-3511 for sharps containers.

Biosafety Levels

The Centers for Disease Control (CDC) and the National Institutes of Health (NIH) have developed standard procedures providing protection against biological hazards. The publication *Biosafety in Microbiological and Biomedical Laboratories* provides specific descriptions of combinations of microbiological practices, laboratory facilities, and safety equipment, and recommends their use in four biosafety levels of operation with infectious agents. These biosafety levels are described below.

The biosafety levels described in the *NIH Guidelines for Research Involving rDNA Molecules* are based on and consistent with the biosafety levels presented here. A biosafety level (BSL) is based on the potential hazard of the agent and the functions of the lab. BSL1 is for work with agents that pose the least hazard and BSL4 is for work with agents that pose the greatest hazard. Only BSL1 through 3 are included here because there are not any BSL4 labs at the university. Included are examples of organisms which fall within a particular biosafety level. Keep in mind that the biosafety level used for a particular organism may change based on the procedures being performed and the amount of cultures involved.

All work with microbiological agents should follow the CDC/NIH guidelines for the appropriate BioSafety Level. At a minimum, research and instructional labs conducting work with microbiological agents should follow the guidelines for BioSafety Level 1 (BSL1). If you are uncertain concerning

which biosafety level your work should be considered, call the EHS Biological and Laboratory Safety Coordinator at 471-3511 for assistance.

Biosafety Level 1

BSL1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

Examples of BSL1 Agents

E. Coli K-12
S. cerevisiae

The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be 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 BSL1:

Standard Microbiological Practices (BSL1)

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designed and used for this purpose.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - Used disposable needles and syringes 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.
  - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- Perform all procedures to minimize the creation of splashes and/or aerosols.

- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.
  - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.
  - Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
- An effective integrated pest management program is required.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Special Practices (BSL1)

None.

Safety Equipment (Primary Barriers and Personal Protective Equipment) (BSL1)

- Special containment devices or equipment such as a biological safety cabinet are generally not required.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

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

Laboratory Facilities (Secondary Barriers) (BSL1)

- Laboratories should have doors for access control.
- Each laboratory must contain a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Rugs in laboratories are not appropriate.
- Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Laboratory windows that open to the exterior should be fitted with screens.

Biosafety Level 2

BSL2 builds upon BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

Examples of BSL2 Agents

Influenza virus
Shigella spp.

It *differs* from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures, (2) access to the laboratory is restricted when work is being conducted, and (3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

In addition to all the requirements for BSL1, work at BSL2 requires:

Special Practices (BSL2)

- All persons entering the laboratory must be advised of the potential hazard and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
- Potentially infectious materials must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes,

or other potential contamination.
    - Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    - Equipment must be decontaminated before repair, maintenance, or removal from the facility.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained (see Medical Program, Chapter C.4 of this manual).
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

Safety Equipment (Primary Barriers and Personal Protective Equipment) (BSL2)

- 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 include pipetting, centrifuging, grinding, blending, shaking or mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
    - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use must be worn while working with hazardous materials. This protective clothing must be removed before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
- Eye and face protection (goggles, mask, face shield, or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:
    - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
    - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
    - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers) (BSL2)

- BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
- Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station must be readily available.
- There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
- A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

BSL3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure.

Examples of BSL3 Agents

Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

A BSL-3 laboratory has special engineering and design features.

It is recognized that many existing facilities may not have all the facility safeguards recommended for BSL3 (e.g., access zone, sealed penetrations, and directional airflow). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in BSL2 facilities. However, the recommended Standard Microbiological Practices, Special Practices, and Safety Equipment for BSL3 must be rigorously followed. The decision to implement this modification of BSL3 recommendations should be made only by the laboratory director.

In addition to all the requirements for BSL2, work at BSL3 requires:

Safety Equipment (Primary Barriers and Personal Protective Equipment) (BSL3)

- All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
- Protective laboratory clothing with a solid-front such as tie-back or wrap-around gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
- Eye, face, and respiratory protection must be used in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers) (BSL3)

- Laboratory doors must be self-closing and have locks in accordance with the institutional policies.
  - The laboratory must be separated from areas that are open to unrestricted traffic flow within the building.
  - Access to the laboratory is restricted to entry by a series of two self-closing doors.
  - A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door.
  - If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone.
  - Additional sinks may be required as determined by the risk assessment.
- The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
  - Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
  - Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
  - Ceilings should be constructed, sealed, and finished in the same general manner as walls.
  - Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.
- All windows in the laboratory must be sealed.
- Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
- A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
  - Laboratory personnel must be able to verify directional airflow. A visual monitoring

device which confirms directional airflow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of airflow disruption.
    - The exhaust air must not re-circulate to any other area of the building.
    - The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
- A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
- Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
- Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.
- The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Physical/Engineering Controls

Building Controls

Sinks

Ventilation

Biosafety Cabinets

A biological safety cabinet (BSC) is used as a primary barrier against exposure to infectious biological agents. A BSC has High Efficiency Particulate Air (HEPA) filters. The airflow in a BSC is laminar, i.e. the air moves with uniform velocity in one direction along parallel flow lines. A BSC

must be used in conjunction with safe laboratory techniques, because potentially dangerous aerosols can still escape.

Depending on the design, a BSC may be vented to the outside or the air may be exhausted into the room. BSCs are not chemical fume hoods. A percentage of the air is re-circulated in most types of BSCs. Therefore, the levels of explosive, flammable, or toxic materials will be concentrated within the cabinet. HEPA filters only trap particulates, allowing any contaminant in non-particulate form to pass through the filter.

Class I BSCs
In Class I BSCs, the exhaust air is HEPA filtered so the user and the environment are protected, but the product inside the cabinet is not. With a Class I cabinet, the user's hands and arms while inside the cabinet are exposed to the infectious materials. The Class I BSC is designed for general microbiological research with low to moderate risk agents, and is useful for containment of mixers, blenders, and other equipment.

Class II BSCs
There are different types of Class II BSCs, but they all offer HEPA filtered supply and exhaust air. This type of cabinet will protect the user, environment, and the product and is suitable for work assigned to Biosafety Levels 1, 2, or 3. Class II cabinets are the class most commonly used.

Class III BSCs
These cabinets are often referred to as Gloveboxes. The Class III cabinet is gas-tight and under negative pressure. All work in the cabinet is performed through rubber gloves attached to entry portals. The Class III cabinet offers the highest level of protection from infectious aerosols. Class III cabinets are most suitable for work with agents that require BSL3 or BSL4 containment.

Proper Use of BSCs

- Before and after use, wipe the surface of the BSC with a suitable disinfectant, e.g., 70% alcohol or a 1:10 bleach solution.
- Place everything you will need inside the cabinet before beginning work, including a waste container. You should not have to penetrate the air barrier of the cabinet once work has begun.
- Do not place anything on the air intake grilles as this will block the air supply.
- A sign can be posted on the door of the room stating that the cabinet is in use.
- You should prevent unnecessary opening and closing of doors as this will disrupt the airflow of the cabinet.
- Always wear a lab coat while using the cabinet and conduct your work at least four inches inside the cabinet.
- Place burners to the rear of the cabinet to reduce air turbulence.
- Place a disinfectant-soaked towel on the work surface to contain any splatters or small spills that might occur.
- Do not work in the BSC while the ultraviolet light is on. Ultraviolet light can quickly injure the eye.
- When finished with your work procedure, cover the waste container and decontaminate the surfaces of any equipment that is not enclosed.
- Operate the cabinet for five minutes before and after performing any work in it in order to purge airborne contaminants.

- Remove the equipment from the cabinet and decontaminate the work surface.
- Thoroughly wash your hands and arms.

Certification of BSCs

A BSC must be certified annually and after it has been newly installed, moved, or had a filter replaced. There are several companies in the area which provide this service. For further information, contact EHS at 471-3511.

Potentially Hazardous Equipment

All of these instruments can create aerosols and this must be considered with each use. The necessary precautions taken will depend upon what is being used in these instruments. If hazardous materials such as carcinogens, highly toxic, or infectious agents will be placed in any of these instruments, then precautions must be taken to prevent an exposure of lab personnel to aerosols or liquids.

Blenders

Depending on the nature of the material being used in these instruments, it may be necessary for them to be used or opened only in a biological safety cabinet.8

When working with infectious agents, blenders should have leak proof bearings and a tight-fitting, gasketed lid. Inspect the lid and gaskets routinely to ensure they are in good condition. Household blenders do not prevent the spread of aerosols.

Bunsen Burners

Centrifuges

Centrifuges that have sealed buckets, safety trunnion cups, or sealed heads are effective at preventing the escape of aerosols and liquids. The potential for exposing people to a hazardous material used in a centrifuge is great if the centrifuge tube breaks without the use of the safety features mentioned above.

Routinely inspect your centrifuge to ensure leakage is not occurring. An indicator such as fluorescein can be used to detect leaks. The fluorescein can be added to water and then centrifuged as you would other materials. An ultraviolet light can then be used to detect the fluorescein's presence on work surfaces, floors, and walls.

Clean Benches

Clean benches (a.k.a. laminar flow hoods) are not considered laboratory safety equipment. However, they deserve mention because they may be confused with BSCs. A clean bench is designed to protect the product from contamination, but it does *not* protect the user. The direction of airflow in a clean bench is toward the user.

Cryostats
Flow Cytometers

French Press
Fermentors

Homogenizers

Depending on the nature of the material being used in these instruments, it may be necessary for them to be used or opened only in a biological safety cabinet.

Loop Sterilizers
Lypholizers and Ampoules

Pipettes

In the past, some lab personnel were taught to mouth pipette. This practice has been known to result in many laboratory acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited. Mouth pipetting should never be used, even for innocuous materials, because you may at some time mistakenly mouth pipette something that is hazardous. To minimize aerosol production, a pipette should be drained with the tip against the inner wall of the receiving vessel. Never forcibly expel any hazardous material from a pipette.

Sharps (Needles, scalpels, razors, microtome, microcapillary tubes, dissecting pins)

Sonicators

Depending on the nature of the material being used in these instruments, it may be necessary for them to be used or opened only in a biological safety cabinet. Hearing protection may be required while using a sonicator.

Tissue Grinders
Vacuum Aspirators
Vortexers

Work Practice Controls

Microbiological Technique
Broken Glass
Food and Drink
Handwashing
Housekeeping
Laundry

Personal Protective Equipment (Gloves, Lab Coats, Eyewear, Faceshields, Respirators)

The type of personal protective clothing required in microbiological labs will depend upon the assigned Biosafety Level for that lab (see Section 2 of this chapter regarding Biosafety Levels). The protective clothing suitable for a typical undergraduate microbiology lab is a lab coat, to prevent street clothes from getting soiled, and latex or vinyl gloves. Long hair must be restrained if Bunsen burners are in use.

For a typical graduate level teaching or research microbiology lab (which are often a BSL2), lab coats or similar protective clothing should be worn while in the lab, and gloves must be worn while handling any infectious materials. Additionally, if the work involves human blood, a face shield, safety glasses or goggles, and a mask may be required if there is a potential for splash.

A research lab that is assigned a Biosafety Level 3 has additional requirements for personal protective clothing: laboratory clothing that protects street clothing must be worn, e.g., a solid-front or wrap-around gown. Typical lab coats which button down the front are not acceptable because they do not provide full protection. (See the EHS Website: Biosafety for information about Lab Coats.) Gloves must be worn in the lab, and respirators worn in rooms containing infected animals.

Whenever personal protective clothing becomes contaminated, it must be removed and replaced. Leave protective clothing in the lab and do not wear it to other non-lab areas. Disposable gloves are meant to be used only once and should then be discarded. In between glove changes, thoroughly wash your hands and arms.

Pest Control

Disinfection and Disposal

Waste Disposal Policy

There are many types of waste generated in a microbiological lab and all need to be handled, treated, stored, and disposed of properly (refer to EHS Procedures for Disposal of Hazardous Waste manual for more detailed information).

Autoclaves
Chemical
Radiation
Decontamination of Equipment

Emergency Procedures

Some biological materials when spilled or released can lead to significant infection exposures of personnel. This is particularly hazardous when the agent spilled or released is classified as a BSL2 agent or higher. The following emergency procedures that must be followed are determined by the Biosafety Level of the agent involved. Unless very minor, all spills and exposures should be reported to EHS and to the PI in charge of the lab.

a. Spills or Releases Involving BSL1 Agents

- Wear a lab coat and disposable gloves.
- Soak a paper towel(s) in an appropriate disinfectant such as a fresh 1:10 bleach solution and place over the spill area.

- Place the paper towels and gloves into a biohazard bag (available from EHS) for disposal by EHS or autoclave the materials.

b. Spills or Releases Involving BSL2 Agents

- If an accident occurs that may generate aerosols or droplets of an infectious agent, leave the area, close the door, decontaminate clothing, and shower. Allow at least 30 minutes for the droplets to settle and for the aerosol concentration to decrease.
- Wear appropriate personal protective clothing such as gloves, lab coat, and approved respiratory equipment, if needed.
- Cover the spill area with paper towels, pour a 1:10 bleach solution around the edges of the spill and then into the spill. Allow 10 minutes contact time.
- Working from the outer edges into the center, use paper towels to clean the area. Clean the spill area with fresh towels soaked in a disinfectant. Be sure to decontaminate any areas or surfaces that you suspect may have been affected by the spill. Place all clean up materials and gloves into a bag for decontamination, preferably by autoclaving. Wash thoroughly.
- A small spill of material that did not result in a significant generation of aerosols, or contamination of a person, can be cleaned up following steps two through four above.

c. Spills or Releases Involving BSL3 Agents

- If the spill occurs in a biological safety cabinet, keep the cabinet running, and clean the spill following steps two through four from *Spills or Releases Involving BSL2 Agents*, except that personal protective clothing appropriate for a BSL3 lab should be worn. If the spill in the cabinet is quite substantial, it may be necessary to decontaminate the cabinet's fan, filters, and airflow plenums. This should be done by a qualified outside company. Call the EHS Biological and Laboratory Safety Coordinator for assistance.
- If a minor spill occurs outside of a biological safety cabinet, follow steps two through four from *Spills or Releases Involving BSL2 Agents*, except that personal protective clothing appropriate for a BSL3 lab should be worn.
- If anything other than a minor spill occurs outside of a biological safety cabinet, leave the area immediately and notify appropriate personnel, including the EHS Biological and Laboratory Safety Coordinator. A specially designed decontamination procedure may be necessary.

Note: Whenever bleach is used to clean up spills of an infectious agent, a fresh solution should be prepared. After a few days, a bleach and water solution will lose its effectiveness for decontamination.

Notification
Animals
Biosafety Recommendations
Exposure to Biological Agents
Biological Toxins
Select Toxins
LPS
TAT

Recombinant DNA
- Transgenic Animals
- Transgenic Insects
- Transgenic Plants
- Viral Vectors

Special Biological Hazards

- Allergens

Bloodborne Pathogens

In December 1991, the Federal Government published the final rule governing occupational exposure to bloodborne pathogens which became effective March 6, 1992. The Texas Administrative Code: Title 25, Part 1, Chapter 96 – Bloodborne Pathogen Control applies to public employers within Texas.

The objective of these standards are to provide guidelines to eliminate or minimize employee exposure to human bloodborne pathogens. A human bloodborne pathogen is a pathogenic microorganism, present in human blood, that can cause disease in humans. The standard includes the Centers for Disease Control (CDC) guidelines referred to as Universal Precautions.

If during the course of work a potential exists for coming in contact with human blood or other potentially infectious materials, you must receive training on bloodborne pathogens. Contact EHS at 471-3511 for information regarding Bloodborne Pathogens Training.

The CDC Universal Precautions are used as an approach to infection control. The concept behind Universal Precautions is to treat all human blood and certain human body fluids as if known to be infected with HIV, Hepatitis B, and other bloodborne pathogens. The Universal Precautions are summarized below and should be practiced whenever coming in contact with human blood:

a. Use appropriate barrier precautions to prevent skin and mucus membrane exposure when contact with blood is anticipated. Always wear gloves. Wear masks and protective eyewear or face shields to prevent exposure to the eyes, mouth, and nose during procedures that are likely to result in droplets of blood. Wear gowns or aprons during procedures that are likely to result in splashes of blood. Remove all protective clothing before leaving the laboratory.
b. Wash hands and other skin surfaces immediately if contaminated with blood and after the removal of gloves.
c. Limit the use of needles to where there is no alternative and take precautions to prevent injuries by needles and other sharps. To prevent needle-stick injuries, needles should not be recapped, bent, removed from the syringe, or otherwise manipulated by hand. Place needles and other sharps into puncture-resistant containers.
d. Keep all specimens of blood in well constructed containers with a secure lid to prevent leakage during transport.
e. Use biological safety cabinets whenever procedures that have a high potential for generating droplets are conducted.
f. Never mouth pipette.
g. Decontaminate laboratory work surfaces after a spill of blood and when work activities are completed.

Some animals can also carry pathogens that can be transmitted to humans through contact with their body fluids, similar to human bloodborne pathogens. This contact can occur through biting, spitting, or contamination of broken skin or mucus membranes with bodily secretions from the animal. An example of a disease transmitted this way is the B-virus infection. B-virus is a naturally occurring alpha-herpes virus infecting macaques. Human infection has been documented in 25 instances, 16 of those resulting in death.

When working with animals such as macaques that are capable of transmitting disease to humans, take necessary precautions to protect yourself. Wear gloves, masks, and laboratory coats whenever entering an area where these animals are housed. Guidelines are available for safely working with macaques and can be obtained by calling EHS.

- Oncogenic Agents
- Reproductive Hazards
- Large Scale Research

Working with Human and NHP Specimens
- Body Fluids
- Clinical Laboratories

Tissue Culture
- Human Cell Line Policy

Select Agents and Toxins

Medical Surveillance
- Exams
- Vaccination
- Exposures
- Respirators

Access and Security

Transportation and Shipping
- On-Campus
- Off-Campus
- Importing Material
- Exporting Material

Inspection Form

Lab Signs

Manual Templates
- BSL-1
- BSL-2

References:

Biosafety in the Laboratory, Prudent Practices for the Handling and Disposal of Infectious Materials. National Research Council. 1989.

Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition. Centers for Disease Control and National Institutes of Health. 2009.

Texas Administrative Code: Title 25, Part 1, Chapter 96 – Bloodborne Pathogen Control