

Biological Safety Manual

Introduction

The hazards posed by biological materials, plus infections resulting from exposure to infectious materials, are important considerations when working in a biological laboratory. Several deaths have resulted from infections acquired in labs where etiological agents have been in use.

Controlling exposures (and the resulting infections) requires an understanding of the factors involved in disease transmission in the laboratory. The most common routes of exposure are ingestion, inhalation, and self-inoculation. The development of an infection subsequent to an exposure to an infectious agent depends upon individual susceptibility, the size of the dose, and the pathogenicity of the organism. The only one of these three factors within the control of the investigator is the size of the dose. If all exposures can be kept below the infectious dose, the risk of infection is greatly minimized. This is the basis for safety in the biological laboratory.

This document was based in part on the CDC/NIH publications “Biosafety in the Microbiological and Biomedical Laboratories, 4th Edition” and “Primary Containment for Biohazards: Selection, Installation and Use of Biosafety Cabinets”, the American Public Health Association publication “Control of Communicable Diseases, 17th Edition”, the American Industrial Hygiene Association publication “Biohazards Reference Manual, 2nd Edition”, and on the National Research Council publication “Biosafety in the Laboratory: Prudent Practices for the handling and disposal of Infectious Materials”. The biosafety cabinet graphics are from the CDC/NIH publication “Primary Containment...” and were scanned in by the ORCBS at Michigan State University. Employees desiring more information than is presented here should refer to these references, or consult an Environmental Health and Safety (EHS) staff member.

General Precautions

Employees who work with or around an agent for which there is a vaccine should consult EHS or the Occupational Health Nurse for information about immunization for that particular agent. Please inform EHS of the receipt of any material classified as Biosafety Level 2 (BSL2) or above; include information about the location, storage, use, precautions, and emergency procedures. Use a biohazard warning symbol to designate labs or storage areas housing human blood, blood products, or tissues, and any pathogenic agents. If a laboratory is conducting work at BSL2 level or above, a warning sign identifying the agent, emergency contact, and any special precautions required, must be posted on the laboratory door as well. See the appendices for a summary of biosafety levels, and listings of agents.

Note that receipt of any material deemed by the CDC/USDA as a Select Agent requires prior approval; work with BSL2 Agents or above require submitting a protocol for approval with the Institutional Biosafety Committee.

Biological laboratories must be reviewed annually by the Department Head, Primary Investigator or other responsible individual, and copies of the reviews submitted to EHS (see Appendix D for a copy of a Biological Laboratory checklist). EHS will conduct random inspections of biological laboratories.

Please observe the following basic precautions while working in a biological laboratory:

- Do not eat, drink, apply cosmetics or lip balm, store food, or smoke in the laboratory.
- Never mouth pipette.
- Wear disposable, high cuff, latex or nitrile gloves when working with biohazards (remember that latex gloves are permeable to organic solvents, including ethanol).
- Thin gloves offer little protection against cuts, bites, scratches, etc. Use the thickest gloves allowed by your work (i.e. do not sacrifice the dexterity required by your work).
- Wearing two pairs of thinner gloves permits the safe removal of the outer pair in case of inadvertent contamination.
- Wear a lab coat while in the laboratory. The lab coat should be closed, and the sleeves tucked into the gloves or otherwise restrained. Disposable Tyvek lab coats are available and recommended for work conducted in biosafety cabinets.
- Do not wear lab coats outside of the laboratory.
- Use at least a Class II biosafety cabinet for work with biohazardous materials.
- Procedures which might generate aerosols should be performed in the rear third of the biosafety cabinet.

- Avoid the use of needles, scalpels, or other sharp instruments whenever possible. If needles and syringes must be used, cover the tip with absorbent material when adjusting the volume or withdrawing the tip from a septum or injection site.
- Dispose of sharps in a puncture resistant container. Do not re-sheath or remove used needles; insert the whole assembly into the container. All punctures should be washed with soap and water and reported to your supervisor, Compendium, Risk Management, and EHS.
- If experimentation requires the use of pathogens, first develop and test all procedures using nonpathogenic agents (i.e. perform “dry runs”).
- Use disposable glass or plasticware. If non-disposable glassware must be used, disinfect contaminated items before cleaning.
- Clean up spills immediately with a fresh solution of chlorine bleach diluted 1:10 with water.
- **All waste materials must be decontaminated before disposal.**

Discard non-sharp disposable materials (e.g., gloves, pipettes, pipette tips, and plastic tubes) that come in contact with potentially infectious materials in polyethylene biohazard bags, and then autoclave or incinerate. Treat blood and other potentially infectious fluids with 10% chlorine bleach and decant down the drain. Do not dispose of blood or sharps with the regular laboratory trash.

Experiments Using Blood, Blood Products, Human Secretions or Other Potentially Infectious Materials

Persons who work with blood or blood products are at increased risk of contracting hepatitis. Hepatitis B vaccination is recommended for all individuals working with blood or potentially HBV containing material (note: OSHA requires all potential HBV exposed personnel to have either an HBV vaccination, or a signed declination on file). The most important way for personnel to protect themselves from Hepatitis B infection (as well as from other blood-borne infections) is to follow Universal Precautions (handling all blood, blood products, secretions and other potentially infectious material as if infectious).

If an exposure to potentially infectious material occurs, report it immediately to EHS for evaluation and possible treatment. See the Clemson University Exposure Control Plan for more information.

Recombinant DNA Experiments

If you plan to conduct experiments involving recombinant DNA, be aware that the Clemson University Institutional Biosafety Committee (IBC) does not recognize any of the exemptions detailed in the NIH rDNA regulations. Any recombinant DNA work by a Clemson Investigator or conducted in Clemson facilities must seek IBC approval prior to initiation of work. Contact the Office of Research Compliance for the proper application forms.

Work With Potentially Infectious Materials

For most biohazardous agents the routes of potential infection are inoculation, ingestion and inhalation. Wearing gloves, a lab coat, safety glasses, and using a biological safety cabinet (if appropriate) greatly minimizes an individual's exposure.

Decontamination of wastes can be accomplished for all these agents by autoclaving, or 30-minute exposure to fresh 10% chlorine bleach. Note that the color change associated with oxidation of media is not a good indicator of inactivation.

All human specimens should be regarded and handled as infectious. The risk from human specimens is not restricted to hepatitis or AIDS, but includes many others which may be found in blood, blood products, urine, feces, amniotic fluid, etc. Researchers frequently receive blood that has been designated "not for transfusion" and other fresh specimens that have not been screened for these agents. Research with human specimens is generally BSL 2, and the procedures set forth by the CDC/NIH for those biosafety levels should be followed.

Cell Lines

While information on specific cell lines is not included, it is important to recognize that there is no "normal" cell line; many reputedly "normal" lines harbor viruses and potentially hazardous gene sequences. Handle these materials as if infectious and decontaminate culture wastes prior to disposal. All cells should be fixed before subjecting them to an aerosol generating process (e.g., flow cytometers).

Viruses

Fluids, tissues, isolates and cell cultures containing infectious viruses pose a risk following exposure by ingestion, percutaneous or parenteral inoculation, and droplet or aerosol contamination of the mucous membranes of the eyes, nose, mouth, or broken skin. The aerosol risk from handling large volumes and concentrated stocks is great since some viruses are stable at ambient temperatures and withstand drying. Variation in viral structures results in differential susceptibility to "germicidal" agents and detergents; however, autoclaving and chlorine bleach treatment are usually effective. See the appendix at the end of this section for a chart showing the relative risk from oncogenic viruses.

Bacteria

Many bacteria are ubiquitous, but some of these such as *Staphylococcus aureus* and group A streptococci, are responsible for serious infections in man. The potential routes of exposure are as discussed above for viruses. Aerosols are of major concern when working with large volumes or concentrated stocks and with pathogenic spore forming species since spores resist adverse or extreme conditions. Safety glasses must be worn when handling bacteria, especially those that infect the conjunctiva, e.g., *N. gonorrhoea*. Work at a higher biosafety level is required when large volumes, highly concentrated stocks, or aerosol generating procedures are employed with infectious bacteria. All wastes must be decontaminated prior to disposal; autoclaving and chlorine bleach treatment are effective.

Parasites

Infectious stages of protozoal parasites of humans may be present in blood, feces, lesion exudates, and infected arthropods. Depending on the parasite, accidental parenteral inoculation, transmission by arthropod vectors, skin penetration (including bites from infected animals), and ingestion are the primary laboratory hazards. Aerosol or droplet exposure of the mucous membranes of the eyes, nose, or mouth with trophozoites are potential hazards when working with cultures of *Leishmania* and *Trypanosoma* species. All exposures should be reported to EHS and treated immediately, e.g., wipe bite with 70% ethanol or irrigate eye with distilled water. In general protozoa are very fragile, sensitive to drying, and, with notable exceptions such as *T. cruzi*, lysed even by water; however, all spills and waste must be actively treated. BSL2 containment procedures are recommended for work with all parasites except *Babesia*, which require BSL3 containment procedures.

Fungi

Fungi are generally not significant causes of human disease. Transmission of fungal diseases from person to person is extremely rare. Fungal spores however are generally very allergenic and some of the fungal constituents and by-products can be highly toxic, such as aflatoxin B. The more common hazardous fungi used in laboratories include: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Sporothrix schenckii*. All of these agents should be handled at BSL2 levels.

A classification of microorganisms according to hazards is presented in Appendix B of the Guidelines for Recombinant DNA Research (available from the Office of Research Compliance). Note that agents of class 1-4 should be handled according to the corresponding biosafety containment level and that there are restrictions against importation of class 5 agents.

Work with Laboratory Animals

All vertebrate animal experimentation requires the approval of the Animal Research Committee. Animals are to be housed only in accredited animal facilities and are not to be kept in laboratories for more than 24 hours. Users of laboratory animals must recognize that virtually all laboratory animal species can carry pathogens that are infectious to humans. Inoculated animals readily transmit viruses to cagemates by inhalation and contact with urine, feces, sputum, etc. Caution should be taken when working with any animal. Concern for the health of others who do not work directly with animals should be paramount when laboratory animals are transported or used in general laboratory areas outside of an animal facility.

Zoonoses

Although animal diseases do not commonly affect humans, some few do. A few of the more important animal reservoirs are:

- Primates. Diseases such as tuberculosis, shigella, campylobacter and salmonella can be serious health threats.

Herpesvirus B carried by rhesus, cynomolgus, and other Old World monkeys can cause fatal encephalitis in man.

- Dogs and Cats. Bite wound infections, cat scratch disease, visceral larval migrans and sarcoptic mange from dogs and toxoplasmosis and fungi's (such as ringworm) from cats are common.
- Rodents. Precautions should be taken against toxoplasmosis, lymphocytic choriomeningitis, salmonella and shigella and ringworm. Toxoplasmosis is one of the most commonly acquired parasitic diseases in the laboratory.
- Rabbits, Sheep, Swine, and Birds can be the source of tularemia, Q fever, Erysipelas, and Chlamydia (psittacosis), respectively.

A more complete list of agents is given in Appendix C.

Some requirements for the use of animals:

- Protocols involving the use of hazardous chemicals or biohazardous agents in animals must be reviewed with the ARC, IBC, and EHS prior to initiation. Use of radioisotopes also requires permission of the Radiation Safety Committee (see the Radiation Safety Manual).
- All users of laboratory animals must have an active tetanus immunization and other immunizations as appropriate, e.g., rabies, HBV.
- Bites or scratches that break the skin should be washed thoroughly with soap and water and reported to your supervisor, Compendium, Risk Management, and EHS.
- Wearing a facemask, gloves and a lab coat is required of users of animals to reduce aerosol, direct contact or inadvertent oral and nasal contact with contaminated materials.
- A full-face respirator is required for those at high risk.
- Lab coats should be changed and hands thoroughly washed if an animal, its fluids, or feces is touched.

Allergic responses to laboratory animals are the most common cause of human disease related to the use of animals in research. Allergy results from the direct or indirect exposure to allergens such as skin contact or inhalation of fur, dander, saliva, urine, serum, etc. Symptoms can vary from wheezing, sneezing and rhinitis to itching eyes and skin, obvious rashes, and asthma. Do not ignore the symptoms. Continued exposure can lead to anaphylaxis and is life threatening.

Pregnant employees should not expose themselves to feces, dander, or biohazard areas, and should suspend work involving the handling of cats and monkeys. Likewise, pregnant women without immunity to toxoplasmosis should avoid cat contact to avoid the possibility of congenital disease and fetal death.

Aerosol-Generating Processes

Aerosols (dispersions of particles in air) can result from the use of blenders, mixers, sonicators, cell disrupters, centrifuges, syringes, pipettes, aspirators, test and centrifuge tube caps. (The hazards associated with the use of centrifuges are discussed under precautions for laboratory equipment and devices in General Laboratory Safety).

Several well-documented studies have made it clear that great attention must be given to prevent contamination of room air with the suspension of liquid or solid particles containing hazardous materials including radioisotopes, infectious agents (viruses and mycoplasma from “normal” cells), as well as toxic chemicals and carcinogens.

Particle size is a factor in determining the path an aerosol will follow. Particles in the range of 1 to 5 microns present the greatest hazard to the laboratory worker, since they more readily penetrate the respiratory tract than larger particles and are more readily retained than smaller or larger particles. Many laboratory procedures produce aerosols with particles in this range. Particles larger than 10 microns fall out on surfaces or are impinged on materials with an opposite electrostatic charge. In the respiratory tract, larger particles do not penetrate into the lower spaces but are removed by interception and impaction in the upper respiratory tract and subsequently expelled or swallowed. Large droplets that fall out on surfaces dry quickly, and secondary aerosols of the dry particles can be created by air currents or laboratory activity.

Significant settling of larger particles from an aerosol can occur in five minutes; however, most of the remaining small particles require 30 minutes to an hour to settle, assuming that fresh currents of air do not prevent their settling. This is why it is best to wait before cleaning up a spill of infectious virus, or other biologically hazardous material. Besides the direct effects of aerosols, they may contaminate surfaces of the skin or equipment and subsequently enter the body as a result of hand-to-mouth contact and ingestion or through abrasions of the skin.

In addition to avoiding the creation of an aerosol, three general approaches are recommended to decrease the hazards of aerosols associated with research on tumor specimens, cell and virus cultures and concentrates, and toxic chemical materials:

- Reduce the extent or concentration of the aerosol.
- Contain the aerosol in a primary barrier system.
- Use personal respiratory protection and protective laboratory clothing.

Aerosol Generating Activities

The following is an example of some aerosol generating activities:

- Forced expulsion of the last drop of liquid or mixing of liquid by alternately sucking and blowing with the pipette, creating splashes and bubbles.
- Removing the cap or stopper from bottle after vigorous shaking to mix, wash or re-suspend material.

- Blending materials to disrupt cells, release enzymes or viruses, to homogenize suspensions, etc., without aerosol tight cover seals or leak-proof rotor bearings.
- Sonic disruption of cells or organelles.
- Grinding tissue with mortar and pestle, glass tissue grinder, or ball mill.
- Pouring hazardous materials from one container to another, e.g., decanting supernatants.
- Sterilizing a wire loop or needle in a flame, creating splatter.
- Withdrawing a syringe needle as from a vaccine bottle or following inoculation of experimental animals.
- Weighing dry hazardous materials.
- Opening a freeze-dried preparation.
- Removing plugs from flasks and tubes.
- Handling cages that held infected animals or large animals in open areas or unventilated cages.

Measures to Decrease Hazards From Aerosols

- Use gravity flow of liquid with pipette calibrated for mark-to-mark drain-to-tip delivery and with pipette tip in contact with container wall.
- Use swirling motion rather than shaking, allow aerosol to settle for a few minutes after bubbles disappear before removing cap or stopper.
- Use special safety containers with seals to prevent escape of aerosols; use drain/siphon system to remove contents without removing cover.
- Use cup or chamber that is aerosol tight; allow aerosol to settle before opening cup. Place sonicator in fume hood or laminar flow cabinet
- Use slow speeds; use a clear plastic or inflatable glove bag to further contain the operation within the safety cabinet; allow aerosol to settle before removing cover.
- Use transfer pipettes or closed siphon or vacuum technique.
- Gradually dry loop or needle near flame, or use specially designed incinerator for loops and needles.
- Use sterile cotton gauze to enclose needle; if experiment permits, use disinfectant with cotton or gauze.
- Use draft-free, low-humidity enclosure for balance; discharge static electricity; use tared weighing containers not open weighing dishes or papers.
- If material is in an ampoule, nick the ampoule with a file, cover its neck with sterile gauze. If material is in a rubber-stoppered bottle, first relieve vacuum with a hypodermic needle. If material is to be dissolved or suspended in liquid, introduce the liquid with a syringe and cover needle with gauze wetted with disinfectant.

- Avoid disturbing cage contents; if animals are held in open areas, use liquid disinfectants during cage cleaning; keep area clean; use personal respirator.

Biological Safety Cabinets

BSCs are only one part of an overall biosafety program. Detailed descriptions of acceptable work practices, procedures, and facilities, described as biosafety levels 1 through 4, are presented in the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition (BMBL). The following information on BSCs was excerpted from the NSF/ANSI publication *Class II Biosafety Cabinetry* (NSF 49-2002).

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III have been developed to meet varying research and clinical needs.

The similarities and differences in protection offered by the various classes of biosafety cabinets are reflected in the following table:

Biological Risk Assessed	Protect Personnel	Protect Product	Protect Environment	BSC Class
BSL 1-3	YES	NO	YES	I
BSL 1-3	YES	YES	YES	II (A, B1, B2, B3)
BSL 4	YES	YES	YES	III B1, B2

The Class I BSC

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained (5) through the front opening. With the product protection provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures (e.g. cage dumping, aerating cultures or homogenizing tissues) with a potential to generate aerosols.

HEPA filters remove particles equal to, greater than, and less than 0.3um (which essentially includes all bacteria, spores and viruses) with an efficiency of 99.97%. A detailed explanation of HEPA filter efficiency and the mechanics of particle collection have been well documented; for more information contact EHS.

The Class I BSC is hard-ducted to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the exhaust plenum. A second HEPA filter may be installed at the terminal end of the exhaust.

Some Class I BSCs are equipped with an integral exhaust blower; the cabinet blower must be interlocked with the building exhaust fan. In the event that the building exhaust fan fails, the cabinet exhaust blower must turn off so that the exhaust ducts are not pressurized. Filters should be installed on the intake side of the fan. Also note that use of two filters increases the static pressure on the fan. If the ducts are pressurized and the HEPA filter develops a leak, contaminated air could be discharged into other parts of the building or the environment.

A steel panel with armholes to allow access to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, thereby increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel.

The Class II BSC

The Class II (Types A1, A2 (formerly B3), B1, B2) biological safety cabinets provide personnel, environmental and product protection. Airflow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A BSC) or ducted out of the building (Type B BSC).

HEPA filters are effective at trapping particulates and infectious agents, but not at capturing volatile chemicals or gases. Only BSCs that are ducted to the outside may be used when working with volatile toxic chemicals.

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. Class II cabinets provide the microbe-free work environment necessary for cell culture propagation, and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.

THE CLASS II, TYPE A1

This class was formerly known as Class II, Type A. An internal blower draws sufficient room air through a front grille to maintain a minimum inflow velocity of at least 75 lfpm at the face opening of the cabinet. The supply air flows through a HEPA filter and provides particulate-free air to the work surface. Laminar airflow reduces turbulence in the work zone and minimizes the potential for cross-contamination.

The downward moving air “splits” as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille. Although there are variations among different cabinets, this split generally occurs about halfway between the front and rear grilles, and two to six inches above the work surface.

The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Due to the relative size of these two filters, approximately 30% of the air passes through the exhaust HEPA filter and 70% recirculates through the supply HEPA filter back into the work zone. Most Class II, Type A cabinets have dampers to modulate this 30/70 division of airflow.

An unducted Class II Type A1 BSC is not to be used for work involving volatile or toxic chemicals. The buildup of chemical vapors in the cabinet (by recirculated air) and in the laboratory (from exhaust air) could create health and safety hazards.

It is possible to duct the exhaust from a Type A cabinet out of the building. However, it must be done in a manner that does not alter the balance of the cabinet exhaust system, thereby disturbing the internal cabinet airflow. The typical method of ducting a Type A1 cabinet is to use a “thimble”, or canopy hood, which maintains a small opening (usually 1 inch) around the cabinet exhaust filter housing. The volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing (contact manufacturers for any additional specifications).

The thimble must be removable or be designed to allow for operational testing of the cabinet. The performance of a cabinet with this exhaust configuration is unaffected by fluctuations in the building exhaust system.

“Hard-ducting” (i.e., direct connection) of Class II Type A1 cabinets to the building exhaust system is not recommended. The building exhaust system must be precisely matched to the airflow from the cabinet in both volume and static pressure. However, fluctuations in air volume and pressure that are common to all building exhaust systems make it difficult, if not impossible, to match the airflow requirements of the cabinet.

THE CLASS II, TYPE A2

Formerly known as the Class II Type B3. This biological safety cabinet is typically a ducted Type A cabinet having a minimum inward airflow of 100 lfpm, although it may be exhausted into the lab space. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment. A connection to the building exhaust system required.

THE CLASS II, TYPE B1

Room air is drawn through the face opening of the cabinet at a minimum inflow velocity of 100 lfpm. As with the Type A cabinet, there is a split in the down-flowing air stream just above the work surface. In the Type B cabinet, approximately 70 percent of the downflow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the downflow air is drawn through the front grille. Since the air which flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted towards the rear of the cabinet.

Type B1 cabinets must be hard-ducted, preferably to their own dedicated exhaust system, or to a properly designed laboratory building exhaust. As indicated earlier, blowers on laboratory exhaust systems should be located at the terminal end of the ductwork. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor should be installed to sound an alarm and shut off the BSC supply fan, should failure in exhaust airflow occur. Since all cabinet manufacturers do not supply this feature, it is prudent to install a sensor in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.

THE CLASS II, TYPE B2

This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides simultaneous primary biological and chemical containment. The supply blower draws in room air or outside air at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building or cabinet exhaust system draws air through both the rear and front grilles, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter) prior to discharge to the outside. Exhausting as much as 1200 cubic feet per minute of conditioned room air makes this cabinet expensive to operate.

Should the building or cabinet exhaust fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system installed by the manufacturer to prevent the supply blower from cease operating whenever the exhaust flow is insufficient. Presence of such an interlock system should be verified; systems can be retrofitted if necessary. A pressure-independent device should monitor exhaust air movement.

SPECIAL APPLICATIONS

Class II BSCs can be modified to accommodate special tasks. For example, the front sash can be modified by the manufacturer to accommodate the eyepieces of a microscope, or the work surface can be designed to accept a carboy, a centrifuge, or other equipment that requires containment. A rigid plate with armholes can be added if needed. Good cabinet design, microbiological aerosol tracer testing of the modification, and appropriate certification are required to ensure that the basic systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures.

The Class III BSC

The Class III biological safety cabinet was designed for work with biosafety level 4 microbiological agents, and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank (that is accessible through the cabinet floor) or double-door pass-through box (such as an autoclave) that can be decontaminated between uses. Reversing that process allows for safe removal of materials from the Class III biosafety cabinet. Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (usually about 0.5 inches of water pressure). A connection to the building exhaust system required.

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

Several Class III cabinets can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom-built; the equipment installed within the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators, etc.) is generally custom-built as well. Furthermore, Class III cabinets are usually only installed in maximum containment laboratories that have controlled access and require special ventilation or other support systems.

Laminar flow "clean benches"

Laminar flow clean air benches (horizontal or vertical) are **not** BSCs. These benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker can be exposed to materials (including proteinaceous antigens) being manipulated on the clean bench, which may cause hypersensitivity. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices.

Horizontal units discharge HEPA-filtered air across the work surface and toward the user. Horizontal clean air benches should never be used as a substitute for a biological safety cabinet in research, biomedical, or veterinary laboratories.

Vertical laminar flow clean benches may be useful in pharmacies when a clean area is needed for preparation of intravenous drugs. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems as the horizontal laminar flow clean benches.

Chemicals in BSCS

Work with infectious microorganisms often requires the use of various chemical agents, and many commonly used chemicals vaporize easily. Therefore, evaluation of the inherent hazards of the chemicals must be part of the risk assessment when selecting a BSC. Volatile or toxic chemicals should not be used in unducted Class II, Type A1 cabinets since vapor buildup inside the cabinet presents a fire hazard. In order to determine the greatest chemical concentration which might be entrained in the air stream following an accident or spill, it is necessary to evaluate the quantities to be used.

The electrical systems of Class II cabinets are not spark-proof, so no chemical concentration should be allowed that would approach the lower explosive limits of the compound. Furthermore, since Class II, Type A1 cabinets return chemical vapors to the cabinet workspace and the room, they may expose the operator and other room occupants to toxic chemical vapors.

Rather than a BSC, consider using a chemical fume hood, which is designed for work with volatile chemicals. Chemical fume hoods are connected to the building exhaust system and operate with single-pass air ducted directly outside the building. They also are used when manipulating chemical carcinogens. However, because they also are ducted to the outside, Class I and Class II, Type B2 biological safety cabinets can be used when manipulating small quantities of volatile chemicals as an adjunct to microbiological studies. The Class II, Type B1 cabinet also may be used with minute or tracer quantities of nonvolatile chemicals.

Many virology and cell culture laboratories use diluted preparations of chemical carcinogens and other toxic substances. Prior to maintenance, careful evaluation must be made of potential problems associated with decontaminating the cabinet and the exhaust system. Air treatment systems, such as a charcoal filter in a bag-in/bag-out housing, may be required so that effluents meet applicable emission regulations.

Radiological Hazards in the BSC

Volatile radionuclides such as I^{125} should not be used within Class II, Type A cabinets (see Table 2). When using nonvolatile radionuclides inside a BSC, the same potential inherent hazards exist as when working with radioactive materials on the bench top. Work that has the potential for splatter or aerosolization can be done within the BSC, and monitoring for radioactivity must be done. BSCs should be decontaminated as needed. A straight vertical (not sloping) beta shield may be used inside the BSC to provide worker protection when appropriate.

BSC Use: Work Practices and Procedures

Preparing for Work Within a Class II BSC

Preparing a written checklist of materials necessary for a particular activity and placing necessary materials in the BSC before beginning work serves to minimize the number of arm-movement disruptions across the fragile air barrier of the cabinet.

The rapid movement of a worker's arms in a sweeping motion into and out of the cabinet will disrupt the air curtain and may compromise the partial barrier containment provided by the BSC. Moving arms in and out slowly, perpendicular to the face opening of the cabinet, will reduce this risk. Other personnel activities in the room (e.g., rapid movement, open/closing room doors, etc.) may also disrupt the cabinet air barrier.

Laboratory coats must be worn buttoned over street clothing; latex gloves are worn to provide hand protection. A solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside. Elasticized sleeves can also be worn to protect the investigator's wrists.

Before beginning work, the user should adjust the stool height so that his/her face is above the front opening. Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants. When the user's arms rest flatly across the front grille, room air may flow directly into the work area, rather than being drawn through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed at least four "4" inches from the front grille on the work surface.

Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

BSCs are designed to be operated 24 hours per day, and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration. In some instances, room exhaust is balanced to include air discharged through ducted BSCs.

Cabinet blowers should be operated at least three to five minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet. The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with non-sterile water may recontaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

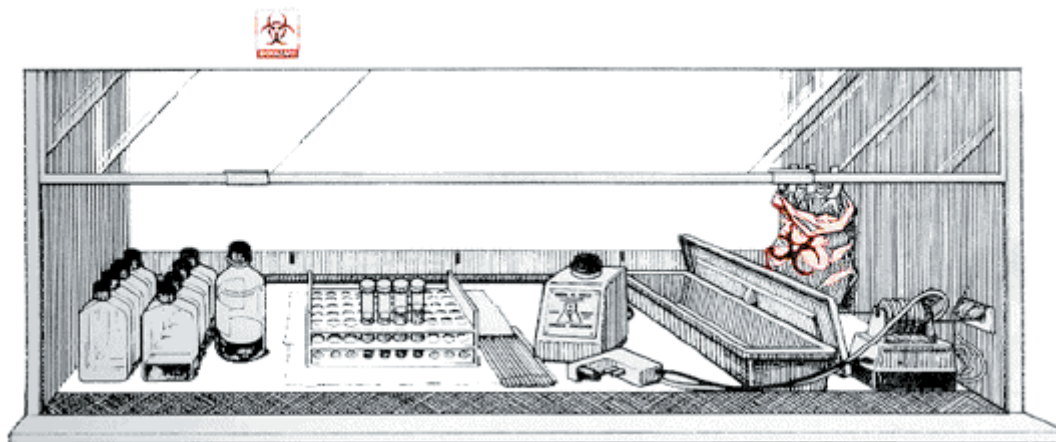
Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% ETOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Material Placement Inside the BSC

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill. It then can be folded and placed in an autoclavable biohazard bag when work is completed.

All materials should be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet (Figure 11). Similarly, aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split described in Section III. Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet.

Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right). This arrangement is reversed for left-handed persons.



Certain common practices interfere with the operation of the BSC. The autoclavable biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet.

Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or for other decontamination treatment.

Operations Within A Class II BSC

Many common procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within the cabinet. Class II cabinets are designed so that horizontally nebulized spores will be captured by the downward flowing cabinet air within fourteen inches of travel. Therefore, as a general rule of thumb, keeping clean materials at least one foot away from aerosol-generating activities will minimize the potential for cross-contamination.

The general workflow should be from “clean to contaminated (dirty)”. Materials and supplies should be placed in such a way as to limit the movement of “dirty” items over “clean” ones.

Several measures can be taken to reduce the chance for cross-contamination when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the towel. Items should be recapped or covered as soon as possible.

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of air supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric “furnaces” are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter (see Figure 12). This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of appropriately as noninfectious waste.

Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work. When chemical means are appropriate, suitable liquid disinfectant should be placed into the discard pan before work begins. Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions. Alternatively, liquids can be autoclaved prior to disposal. Contaminated items should be placed into a biohazard bag or discard tray inside the BSC. Water should be added to the bag or tray prior to autoclaving.

When a steam autoclave is to be used, contaminated materials should be placed into a biohazard bag or discard pan containing enough water to ensure steam generation during the autoclave cycle. The bag should be taped shut or the discard pan should be covered in the BSC prior to removal to the autoclave. The bag should be transported and autoclaved in a leakproof tray or pan.

Decontamination

Surface Decontamination

All containers and equipment should be surface decontaminated and removed from the cabinet when work is completed. At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass. If necessary, the cabinet should also be monitored for radioactivity and decontaminated when necessary. Investigators should remove their gloves and gowns and wash their hands as the final step in safe microbiological practices.

Small spills within the BSC can be handled immediately by removing the contaminated absorbent paper toweling and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the cabinet interior, should be immediately wiped with a towel dampened with decontaminating solution. Gloves should be changed after the work surface is decontaminated and before placing clean absorbent toweling in the cabinet. Hands should be washed whenever gloves are changed or removed.

Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination. All items within the cabinet should be surface decontaminated and removed. After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.

Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent. Manufacturer's directions should be followed. The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag. The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation. The drain pan should be flushed with water and the drain tube removed.

Should the spilled liquid contain radioactive material, a similar procedure can be followed. EHS should be contacted for specific instructions.

Gas Decontamination

BSCs that have been used for work involving infectious materials must be decontaminated before HEPA filters are changed or internal repair work is done. Before a BSC is relocated, a risk assessment which considers the agents manipulated within the BSC must be done to determine the need for decontamination. The most common decontamination method uses formaldehyde gas.

Certification of Biological Safety Cabinets

Containment Standards

The National Sanitation Foundation (NSF International) Standard No. 49 for Class II (Laminar Flow) Biohazard Cabinetry is the Standard for biological safety cabinets, and establishes performance criteria for biological safety cabinets and provides the minimum requirements that are accepted in the United States. This standard replaces the older NIH specifications which had been used by other institutions and organizations previously. NSF Standard 49 incorporates specifications regarding design, materials and construction. Cabinets which meet the standard and were certified by the NSF bear an NSF 49 Seal.

The operational integrity of a new BSC must be validated by certification before it is put into service or after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC must be tested and certified at least **annually** to ensure continued proper operation.

On-site testing following the recommendations for field testing (NSF Standard 49) must be performed by experienced, qualified personnel. Some basic information is included here to assist in understanding the frequency and kinds of tests to be performed. The importance of proper certification cannot be emphasized enough, Since persons who manipulate infectious microorganisms are at increased risk of acquiring an occupational illness when their BSCs are functioning improperly.

General Biosafety Issues

Biological Stains

Fixatives and stains used for the preparation of tissues and cellular materials often have toxic properties (e.g. methylene blue and trypan blue are teratogens), requiring the use of impermeable gloves and appropriate ventilation. In addition several dyes used in conjunction with flow cytometry and visualization of nucleic acids are suspect carcinogens. Be sure the precautions you are taking are adequate. If in doubt, consult with your supervisor or EHS.

Incubators

Incubators can become the inadvertent and undesired repositories of microorganisms. Although they may present a hazard to laboratory workers, most often they are a source of contamination of laboratory cultures.

Besides the moist surfaces, rubber gaskets, the humidity trough (if present), and fan mechanism are areas in which contaminating microorganisms concentrate. It is recommended that an anti-microbial agent, such as Zephiran Chloride be added to the humidity source water; do not use sodium azide. In addition, the inner panels, trays, and the other removable parts should be autoclaved and the gaskets and non-removable parts wiped thoroughly with 70% ethanol every two months.

Freezer and Liquid Nitrogen Storage

Freezers containing potentially hazardous biological materials and toxins should be labeled accordingly.

These freezers should be defrosted at least annually to prevent the accumulation of broken vials and excessive frost. Note that "frost-free" freezers allow small samples to thaw during warming cycles.

Ethanol and other flammable solvents may not be placed in refrigerators or freezers that are not designed for flammable solvent storage. Moving the controls of a standard refrigerator or freezer to the outside is not acceptable, and does not allow for the storage of flammable solvents inside the altered unit. If you must refrigerate flammable solvents, use only a refrigerator or freezer meeting OSHA requirements for flammable solvent storage.

Cells and virus stocks should be stored in sealed ampules and not in screw cap glass vials. Screw cap glass vials are permeable to liquid nitrogen (approximately 50% of the time) and therefore represent a source of contamination in the storage tank. Plastic screw cap ampules also leak and must be used with a heat sealed sleeve to prevent contamination of the liquid nitrogen and other samples. Upon thawing, screw cap vials may explode, producing an aerosol of glass and cell debris.

If freezing manually, place ampules in the bottom of a beaker, cover with methanol and a dye, e.g., methylene blue, and transfer the entire beaker from refrigerator to freezer. The methanol provides even freezing and the dye will penetrate imperfectly sealed vials permitting their identification and elimination.

When adding samples to liquid nitrogen storage repositories, be aware that the liquefied nitrogen may boil vigorously as warmer materials are added. Use only in a well-ventilated area. Liquefied nitrogen is a cryogenic gas and expands 700-fold upon vaporization; this may result in a rapid displacement of air. See the Chemical Hygiene Plan for SOPs regarding required safety equipment when working with cryogenic liquids.

Ampoules to be thawed should be dropped into a plastic beaker containing 70% ethanol at 37C within a spongy bucket and covered immediately. Confirm the identification of the sample. Open the vial in a biological safety cabinet, by nicking the ampoule with a file near the neck. Wrap it in ethanol wetted material and, holding the vial upright, snap the ampoule open at the nick. Add liquid slowly to dried material. Withdraw the suspension and mix in another vessel.

Biological Spills and Decontamination

Spills

The following guide is to be followed in the event of a small, contained spill of biological materials or until assistance from EHS is obtained:

- If the substance is dry or nonvolatile, shut off hoods, close windows and doors, and vacate rooms. Label door with appropriate warning. Allow the aerosol to settle for at least 30 minutes before reentering room.
- If the substance is volatile, leave on ventilation and vacate room, closing door. Label door with appropriate warning.
- Notify your laboratory supervisor and EHS.
- Assemble materials necessary for decontamination and don appropriate protective clothing, i.e., disposable lab coat, impermeable gloves. Note that surgical latex gloves are permeable to alcohol and that a respirator may be required if the substance is volatile. If you feel unsure of your ability to respond appropriately to the spill, or if you are not approved by EHS for a respirator, do not attempt clean up until EHS arrives.
- For a liquid biological spill: pour the appropriate decontaminating solution (see “Decontamination” below) on the spill, working from the perimeter inward.

Decontamination

Keeping biological waste separate from other waste streams is essential for any management program. Ideally, it should be treated and destroyed on-site. Disposal of biological (medical) waste, subject to federal, state and local laws, is becoming increasingly more regulated and costly—consult with EHS before disposing of biological waste.

All culture materials and biological specimens, including that from “normal” cultures and primary tissue, should be collected inside the biological safety cabinet. These materials should be autoclaved or otherwise chemically inactivated on at least a daily schedule.

Do not leave untreated waste in a corridor or public area.

Hypochlorites or any other strong oxidizing material must not be autoclaved with organic material such as paper, cloth, oils, or volatile solvents as this may produce toxic vapors or an explosion. Therefore, do not autoclave waste that has been treated with chlorine bleach.

Do not autoclave materials contaminated with radioisotopes or toxic chemicals. These materials may volatilize and contaminate the autoclave and expose workers.

The biological safety cabinet should be wiped down with an appropriate disinfectant (see below) prior to and at the end of each session.

Waste for autoclaving should be placed in autoclavable bags, and the name of the generator should be clearly marked on the bag. The bags should be no more than two-thirds full and tied or taped closed. To prevent piercing the bags, place all sharp objects in puncture-proof containers. Up to a liter of either absorbed or contained liquid (i.e. on cultureware) may be placed in each bag. Materials should be autoclaved for 60 minutes at 121C and 15 PSI. Autoclave in a shallow plastic tray or other vessel suitable to contain possible leakage from the bag.

To monitor autoclave performance in various locations of the autoclave, include spore strips routinely and ampules of *Bacillus stearothermophilus* monthly in waste bags. When unloading autoclave, wear loose fitting thermal gloves; remove these immediately if they get wet. Do not remove liquids immediately following completion of the cycle, as they may be super heated and boil vigorously. Note that dry heat is much less effective than moist heat for sterilization and is not appropriate for waste treatment. For example, a dry heat oven set at 165C requires 5-6 hours to effectively sterilize glassware that can be sterilized by autoclave at 121C in 20 minutes. Hot air is a less effective heat conductor than steam; in addition, the dry oven usually requires a much longer time to reach temperature.

Disinfectants

Alcohol

Isopropyl and ethyl alcohols in 70-90% concentrations may be germicidal against lipid-containing agents but are not effective against spores and infectious DNA. Note that 100% ethanol is not a good disinfectant. The major advantage of alcohols is that they are fast acting, evaporate rapidly, and leave no residue. Moreover, they can be combined with other disinfectants (quaternaries, phenolics, and iodine) to form tinctures further enhancing cidal action.

Chlorine

A very active disinfectant, chlorine is cidal against a wide variety of gram-negative and gram-positive bacteria, bacterial spores and most viruses. Disinfect media with a 10% solution of chlorine bleach (5.25% hypochlorite or 52,500 ppm) for 15 to 30 minutes. Note that solutions deteriorate with age and are rapidly neutralized by organic matter.

Its effectiveness may be enhanced by the addition of 0.1% solution of an ionic detergent. If used directly on a stainless steel surface, rinse thoroughly with water to prevent tarnishing and decomposition. Do not autoclave chlorine solutions.

Iodophor

Characteristics of chlorine and iodine are similar. Iodophors are effective against gram-positive and gram-negative organisms, mycobacteria, and some viruses, and are most effective in acid solutions. Organic matter reduces effectiveness, but iodophors are less affected than hypochlorites. Do not autoclave since iodophors vaporize at 120F. Iodophors are stable in storage if kept cool and tightly covered.

Ethylene Oxide

Due to its acute toxicity (skin, eye, respiratory and mucous membrane irritation, vomiting, and diarrhea), chronic toxicity (respiratory irritation, secondary respiratory infection, anemia), and status as a suspected carcinogen and mutagen, Ethylene oxide should be used for decontamination only when no other agent or method is effective. Ethylene oxide sterilizers are commonly used for decontamination and sterilization of heat-sensitive or moisture-sensitive apparatus.

In the event of an ethylene oxide leak, evacuate the area, and call 911. Avoid all skin contact with ethylene oxide. The room should have adequate ventilation and the sterilizer should have dedicated ventilation. Respiratory protection should be readily accessible to the gas cylinder storage and hook-up areas but do not store the respirators in these areas.

Splashes of liquid ethylene oxide or a solution of ethylene oxide should be treated immediately by removing any contaminated clothing and flushing the affected areas with copious amounts of water. Contaminated clothing, especially leather items such as shoes, must be bagged and aerated for at least 8-12 hours and then thoroughly laundered before reuse.

If inhalation occurs, leave the area immediately and move into an area with fresh air. Contact EHS. If overexposure symptoms develop (vomiting or nausea) contact a physician. Note that symptoms may not develop until up to 6 hours after the exposure.

When working with liquid ethylene oxide, its solution or the gas cylinders, wear heavy butyl or nitrile gloves, and goggles or a face shield. Other garments, e.g., sleeves, lab coats, should be made of polyethylene-coated disposable materials, e.g., Tyvek.

Items must be thoroughly cleaned before treatment with ethylene oxide. Residual organic matter or debris protects microorganisms from exposure to the gas and the residual materials (e.g., proteins, salts, solutions) may actually contaminate the sterilizer and the aerator.

The sterilizer equipment and room must be monitored to ensure that exposure limits are below OSHA Permissible Exposure Limits (PELs); if you are using ethylene oxide for sterilization, notify EHS.

Any area where exposure to ethylene oxide may exceed the PEL must be designated a regulated area and access restricted to authorized personnel. The area must be posted:

DANGER - ETHYLENE OXIDE
CANCER HAZARD AND REPRODUCTIVE HAZARD
AUTHORIZED PERSONNEL ONLY
RESPIRATOR AND PROTECTIVE CLOTHING MAY BE
REQUIRED TO WORK IN THIS AREA

Contact EHS for information on emergency procedures, training, and environmental monitoring.

Bio-Hazardous Waste Management

Bio-hazardous waste is defined in SCDHEC "Infectious Waste Management Regulations R.61-105". Copies and other information on this subject are available at the SCDHEC Infectious waste web site (link at the end of this section)

Here are some simple instructions and generalizations derived from this regulation and our local landfill restrictions. For all practical purposes Non Infectious and Infectious Biological wastes are managed the same but collected separately. Petri Dishes and other items must be rendered non-hazardous by treatment on site and rendered unrecognizable before sent to normal landfill. Use of Biological Symbols on Red bags is not allowed in this instance. No perishable or sharp items should be considered for this route of disposal. Any treatment on site is subject to the requirements in Autoclave Maintenance and Procedures section. It is the responsibility of the department who operates such devices to review and initiate all these proper procedures in order to conduct treatment by Autoclave. And to deface all items before sending to solid waste landfill.

1. All small animal tissues are bio-hazardous waste and should be declared to Environmental Health and Safety for removal. Large animal tissue disposal is also regulated by SCDHEC and you should call us for instructions on these items.
2. All animal tissues fixed in Ward Safe, Caro Safe and any other non-hazardous fixative should be considered Non infectious bio-hazardous waste with all free liquids removed and the solids properly packaged in proper shipping containers supplied by Environmental Health & Safety.
3. All Human Blood and tissue, animal blood and bedding potentially contaminated with a zoonotic microbe and any wastes from recombinant DNA experiments are bio-hazardous waste and should be declared to Environmental Health and Safety for removal.
4. All non-controlled pharmaceuticals, vaccines, & enzymes are Non infectious bio-hazardous waste and should be declared to Environmental Health and Safety for removal.

5. All filters and apparatus that have been used to filter or contain Human blood and tissue, recombinant DNA or zoonotic microbes are bio-hazardous waste unless properly deactivated as described in R.61-105 and should be declared to Environmental Health and Safety for removal.
6. All items determined by protocol review from the University Bio-Safety Committee or the department of Environmental Health and Safety to be bio-hazardous are such and will be managed as bio-hazardous wastes.
7. All needles, scalpels and any other sharp item that could be defined as "Medical Waste" should be placed in an appropriate sharps container obtained from Environmental Health and Safety
8. No waste should be stored for more than 96 hours with out refrigeration or no more than 30 days under proper refrigeration conditions described in R. 61-105 as below 42 degrees Fahrenheit.

Proper Disposal methods of infectious and non infectious Biological wastes and related online forms for scheduling pickup of wastes can be found on the Clemson Web server at: <http://ehs.clemson.edu>

Current SCDHEC Infectious waste regulations R. 61-105 can be found at the following SCDHEC web site: <http://www.scdhec.net/lwm/html/infect.html>

Appendix A - Select Agents

HHS Select Agents

Crimean-congo haemorrhagic fever virus
Ebola viruses
Lassa fever virus
Marburg virus
Rickettsia prowazekii
Rickettsia rickettsii
South american haemorrhagic fever viruses
Tick-borne encephalitis complex viruses
Variola major virus (smallpox virus)
Viruses causing hantavirus pulmonary syndrome
Yellow fever virus
Yersinia pestis
Abrin
Conotoxins
Diacetoxyscirpenol
Ricin
Saxitoxin
Tetrodotoxin

USDA-HHS Overlap Agents

Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia (pseudomonas) mallei
Burkholderia (pseudomonas) pseudomallei
Clostridium botulinum
Coccidioides immitis
Coxiella burnetii
Eastern equine encephalitis virus
Equine morbillivirus (hendra virus)

Francisella tularensis
Rift valley fever virus
Venezuelan equine encephalitis virus
Aflatoxins
Botulinum toxins
Clostridium perfringens epsilon toxin
Shigatoxin
Staphylococcal enterotoxin
T-2 toxin

USDA High Consequence Livestock Pathogens and Toxins

African horse sickness virus
African swine fever virus
Akabane virus
Avian influenza virus (highly pathogenic)
Blue tongue virus (exotic)
Bovine spongiform encephalopathy agent
Camel pox virus
Classical swine fever virus
Cowdria ruminantium (heartwater)
Foot and mouth disease virus
Goat pox virus
Japanese encephalitis virus
Lumpy skin disease virus
Malignant catarrhal fever virus
Menangle virus
Mycoplasma capricolum/m.f 38/m.mycoides capri (contagious caprine pleuropneumonia agent)
Mycoplasma mycoides mycoides (contagious bovine pleuropneumonia agent)
Newcastle disease virus (exotic)
Nipah virus
Peste des petits ruminants virus
Rinderpest virus

Sheep pox virus

Swine vesicular disease virus

Vesicular stomatitis virus

APHIS plant pathogens

Ralstonia solanacearum race 3

Synchytrium endobioticum

Xanthomonas oryzae pv. Oryzicola

Phakopsora pachyrhizi

Peronosclerospora philippinensis

Sclerophthora rayssiae var zeae

Liberobacter africanus

Liberobacter asiaticus

Xylella fastidiosas (citrus variegated chlorosis strain)

Plum pox potyvirus

Appendix B – Summary of Biosafety Levels

From the CDC/NIH publication Biosafety in the Microbiological and Biomedical Laboratory, 4th Edition.

Biosafety Level 1 (BSL1)

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.

Biosafety Level 2 (BSL2)

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

Biosafety Level 3 (BSL3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features.

Note that few Clemson University laboratories meet the design requirements for working with BSL3 Agents.

Biosafety Level 4 (BSL4)

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

The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment.

Note that there are no Clemson University facilities suitable for working with BSL4 agents; thus, work with such agents at Clemson is prohibited.

Appendix C – Summary of Agents

Note that this is not all-inclusive; contact EHS for assistance with determining the Biosafety Level for agents not on this list. Note that working with high titers or large volumes (> 1liter) of these agents require higher levels of BSL facilities and procedures. Contact EHS for assistance.

From the CDC/NIH publication Biosafety in the Microbiological and Biomedical Laboratory, 4th Edition; more detailed summaries of these agents are available there.

BSL2 Bacterial Agents

Bacillus anthracis

Bordetella pertussis

Brucella (B. abortus, B. canis, B. melitensis, B. suis)

Burkholderia pseudomallei (Pseudomonas pseudomallei)

Campylobacter (C. jejuni/C. coli, C. fetus subsp. fetus)

Chlamydia psittaci, C. pneumoniae, C. trachomatis

Clostridium botulinum

Clostridium tetani

Corynebacterium diphtheriae

Escherichia coli (Cytotoxin-producing (VTEC/SLT) organisms)

Francisella tularensis

Helicobacter pylori

Listeria monocytogenes

Legionella pneumophila; other Legionella-like agents

Mycobacterium leprae

Mycobacterium tuberculosis, M. bovis

Neisseria gonorrhoeae

Neisseria meningitidis

Salmonella - all serotypes including typhi

Shigella spp.

Treponema pallidum

Vibronic enteritis (Vibrio cholerae, V. para-haemolyticus)

Yersinia pestis

BSL2 Fungal Agents

Blastomyces dermatitidis

Coccidioides immitis

Cryptococcus neoformans

Histoplasma capsulatum

Sporothrix schenckii

Pathogenic members of the genera Epidermophyton, Microsporum, and Trichophyton

Miscellaneous molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents are Penicillium marneffeii, Exophiala (Wangiella) dermatitidis, Fonsecaea pedrosoi, Ochroconis gallopavum, Cladophialopora bantians, and Ramichlorisium mackenzieim, Bipolaris species. Stachybotrus atra is probably not a cause of infection or toxicosis in humans when the mold or fomites containing the mold are inhaled, although ingestion of moldy grain containing the fungus has poisoned animals.

BSL2 Parasitic Agents

Plasmodium spp. (including P. cynomologi)

Trypanosoma spp (including T. cruzi.)

Leishmania spp.,

Babesia spp.

Toxoplasma spp.

Entamoeba spp

Isospora spp.

Giardia spp.

Sarcocystis spp

Cryptosporidium spp.

Schistosoma spp.

Fasciola spp.

Echinococcus granulosus

Taenia solium (cysticercus cellulosae)

Hymenolepis nana

Ascaris spp.

Strongyloides spp.

Hookworms

Enterobius spp.

BSL2 Prion Agents

Currently, all prions are considered BSL2 agents. Some specific agents:

Scrapie (sheep and goats)

Transmissible mink encephalopathy (TME)

Chronic wasting disease (CWD)

Bovine spongiform encephalopathy (BSE)

Feline spongiform encephalopathy (FSE)

Exotic ungulate encephalopathy (EUE)

Kuru

Creutzfeldt-Jakob disease (CJD)

Gerstmann-Sträussler-Scheinker syndrome (GSS)

Fatal familial insomnia (FFI)

BSL2 Rickettsial Agents

Coxiella burnetii

Rickettsia prowazekii

Rickettsia typhi (R. mooseri)

Orientia (Rickettsia) tsutsugamushi

Rickettsia rickettsii

Rickettsia conorii,

Rickettsia akari

Rickettsia australis

Rickettsia siberica

Rickettsia japonicum

BSL2 Viral Agents (other than arboviruses)

Hantaviruses – Hantaan, Puumala, Seoul, and Sin Nombre (plus others such as the El Moro Canyon virus), BSL2 facilities using BSL3 practices

Hepatitis A Virus, Hepatitis E Virus

Herpesvirus simiae (Cercopithecine herpesvirus [CHV-1], B-virus); large volumes are BSL4

Human Herpes viruses

Influenza

Poliovirus

Poxviruses (smallpox, vaccinia, yaba, tanapox, monkeypox, plus others)

Rabies Virus

Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV)

Vesicular Stomatitis Virus

BSL2 Arboviruses, Arenaviruses, and Related Zoonotic Viruses

Acado	Acara	Aguacate	Alfuy
Almpiwar	Amapari	Ananindeua	Anhanga
Anhembi	Anopheles A	Anopheles B	Apeu
Apoi	Aride	Arkonom	Aroa
Aruac	Arumowot	Aura	Avalon
Abras	Abu Hammad	Babahoyo	Bagaza
Bahig	Bakau	Baku	Bandia
Bangoran	Bangui	Banzi	Barmah Forest
Barur	Batai	Batama	Bauline
Bebaru	Belmont	Benevides	Benfica
Bertioga	Bimiti	Birao	Bluetongue
Boracela	Botambi	Boteke	Bouboui
Bujaru	Bunyamwera	Bunyip Creek	Burg El Arab
Bush bush	Bussuquara	Buttonwillow	Bwamba
Cacao	Cache Valley	Caimito	California enc
Calovo	Candiru	Cape Wrath	Capim
Caraparu	Carey Island	Catu	Chaco
Chagres	Chandipura	Changuinola	Charleville
Chenuda	Chilibre	Chobar gorge	Clo Mor
Colorado tick fever	Corriparta	Cotia	Cowbone Ridge
Csiro Village	Cuiaba	D'Aguilar	Dakar Bat
Dengue-1	Dengue-2	Dengue-3	Dengue-4
Dera Ghazi Khan	East. equine enceph.	Edge Hill	Entebbe Bat
Ep. Hem. Disease	Erve	Eubenangee	Eyach
Flanders	Fort Morgan	Frijoles	Gamboa
Gan Gan	Gomoka	Gossas	Grand Arbaud
Great Island	Guajara	Guama	Guaratuba
Guaroa	Gumbo Limbo	Hart Park	Hazara
Highlands J	Huacho	Hughes	Icoaraci
Ieri	Ilesha	Ilheus	Ingwavuma
Inkoo	Ippy	Irituia	Isfahan
Itaporanga	Itaqui	Jamestown Canyon	Japanaut
Johnson Atoll	Joinjakaka	Juan Diaz	Jugra
Jurona	Jutiapa	Kadam	Kaeng Khoi

Kaikalur	Kaisodi	Kammavanpettai	Kamese
Kannamangalam	Kao Shuan	Karimabad	Karshi
Kasba	Kemerovo	Kern Canyon	Ketapang
Keterah	Keuraliba	Keystone	Kismayo
Klamath	Kokobera	Kolongo	Koongol
KotonKan	Kowanyama	Kunjin	Kununurra
Kwatta	La Crosse	La Joya	Lagos Bat
Landjia	Langat	Lanjan	Las Maloyas
Latino	Le Dantec	Lebombo	Lednice
Lipovnik	Lokern	Lone Star	Lukuni
M'poko	Madrid	Maguari	Manawa
Main Drain	Malakal	Mahogany hammock	Manitoba
Manzanilla	Mapputta	Maprik	Marco
Marituba	Marrakai	Matariya	Matruh
Matucare	Melao	Mermet	Minatitlan
Minnal	Mirim	Mitchell River	Modoc
Moju	Mono Lake	Mont. myotis leuk	Moriche
Mosqueiro	Mossuril	Mount Elgon bat	Murutucu
Mykines	Navarro	Nepuyo	Ngaingan
Nique	Nkolbisson	Nola	Ntaya
Nugget	Nyamanini	Nyando	O'nyong-nyong
Okhotskiy	Okola	Olifantsvlei	Oriboca
Ossa	Pacora	Pacui	Pahayokee
Palyam	Parana	Pata	Pathum Thani
Patois	Phnom-Penh bat	Pichinde	Pixuna
Pongola	Ponteves	Precarious Point	Pretoria
Prospect Hill	Puchong	Punta Salinas	Punta Toro
Qalyub	Quaranfil	Restan	Rio Bravo
Rio Grande	Ross River	Royal Farm	Sabo
Saboya	Saint Floris	Sakhalin	Salehabad
San Angelo	Sandfly fever (Naples)	Sandfly fever (Sicilian)	Sandjimba
Sango	Sathuperi	Sawgrass	Sebokele
Seletar	Sembalam	Serra do Navio	Shamonda
Shark River	Shuni	Silverwater	Simbu
Simian hem. fever	Sindbis	Snowshoe Hare	Sixgun City
Sokuluk	Soldado	Sororoca	Stratford
Sunday Canyon	Tacaiuma	Tacaribe	Taggert
Tahyna	Tamiami	Tanga	Tanjong Rabok
Tataguine	Tehran	Tembe	Tembusu
Tensaw	Tete	Tett nang	Thimiri
Thottapalayam	Tibrogargan	Timbo	Timboteua
Tindholmur	Toscana	Toure	Tribec
Triniti	Trivittatus	Trubanaman	Tsuruse
Turlock	Tyuleny	Uganda S	Umatilla

Umbre	Una	Upolu	Urucuri
Usutu	Uukuniemi	Vellore	Venkatapuram
Vinces	Virgin River	VS-Indiana	VS-New Jersey
Wad Medani	Wallal	Wanowrie	Warrego
West. equine enc.	Whataroa	Witwatersrand	-Wongal
Wongorr	Wyeomyia	Yaquina Head	Yata
Yogue	Zaliv Terpeniya	Zegla	Zika
Zirqa			

Vaccine Strains Of BSL-3/4 viruses which may be handled at BSL2

Chikungunya (Vaccine strain 131/25)

Junin (Vaccine strain Candid #1)

Rift Valley fever (Vaccine strain 20MP-12)

Venezuelan Equine encephalomyelitis (Vaccine strain TC83)

Yellow Fever

BSL3 Bacterial Agents

None

BSL3 Fungal Agents

None

BSL3 Parasitic Agents

None

BSL3 Prion Agents

None

BSL3 Rickettsial Agents

None

BSL3 Viral Agents (other than arboviruses)

Hendra and Hendra-like Viruses (includes virus formerly known as Equine Morbillivirus),
BSL3 facilities, using BSL4 practices

BSL3 Arboviruses and Arenaviruses

Venezuelan equine encephalomyelitis

Rift Valley fever

Chikungunya

Yellow fever

Japanese encephalitis

Louping

West Nile
Lymphocytic choriomeningitis
Orungo
Piry
Wesselsbron
Mucambo
Oropouche
Germiston
Bhanja
Hantaan
Mayaro
Spondweni
Murray Valley encephalitis
Semliki Forest
Powassan
Dugbe
Issyk-kul
Koutango

Arboviruses and Certain Other Viruses Assigned to Biosafety Level 3 (on the basis of insufficient experience)

Adelaide River	Agua Preta	Alenquer	Almeirim
Altamira	Andasibe	Antequera	Araguari
Aransas Bay	Arbia	Arboledas	Babanki
Batken	Belem	Berrimah	Bimbo
Bobaya	Bobia	Bozo	Buenaventura
Cabassou	Cacipacore	Calchaqui	Cananeia
Caninde	Chim	Coastal	Plains
Connecticut	Corfou	Dabakala	Douglas
Enseada	Estero Real	Fomede	Forecariah
Ife	Iguape	Inhangapi	Fort Sherman
Gabek Forest	Gadgets Gully	Garba	Gordil
Gray Lodge	Gurupi	Iaco	Ibaraki
Inini	Issyk-Kul	Itaituba	Itimirim
Itupiranga	Jacareacanga	Jamanxi	Jari
Kedougou	Khasan	Kindia	Kyzylagach
Lake Clarendon	Llano Seco	Macaua	Mapuera

Mboke	Meaban	Mojui Dos Compos	Munguba
Naranjal	Nariva	Nasoule	Ndelle
New	Minto	Ngari	Ngoupe
Nodamura	Northway	Odrenisrou	Omo
Oriximina	Ouango	Oubangui	Oubi
Ourem	Palestina	Palma	Para
Paramushir	Paroo River	Perinet	Petevo
Picola	Playas	Pueblo Viejo	Purus
Radi	Razdan	Resistencia	Rochambeau
Salanga	San Juan	Santa Rosa	Santarem
Saraca	Saumarez Reef	Sedlec	Sena Madureira
Sepik	Shokwe	Slovakia	Somone
Sripur	Tai	Tamdy	Telok Forest
Termeil	Thiafora	Tilligerry	Tinaroo
Tlacotalpan	Tonate	Utinga	Xiburema
Yacaaba	Yaounde	Yoka	Yug Bogdanovac

BSL4 Bacterial Agents

None

BSL4 Fungal Agents

None

BSL4 Parasitic Agents

None

BSL4 Prion Agents

None

BSL4 Rickettsial Agents

None

BSL4 Viral Agents (other than arboviruses)

BSL4 Arboviruses, Arenaviruses and Filoviruses

Central European tick-borne encephalitis

Congo-Crimean hemorrhagic fever

Ebola

Guanarito

Junin

Kyasanur Forest disease

Lassa

Machupo

Marburg

Omsk hemorrhagic fever

Russian Spring-Summer encephalitis

Sabia

Appendix D - Biological Laboratory Inspection Checklist

Contact EHS for advise/assistance

Biosafety Laboratory Inspection Checklist (rev 3/01)

Date: _____

Bldg/Room: _____

PI/Lab Sup: _____

Initial
Personnel:
Semi-annual/quarter

Conditions	Yes	No	NA	Comments
1. Access is limited or restricted				Current posted Entry sign?
2. Dedicated/Available hand washing facility				
3. No eating, drinking, smoking and applying cosmetics				<input type="checkbox"/> No food, frig labeled properly <input type="checkbox"/> Microwave
4. No mouth pipetting				
5. Minimize creation of aerosols and splashes				<input type="checkbox"/> PPE <input type="checkbox"/> centrifuge <input type="checkbox"/> sonicator <input type="checkbox"/> waterbath <input type="checkbox"/> shaker
6. Biohazardous wastes autoclaved				<input type="checkbox"/> Autoclave log <input type="checkbox"/> Biological indicators <input type="checkbox"/> Chemical indicators
7. Insect and rodent control program				
Special Practices				
1. Access is limited or restricted, esp. for immunocompromised personnel				
2. Established entry policies and procedures				
3. Advise of potential hazards				
4. Hazard warning sign				<input type="checkbox"/> Current and Up-to-date
5. Appropriate EHS training				<input type="checkbox"/> Hepatitis B <input type="checkbox"/> Other:
6. Baseline serum samples (if appropriate)				
7. Biosafety manual				<input type="checkbox"/> Biological MSDS <input type="checkbox"/> SOP's
8. Appropriate EHS training				
9. Sharps precautions				
10. Leakage prevention				
11. Appropriate disinfectant				<input type="checkbox"/> Chemical MSDS
12. Spill and accident exposure control plan				<input type="checkbox"/> Exposure Control Plan reviewed within last year
13. No animals allowed				

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9. Sharps precautions				
10. Leakage prevention				
11. Appropriate disinfectant				<input type="checkbox"/> Chemical MSDS
12. Spill and accident exposure control plan				<input type="checkbox"/> Exposure Control Plan reviewed within last year
13. No animals allowed				

Additional Documentation	Yes	No	NA	Comments
1. Diagram of Laboratory				
2. Lab specific Procedural/Safety Manual				
3. Exposure Control Plan				
4. Waste Management Plan				<input type="checkbox"/> Log on Autoclave
5. Contingency Plan				
6. Signature sign-off on conditions				

Notes: