

are not listed above, consult the CDC web page at: <http://www.cdc.gov>. See Appendix for more information on BSL levels.

An additional OSHA Safety and Health Topics page on Pandemic Influenza has been added in response to the 2009 H1N1 influenza pandemic. It can be accessed at: [www.osha.gov/dsg/topics/pandemicflu/index.html](http://www.osha.gov/dsg/topics/pandemicflu/index.html)

#### **4.3 Pathogen Safety Data Sheets (PSDSs) on Infectious Agents**

Although SDSs for chemical products have been available to workers for many years in the U.S. and other countries, Canada is the only country that has developed SDSs for infectious agents. These PSDSs were produced by the Canadian Public Health Agency for personnel working in the life sciences as quick safety reference material relating to infectious microorganisms. These PSDSs on Infectious Agents are organized to contain health hazard information such as infectious dose, viability (including decontamination), medical information, laboratory hazard, recommended precautions, handling information and spill procedures. These PSDSs are available at: <http://www.phac-aspc.gc.ca/msds-ftss>.

#### **4.4 Bloodborne Pathogens**

The OSHA Bloodborne Pathogens (BBP) standard (29 CFR 1910.1030) is designed to protect workers from the health hazards of exposure to bloodborne pathogens. Wright State is subject to the BBP standard since some workers jobs put them at reasonable risk of coming into contact with blood or other potentially infectious materials (OPIM).

Wright State has a written Exposure Control Plan, provides training to exposed workers, and complies with other requirements of the standard, including use of Standard Precautions when dealing with blood and OPIM. In 2001, in response to the Needlestick Safety and Prevention Act, OSHA revised the Bloodborne Pathogens standard. The revised standard clarifies the need for employers to select safer needle devices and to involve workers in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps.

OSHA estimates that 5.6 million workers in the healthcare industry and related occupations are at risk of occupational exposure to bloodborne pathogens, including HIV, HBV, HCV, and others.

All occupational exposure to blood or OPIM places workers at risk for infection with bloodborne pathogens.

OSHA defines blood to mean human blood, human blood components, and products made from human blood.

OPIM means:

- The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;
- Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and
- HIV- or HBV-containing cell or tissue cultures, organ cultures, and HIV or HBV-containing culture medium or other solutions; and
- blood, organs, or other tissues from experimental animals infected with HIV or HBV.

The Centers for Disease Control and Prevention (CDC) notes that although more than 200 different diseases can be transmitted from exposure to blood, the most serious infections are hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Fortunately, the risk of acquiring any of these infections is low. HBV is the most infectious virus of the three viruses listed above. For an unvaccinated healthcare worker, the risk of developing an infection from a single needlestick or a cut exposed to HBV-infected blood ranges from 6-30%. The risk for infection from HCV- and HIV-infected blood under the same circumstances is 1.8 and 0.3 percent, respectively. This means that after a needlestick/cut exposure to HCV-contaminated blood, 98.2% of individuals do not become infected, while after a similar exposure to HIV-contaminated blood, 99.7% of individuals do not become infected.

([http://www.cdc.gov/OralHealth/infectioncontrol/faq/bloodborne\\_exposures.htm](http://www.cdc.gov/OralHealth/infectioncontrol/faq/bloodborne_exposures.htm)).

Many factors influence the risk of becoming infected after a needlestick or cut exposure to HBV-, HCV- or HIV-contaminated blood. These factors include the health status of the individual, the volume of the blood exchanged, the concentration of the virus in the blood, the extent of the cut or the depth of penetration of the needlestick, etc.

#### **4.4.1 Required Training and Practices**

Workers are trained and prohibited from engaging in the following activities:

- Mouth pipetting/suctioning of blood or OPIM, 29 CFR 1910.1030(d)(2)(xii);
- Eating, drinking, smoking, applying cosmetics or lip balm, or handling contact lenses in work areas where there is a reasonable likelihood of occupational exposure to blood or OPIM, 29 CFR 1910.1030(d)(2)(ix); and
- Storage of food or drink in refrigerators, freezers, shelves, cabinets or on countertops or benchtops where blood or OPIM are present, 29 CFR 1910.1030(d)(2)(x).

#### 4.4.2 Employer Responsibilities

Wright State as the employers must ensure that the following are provided:

- Appropriate PPE for workers if blood or OPIM exposure is anticipated, 29 CFR 1910.1030(d)(3); The type and amount of PPE depends on the anticipated exposure.
- Gloves must be worn when hand contact with blood, mucous membranes, OPIM, or non-intact skin is anticipated, or when handling contaminated items or surfaces, 29 CFR 1910.1030(d)(3)(ix).
- Surgical caps or hoods and/or shoe covers or boots must be worn in instances when gross contamination can reasonably be anticipated such as during autopsies or orthopedic surgery, 29 CFR 1910.1030(d) (3)(xii).
- Effective engineering and work practice controls to help remove or isolate exposures to blood and bloodborne pathogens, 29 CFR 1910.1030(d)(2)(i), CPL 02-02-069 (CPL 2-2.69); and
- Hepatitis B vaccination (if not declined by a worker) under the supervision of a physician or other licensed healthcare professional to all workers who have occupational exposure to blood or OPIM, 29 CFR 1910.1030(f)(1)(ii)(A)-(C).

#### 4.4.3 Labels

When any blood, OPIM or infected animals are present in the work area, a hazard warning sign (see graphic) incorporating the universal biohazard symbol, 29 CFR 1910.1030(g)(1)(ii)(A), must be posted on all access doors, 29 CFR 1910.1030(e) (2)(ii)(D).



**Figure 2: Biohazard Symbol**

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#### 4.4.4 HIV/HBV Laboratories

Engineering Controls and Work Practices for All HIV/HBV Laboratories

Employers must ensure that:

- All activities involving OPIM are conducted in Biological Safety Cabinets (BSCs) or other physical-containment devices; work with OPIM must not be conducted on the open bench, 29 CFR 1910.1030(e)(2)(ii)(E);
- Certified BSCs or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, be used for all activities with OPIM that pose a threat of exposure to droplets, splashes, spills, or aerosols, 29 CFR 1910.1030(e)(2)(iii)(A);
- Each laboratory contains a facility for hand washing and an eyewash facility which is readily available within the work area, 29 CFR 1910.1030(e)(3)(i); and
- Each work area contains a sink for washing hands and a readily available eyewash facility. The sink must be foot, elbow, or automatically operated and must be located near the exit door of the work area, 29 CFR 1910.1030(e)(4)(iii).

#### 4.4.5 HIV and HBV Research Laboratories

Additional BBP Standard Requirements Apply to HIV and HBV Research Laboratories

Requirements include:

##### 4.4.5.1 Waste materials:

- All regulated waste must either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens, 29 CFR 1910.1030(e)(2)(i); and
- Contaminated materials that are to be decontaminated at a site away from the work area must be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area, 29 CFR 1910.1030(e)(2)(ii)(B).

##### 4.4.5.2 Access

- Laboratory doors must be kept closed when work involving HIV or HBV is in progress, 29 CFR 1910.1030(e)(2)(ii)(A);
- Access to the production facilities' work area must be limited to authorized persons. Written policies and procedures must be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit

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procedures must be allowed to enter the work areas and animal rooms, 29 CFR 1910.1030(e)(2)(ii)(C);

- Access doors to the production facilities' work area or containment module must be selfclosing, 29 CFR 1910.1030(e)(4)(iv);
- Work areas must be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors must be the basic requirement for entry into the work area from access corridors or other contiguous areas. Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the work area, 29 CFR 1910.1030(e)(4)(i); and
- The surfaces of doors, walls, floors and ceilings in the work area must be water-resistant so that they can be easily cleaned. Penetrations in these surfaces must be sealed or capable of being sealed to facilitate decontamination, 29 CFR 1910.1030(e)(4)(ii).

(These requirements **do not apply** to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissue, or organs, 29 CFR 1910.1030(e)(1).)

#### **4.5 General Precautions For Biological Work**

If you are or will be at risk of infection from an agent for which there is a vaccine, e.g., hepatitis, you should consult a supervisor or the Environmental Health and Safety Department about immunization.

Inform Environmental Health and Safety of receipt of any biohazardous agent or materials containing such agents, include information on the storage location and handling and use precautions, and emergency procedures. Use a biohazard warning symbol to designate the storage location of human blood, blood products and any pathogenic agents. If work is conducted at a Biosafety Level 2 (BL2) or above, a warning sign identifying the agent, emergency contact person, and any special precautions must be posted on the laboratory door as well. (See the appendix at the end of this section for a summary of biosafety levels and a description fo BL2 and BL3 criteria.)



**Figure 3: Biohazard Symbol**

#### **4.6 Standard Microbiological Practices**

Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

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*Do not eat, drink, store food, apply cosmetics or smoke in the laboratory.*

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Never mouth pipet, it is unsafe. Numerous types of pipetting aids are available.

Wear disposable high-cuffed disposable gloves, but note that latex gloves are permeable to organic solvents, including ethanol. Given that thin gloves offer little protection against cuts, bites, and self-inoculation, wear the thickest gloves the dexterity required by your work permits; wearing two pairs of thinner gloves allows for safe removal of contaminated outer gloves. See **GENERAL SAFETY PRACTICES** for more information on selection and use of gloves.

Wear lab coats. The primary reason for wearing a lab coat is to protect yourself from contact with hazardous materials. However, the liberation of microorganisms from human skin plays an important role in transmission of airborne infection to humans or experimental materials, wearing a lab coat can help minimize this transmission. To prevent clothing from acting as a bellows, the front of the lab coat should be closed and sleeves should be tucked inside gloves or taped at the wrists. Disposable, Tyvec™ lab coats are also available and recommended for work in biological safety cabinets.

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**Do Not** wear lab coats outside the laboratory environment.

In general, a Class II biological safety cabinet should be used for work with biohazards.

Aerosol generating procedures should be performed in an appropriate enclosure, e.g., the rear third of a biosafety cabinet, (see “Aerosol-Generating Processes” later in this manual). Remember that even a drop falling onto a hard surface can generate an aerosol. Using fluorescein and black light, one can test for aerosol escape.

Avoid the use of needles, scalpels, and other sharp implements. 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, leak resistant container. Do not resheath or remove used needles; insert the whole assembly into the container. These containers must be tightly closed to prevent loss of contents, must be labeled “**SHARPS**” and be marked with the international biohazard symbol. All punctures should be washed with soap and water and reported to a supervisor or Environmental Health and Safety.

If experimentation requires the use of pathogens, first develop and test all procedures using non-pathogenic agents.

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 solution at a strength of at least fifteen percent.

All waste materials must be accumulated in red or other colored plastic bags labeled with the international biohazard symbol.

Discard non-sharp disposable materials, e.g., gloves, pipets, pipet tips, plastic tubes, that come in contact with blood or potentially infectious materials in red or other colored plastic bags. Treat blood and other potentially infectious fluids with a 15% chlorine bleach solution and decant down the drain. Do not dispose of blood or sharps with the normal laboratory trash.

#### **4.7 Guidelines For Specific Subjects Of Study**

##### **4.7.1 Experiments Using Blood, Blood Products or Human Secretions**

Persons who work with blood or blood products are at increased risk of hepatitis in proportion to the degree of their exposure. Hepatitis B vaccination is recommended for all individuals working with blood or blood products. The most important way for personnel handling blood products to protect themselves from hepatitis B infection (as well as from other blood-borne infections) is to follow the general precautions outlined above. Handle all blood, blood products and human secretions as if infective (see Wright State University’s **EXPOSURE CONTROL PLAN, BLOODBORNE PATHOGENS**).

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If an exposure to blood or blood products occurs, report immediately to the Environmental Health and Safety Department for evaluation and possible treatment with hepatitis immune globulin, which, if administered soon after exposure, may prevent acquisition of hepatitis.

#### 4.7.2 Recombinant DNA Experiments

The vast majority of laboratory experiments are exempt from the NIH guidelines if the recombinant DNA molecules:

- are not in organisms or viruses
- consist entirely of DNA segments from a single nonchromosomal or viral DNA source
- consist entirely of DNA from a prokaryotic or eukaryotic host
- consist entirely of plasmids (excluding viruses) when propagated in that host (or a closely related strain of the same species)
- were transferred to another host by well-established physiological means (prokaryotic DNA only)
- contains less than one-half of any eukaryotic genome that is propagated and maintained in cells in tissue culture
- use *Escherichia coli* K-12 host-vector systems (some exceptions apply)
- use *Saccharomyces cerevisiae* host-vector systems (some exceptions apply)
- use any asporogenic *Bacillus subtilis* strain that does not revert to a sporeformer with a frequency greater than  $10^{-7}$  (some exceptions apply)
- derived entirely from extrachromosomal elements of certain organisms.

#### 4.7.3 Work With Potentially Infectious Agents

In “*The Transforming Principle*”<sup>4</sup>, Dr. McCarty, who along with MacLeod and Avery discovered that DNA was the genetic material, describes the standards set by Professor Avery: “He would then review the protocol...in this manner I was introduced to Avery’s extraordinarily rigorous bacteriological technique...he...had agreed that they would treat all bacterial cultures as though they contained the plague bacillus...it was a common failing to become sloppy in handling nonpathogenic organisms which in turn led to some relaxation of acceptable techniques when dealing with more infectious agents.” Although the advent of the biological safety cabinet has obviated the need for a flame, in fact made its use undesirable, the rigors of Avery’s protocol review and careful approach are still highly recommended even when handling “normal” cell lines, some of which may actually present risks to investigators as well as compromise their experiments. For most biohazardous agents the routes of potential infection are inoculation, ingestion and inhalation. The general laboratory procedures detailed in **GENERAL SAFETY PRACTICES** should be used to reduce exposure to biological agents. This includes wearing gloves, a lab coat and safety glasses if the organism is able to infect the eye, using a biological safety cabinet if



appropriate as outlined below, and decontaminating all biological wastes before disposal. Decontamination of wastes can be accomplished for all these agents by autoclaving, which may require 60 minutes for a full load, or 30 minute exposure to fresh 15% chlorine bleach. Note that the color change associated with oxidation of media is not a good indicator of inactivation.

The Biosafety Levels (BL) cited below represent a set of standards, special practices, containment equipment, etc., assigned by the CDC (Table 6: CDC Summary of Recommended Biosafety Levels for Infectious Agents). The BL number increases with increasing hazard. Often work at a higher BL is recommended when large volumes, highly concentrated stocks, or aerosol generating procedures are employed. All human specimens should be regarded and handled as infective. The risk from human specimens is not restricted to hepatitis or AIDS but includes many other agents, including some of those listed below, which may be found in blood, blood products, urine, feces, amniotic fluid, etc. Researchers frequently receive blood which has been designated “not for transfusion” and other fresh specimens which have not been screened for these agents. Research with human specimens is BL 2/3 (see the appendix at the end of this section for the definition of BL conditions), and the procedures outlined for these levels should be followed. Personnel who will be exposed to blood or blood products should first be immunized with the Hepatitis B vaccine.

**Table 6: CDC Summary of Recommended Biosafety Levels for Infectious Agents**

Biosafety Level	Agent Characteristics	Practices	Safety Equipment	Facilities (secondary barriers)
BSL-1	Not known to consistently cause disease in health adults	Standard microbiological practices	None	Open bench top sink
BSL-2	Associated with human disease, hazard from percutaneous injury, ingestion, mucous membrane exposure	Standard microbiological practices Limited access Biohazard warning signs Sharps precautions Biosafety manual defining any needed waste	Class I or II biosafety cabinets (BSCs) or other containment devices used for all agents that cause splashes or aerosols of infectious materials	Open bench top sink Autoclave

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		decontamination or medical surveillance policies.	Laboratory coats and gloves  Face protection as needed	
BSL-2	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	All BSL-2 practices  Controlled access  Decontamination of all waste  Decontamination of laboratory clothing before laundering  Baseline serum	Class I or II BSCs or other physical containment devices used for all open manipulations of agents  Protective lab clothing and gloves  Respiratory protection as needed	Open bench top sink  Autoclave  Physical separation from access corridors  Self-closing, double-door access  Exhaust air not recirculated  Negative airflow in laboratory
BSL-4	Dangerous/exotic agents which pose high risk of life-threatening disease; aerosol-transmitted lab infections; or related agents with unknown risk of transmission	All BSL-3 practices  Clothing change before entering  Shower on exit  All material decontaminated on exit from facility	All procedures conducted in Class III BSCs, or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus:  Separate building or isolated zone  Dedicated supply and exhaust, vacuum, and decontamination systems  Other requirements outline the text

#### 4.7.4 Cell Lines

While data on specific cell lines have been omitted, it is important to recognize that there is no “normal” cell line; many reputedly “normal” lines harbor viruses

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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.

#### **4.7.5 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 or mouth or of 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.

#### **4.7.6 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 should be worn when handling bacteria which infect the conjunctiva, e.g., *N. gonorrhoea*. Work at a higher biosafety level is recommended when large volumes, highly concentrated stocks, or aerosol generating procedures are employed with infectious bacteria. All wastes must be decontaminated prior to disposal; chlorine bleach treatment is effective.

#### **4.7.7 Parasites**

Infective 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 exposure should be reported to a supervisor and Environmental Health and Safety 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. BL2 containment and procedures are recommended for work with all parasites except *Babesia*.

#### 4.7.8 Fungi

Fungi are in general 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 the well-know aflatoxin B. The more common hazardous fungi used in laboratories include: *Blastomyces dermatitides*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Sporothrix schenckii*. All of these agents should be handled at BL2 levels.

A classification of microorganisms according to hazards is presented in Appendix B of the Guidelines for Recombinant DNA Research. Note that agents of class 1-4 should be handled according to biosafety containment levels 1-4 and that there are restrictions against importation of class 5 agents. For annotation of this list see recent National Research Council publications.<sup>5</sup> Additional information can be quickly retrieved from the report of the American Public Health Association.<sup>6</sup>

#### 4.8 Research Animals

All vertebrate animal experimentation requires the approval of the Institutional Animal Care and Use Committee (IACUC). 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 which 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.

All procedures on animals should be performed by properly trained personnel. By using safe work practices and appropriate PPE, 29 CFR 1910.132(a), workers can minimize the likelihood that they will be bitten, scratched, and/or exposed to animal body fluids and tissues.

##### 4.8.1 Possible Injuries/Illnesses

The most common work-related health complaints reported by individuals working with small animals

are the following:

- Sprains;
- Strains;
- Bites; and
- Allergies.

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Of these injuries, allergies (i.e., exaggerated reactions by the body's immune system) to proteins in small animals' urine, saliva, and dander are the greatest potential health risk. An allergic response may evolve into life-long asthma. Because mice and rats are the animals most frequently used in research studies, there are more reports of allergies to rodents than other laboratory animals. Most workers who develop allergies to laboratory animals will do so within the first twelve months of working with them. Sometimes reactions only occur in workers after they have been handling animals for several years. Initially, the symptoms are present within minutes of the worker's exposure to the animals.

Approximately half of allergic workers will have their initial symptoms subside and then recur three or four hours following the exposure.

#### **4.8.1.1 Prevention Practices**

Employers should adopt the following best practices to reduce allergic responses of workers:

- Eliminate or minimize exposure to the proteins found in animal urine, saliva and dander.
- Limit the chances that workers will inhale or have skin contact with animal proteins by using well-designed air handling and waste management systems.
- Have workers use appropriate PPE (e.g., gloves, gowns, hair covers, respirators) to further minimize their risk of exposure.

#### **4.8.2 Requirements**

- Protocols involving the acute and/or chronic use of hazardous chemicals, radioisotopes, or biohazards in animals must be reviewed with the Laboratory Animal Utilization Committee and Environmental Health and Safety prior to initiation.
- Anyone planning to work with live vertebrates must receive documented training in their handling. Proper handling and restraint techniques reduce the chances for bites and scratches, and training is required by law.
- Dosed animals may be transported to or from an animal facility, containment area, or laboratory only in a cage with a cover.
- Experimental materials and specimens must be transported in closed containers inside unbreakable canisters.

#### **4.8.3 Additional Information**

##### **4.8.3.1 Allergic responses**

- Allergic responses to laboratory animals are the most common cause of human disease related to the use of animals in research. Allergies result from the direct or indirect exposure to allergens such as skin contact or inhalation of fur, dander, saliva, urine, serum, etc. Symptoms can vary

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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 can be life-threatening.

#### **4.8.3.2 Immunizations**

- All users of laboratory animals should have an active tetanus immunization and others as appropriate, e.g., rabies.

#### **4.8.3.3 Bites or scratches**

- Bites or scratches that break the skin should be washed thoroughly with soap and water and be reported to a supervisor and Environmental Health and Safety.

#### **4.8.3.4 Personal protective equipment**

- Wearing a face mask, gloves and a lab coat is strongly encouraged for users of animals to reduce aerosol, direct contact, or inadvertent oral and nasal contact with contaminated hands.
- A full-face respirator is recommended for those at high risk.
- Lab coats should be changed and hands thoroughly washed if an animal, its fluids, or feces is touched. **Do Not** wear lab coats outside the lab environment.

#### **4.8.3.5 Pregnant employees**

- 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.

#### **4.8.4 Zoonotic Diseases**

A host of possible infectious agents can be transferred from animals to humans. These infections are referred to as zoonotic diseases. The common routes of exposure to infectious agents are inhalation, inoculation, ingestion and contamination of skin and mucous membranes. Inhalation hazards may arise during work practices that can generate aerosols. These include the following: centrifugation, mixing (e.g., blending, vortexing, and sonication), pouring/decanting and spilling/splashing of culture fluids. Inoculation hazards include needlesticks and lacerations from sharp objects. Ingestion hazards include the following: splashes to the mouth, placing contaminated articles/fingers in mouth, consumption of food in the laboratory, and mouth pipetting. Contamination of skin and mucous membranes can occur via splashes

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or contact with contaminated fomites (e.g., towels, bedclothes, cups, money). Some of the zoonotic diseases that can be acquired from animals are listed below.

#### **4.8.4.1 Zoonotic Diseases – Wild and Domesticated Animals**

Wild rodents and other wild animals may inflict an injury such as a bite or scratch. Workers need to receive training on the correct way to capture and handle any wild animals. While they may carry or shed organisms that may be potentially infectious to humans, the primary health risk to individuals working with captured animals is the development of an allergy. The development of disease in the human host often requires a preexisting state that compromises the immune system. Workers who have an immune compromising medical condition or who are taking medications that impair the immune system (steroids, immunosuppressive drugs, or chemotherapy) are at higher risk for contracting a rodent disease.

Wild rodents may act as carriers for viruses such as Hantavirus and lymphocytic choriomeningitis virus (LCMV) depending on where they were captured. Additionally, each rodent species may harbor their own range of bacterial diseases, such as tularemia and plague. These animals may also have biting insect vectors that can act as a potential carrier of disease (mouse to human transmission).

##### **4.8.4.1.1 Dogs and Cats**

Dogs and Cats: Bite wound infections, cat scratch disease, toxoplasmosis, visceral larval migrans and sarcoptic mange from dogs and fungus such as ringworm from cats are common.

##### **4.8.4.1.2 Rodents**

Rodents: Precautions should be taken against toxoplasmosis, lymphocytic choriomeningitis, *Salmonella*, *Shigella* and ringworm. Toxoplasmosis is one of the most commonly acquired parasitic diseases in the laboratory.

##### **4.8.4.1.3 Rabbits, Sheep, Swine, and Birds**

Rabbits, Sheep, Swine, and Birds can be the source of tularemia, Q fever, *Erysipelas* and *Chlamydia* (psithcosis), respectively.

Examples of zoonotic diseases that can be transmitted from wild and domesticated animals to humans are listed in the following table. ( Table 7: Most common zoonotic diseases in workers)

**Table 7: Most common zoonotic diseases in workers**

Disease	Disease Agent	Cats	Dogs	Birds	Farm Animals	Wild Animals
Brucellosis	<i>Brucella canis</i>		X			
Campylobacteriosis	<i>Campylobacter jejuni</i>	X	X		X	
Cat Scratch Fever	<i>Bartonella henselae</i>	X				
Cryptococcosis	<i>Cryptococcus neoformans</i> and other species			X		
Hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)	<i>Hantavirus</i>					X
Lymphocytic choriomeningitis	<i>Lymphocytic choriomeningitis virus (LCMV)</i>			X		
<i>Pasteurella</i> pneumonia	<i>Pasteurella haemolytica</i>				X	
Histoplasmosis	<i>Histoplasma capsulatum</i>			X		
Orf	Poxvirus				X	
Plague	<i>Yersina pestis</i>					X
Q-fever	<i>Coxiella burnetti</i>				X	
Rabies	Rabies virus	X				
Salmonellosis	<i>Salmonella enterica</i> serovar Typhi				X	
Toxoplasmosis	<i>Toxoplasma</i>	X				

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	<i>gondii</i>					
Tularemia	<i>Tularemia francisella</i>					X

**4.8.4.2 Zoonotic Diseases – Non-human Primates**

It should not be surprising that, given our many similarities, humans and non-human primates are susceptible to similar infectious agents. Because of our differences, the consequences of infection with the same agent often vary considerably. Infection may cause few if any symptoms in one group and may be lethal to the other. Exposures to body fluids from non-human primates should be treated immediately.

In 2003, a report entitled, Occupational Safety and Health in the Care and Use of Non-Human Primates (see References) was published. This report covers topics relevant to facilities in which non-human primates are housed or where non-human primate blood or tissues are handled. The report describes the hazards associated with work involving nonhuman primates and discusses the components of a successful occupational health and safety program, including hazard identification, risk assessment and management, institutional management of workers after a suspected occupational exposure, applicable safety regulations, and personnel training.

Diseases such as tuberculosis, shigella, campylobacter and salmonella can be a serious threat. Herpesvirus B carried by rhesus, cynomolgus, and other old world monkeys can cause fatal encephalitis in man.

**4.8.4.3 Prevention practices**

Laboratory workers need training to adhere to the following good practices to prevent exposure to zoonotic diseases when working with research animals:

- Avoid use of sharps whenever possible. Take extreme care when using a needle and syringe to inject research animals or when using sharps during necropsy procedures. Never remove, recap, bend, break, or clip used needles from disposable syringes. Use safety engineered needles when practical.
- Take extra precautions when handling hooved animals. Due to the physical hazards of weight and strength of the animal, large hooved mammals pose additional concerns for workers. Hooved mammals may resist handling and may require multiple workers to administer medication or perform other functions.
- Keep hands away from mouth, nose and eyes.
- Wear appropriate PPE (i.e., gloves, gowns, face protection) in all areas within the animal facility.

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- A safety specialist may recommend additional precautions, based upon a risk assessment of the work performed.
- Wear tear-resistant gloves to prevent exposure by animal bites. Micro-tears in the gloves may compromise the protection they offer.
- Remove gloves and wash hands after handling animals or tissues derived from them and before
  - leaving areas where animals are kept.
  - Use mechanical pipetting devices (no mouth pipetting).
  - Never eat, drink, smoke, handle contact lenses, apply cosmetics, or take or apply medicine in
  - areas where research animals are kept.
- Perform procedures carefully to reduce the possibility of creating splashes or aerosols.
- Contain operations that generate hazardous aerosols in BSCs or other ventilated enclosures,
  - such as animal bedding dump stations.
- Wear eye protection.
- Wear head/hair covering to protect against sprays or splashes of potentially infectious fluids.
- Keep doors closed to rooms where research animals are kept.
- Clean all spills immediately.
- Report all incidents and equipment malfunctions to the supervisor.
- Promptly decontaminate work surfaces when procedures are completed and after surfaces are soiled by spills of animal material or waste.
- Properly dispose of animal waste and bedding.
- Workers should report all work-related injuries and illnesses to their supervisor immediately.
- Following a bite by an animal or other injury in which the wound may be contaminated, first aid should be initiated at the work site.
  - Contaminated skin and wounds should be washed thoroughly with soap and water for 15 minutes.
  - Contaminated eyes and mucous membranes should be irrigated for 15 minutes using normal saline or water.
- Consult an occupational health physician concerning wound care standard operating procedures (SOPs) for particular animal bites/scratches.

#### **4.8.5 Anesthetic Agents**

The choice of anesthesia should be made with care and after consultation with the animal facility staff. Neither diethyl ether nor chloroform should be used routinely for anesthesia or euthanasia of laboratory animals. Chloroform is a potent hepatotoxin and a suspected human carcinogen. The introduction of ether to cold rooms, refrigerators and freezers and to an incinerator via animal carcasses presents very real hazards due to its explosive characteristics.

Volatile chemicals for anesthesia or euthanasia should be used only in the presence of adequate ventilation, i.e., a fume hood or closed system with scavenger designed for this purpose. This requirement is especially noteworthy when working with halothane derivatives since they have been shown to have very adverse effects on some individuals. If you must use inhalants for anesthesia or euthanasia, be advised that enflurane or isoflurane are less toxic to humans than other halothanes, including methoxyflurane, and they provide excellent control of narcosis. However, frequency of exposure is critical following sensitization; the idiosyncratic response of individuals is difficult to predict and can be fatal.

##### **4.8.5.1 Recommendations**

- Ether and chloroform should not be used for anesthesia because of flammability of the former and toxicity and carcinogenicity of the latter.
- Methoxyflurane is the recommended agent for most brief, bench-top surgeries on rodents. Its low vapor pressure and high lipid solubility permit safe induction in a closed jar and intermittent application of a nose cone for maintenance. Careful attention to safe work practices is required to control exposure of the investigators.
- Isoflurane rather than halothane is recommended for anesthesia when delivered with a precision vaporizer. Isoflurane has an excellent margin of patient safety as well as minimal adverse side effects and occupational health risks.
- While some injectable drug combinations may be appropriate for specific physiological studies involving extended bench-top surgical procedures, avoidance of injectables and the associated risk of needlesticks is recommended.
- Nitrous oxide use with volatile anesthetics should be avoided since it is not essential for animal surgery and is toxic to humans.

#### **4.8.6 Perfusion**

Perfusion of animals should be conducted in a fume hood over a waste collection table/vessel. The waste should be handled as hazardous chemical waste.

## 4.9 Processes And Equipment

### 4.9.1 Aerosol-Generating Processes

Aerosols (dispersions of particles in air) can result from the use of blenders, mixers, sonicators, cell disrupters, centrifuges, syringes, pipets, aspirators, test and centrifuge tube caps. (The hazards associated with the use of centrifuges is discussed under precautions for Aborter equipment and devices in **GENERAL SAFETY PRACTICES**). 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.

The containment of aerosols and aerosol-generating processes is of prime importance. The hazard of an aerosol depends upon the concentration of the material in the suspension, the amount of energy imparted by the equipment creating the aerosol, the degree to which the suspending medium is protective of the material, the degree of danger associated with the material itself, and the susceptibility of the individual to danger from the agent.

Particle size is a factor in determining the path the 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, etc. 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.

#### 4.9.1.1 Aerosol minimization

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:

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

A summary from the National Cancer Institute<sup>7</sup> appears in the following table (Table 8: Summary of Aerosol-Generating Processes).

**Table 8: Summary of Aerosol-Generating Processes**

Potential Aerosol Creating Operation	Measures to Decrease Hazards from Aerosols
<b>Forced expulsion of the last drop of liquid by alternatiely sucking and blowing with the pipet, creating splashes and aerosols.</b>	Use gravity flow of liquid with pipet calibrated for mark-to-mark drain-to-tip delivery and with pipet tip in contact with container wall.
<b>Removing the cap or stopper from bottle after vigorous shaking to mix, wash or resuspend material.</b>	Use swirling motion rather than shaking, allow aerosol to settle for a few minutes after bubbles disappear before removing cap or stopper.
<b>Blending materials to disrupt cells, release enzymes or viruses, to homogenize suspensions, etc. without aerosol-tight cover seals or leak-proof rotor bearings.</b>	Use special safety containers with seals to prevent escape of aerosols; use drain/sipon system to remove contents without removing cover.
<b>Sonic disruption of cells or organells</b>	Use cup or chamber that is aerosol tight; allow aerosol to settle befoe opening cup. Place sonicator in fume hood or laminar flow cabinet.
<b>Grinding tissue with mortar and pestle, glass tissue grinder, or ball mill.</b>	Use slow spreads, use a clear plastic or inflatable glove bag to further contain the operation within the safety cabinet; allow aerosol to settle before removing cover.
<b>Pouring hazardous materials from one container to another; e.g., decanting supernatents.</b>	Use transfer pipets or closed siphon or vacuum technique.
<b>Sterilizing a wire loop or needle in a flame, creating splatter.</b>	Gradually dry loop or needle near flame, or use specially designed incinerator for loops or pipets.
<b>Withdrawing a syringe needle as from a vaccine bottle or following inoculation of</b>	Use sterile cotton gauze to enclose needle; if experiment permits, use disinfectant with

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<b>experimental animals</b>	cotton or gauze.
<b>Weighing dry hazardous materials</b>	Use draft-free, low-humidity enclosure for balance; discharge static electricity; use tared weighing containers not open weighing dishes or pipets.
<b>Opening a freeze-dried preparation</b>	If material is in an ampule, nick the ampule 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.
<b>Removing plugs from flasks and tubes.</b>	Avoid wetting plug; remove plug slowly.
<b>Handling cages that held infected animals or large animals in open areas or unventilated cages.</b>	Avoid disturbing cage contents; if animals are held in open areas; use liquid disinfectants during cage cleaning; keep area clean; use personal respirator.

#### 4.9.2 Biological Safety Cabinets

Biological safety cabinets (BSCs) are the primary means of containment for working safely with infectious microorganisms. BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed.

Biological safety cabinets are divided into three classes based upon the type of protection provided. The selection of a BSC is determined through a risk assessment.

**Table 9: Selection of a Safety Cabinet through Risk Assessment**

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
<b>BSL 1 - 3</b>	Yes	No	Yes	I
<b>BSL 1 - 3</b>	Yes	Yes	Yes	II (A1, A2, B1, B2)

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<b>BSL - 4</b>	Yes	Yes	Yes	III; II - When used in suit room with suit
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Source: BMBL Appendix A

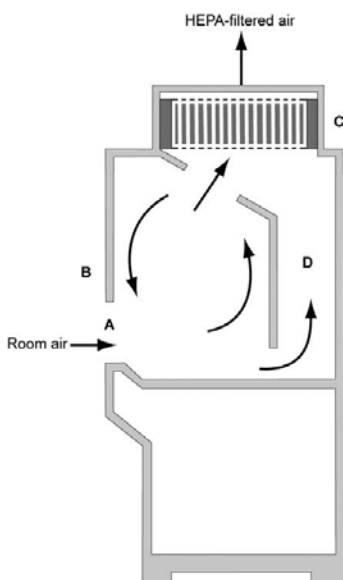
Class I and II cabinets use an air curtain and Class III uses a physical barrier to protect the investigator. Class II and III cabinets filter the air before it is blown onto the work surface, and all three cabinets have filtered exhaust. HEPA (high efficiency particulate air) filters are used since they are efficient in removing at least 99.97% of particles 0.3 microns in diameter. Because of the mechanics of particle filtration, particles of larger and smaller sizes are removed by HEPA filters with even greater efficiency and the efficiency increases slightly as the filter medium becomes loaded with contaminants. As the filter becomes loaded, the resistance to air movement through the filter increases, with the result that the rate of airflow now will decrease. Therefore, airflows must be adjusted periodically to assure proper performance. Also, these cabinets are subject to the same requirements with regard to location as fume hoods are (see **GENERAL SAFETY PRACTICES**). Annual certification of performance is required for these cabinets. Proper maintenance of cabinets used for work at all biosafety levels cannot be over emphasized since they are a primary containment device.

HEPA filters do not remove gaseous contaminants; instead, wet collectors or adsorptive systems are required, e.g., TEDA impregnated charcoal for radioiodine. The performance characteristics of these filters are not as well-defined as those of particulate filters, since their performance can be affected by ambient temperature, relative humidity, chemical concentration, flow rate, dwell time, chemical composition of the filtered air, and available capacity of the filter.

#### 4.9.2.1 Class I Biological Safety Cabinet

The Class I BSC provides personnel and environment protection, but no product protection. The Class I cabinet is the simplest form of biological safety cabinet and consists of an enclosure with a front view panel and a full-width work opening. Room air, drawn into the cabinet through the work opening and into the back wall baffle, prevents airborne contaminants inside the cabinet from escaping into the room, as in a fume hood. Unlike a fume hood, however, the exhaust is HEPA-filtered before entering the duct. Minimum face velocity for a Class I cabinet is 75 ft/min. Since unfiltered room air is drawn across the work area, the Class I cabinet does not protect experimental materials from ubiquitous airborne contamination.

Class I BSCs are used specifically to enclose equipment (e.g., centrifuges), or procedures with potential to generate aerosols (e.g., cage dumping, culture aeration or tissue homogenation).



**Figure 4: Class I BSC**

Optional modes of operation include a front closure panel with access ports which can be placed over the work opening thus reducing the amount of open area and raising the face velocity. Another option is to attach arm-length gloves to the access ports of the closure panel. In this mode, the cabinet serves as a glove box but does not provide containment equivalent to a Class III system.

Since the operator's hands and arms are not protected from contamination, control of contact contamination is dependent upon the use of gloves and other protective clothing. With the caveats cited for fume hoods, Class I cabinets accommodate many routine laboratory operations such as pipetting, blending, and sonicating. Because they lack a sterile work surface, they are not generally recommended. They do, however, provide personal protection during specific applications with low risk oncogenic viruses, and recombinant DNA at Biosafety

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Level 2 (BL2) containment level, as well as for chemical carcinogens, low-level radioactive materials and volatile solvents, provided:

- The face velocity is 100 ft/min.
- Concentrations of the materials being contained will not reach dangerous levels or contaminate the cabinet or associated exhaust system.
- Exhaust air is ducted to the outdoors.
- Quality of the effluent meets emission regulations.

**4.9.2.2 Class II Biological Safety Cabinets**

In the Class II cabinet, commonly known as laminar flow or biosafety hood, room air is drawn into the grille at the front edge of the work surface, passed through a HEPA filter, and recirculated into the cabinet work space through the overhead grille

Concurrently, the cabinet air is drawn from the work space through the grilles at the front and back edge of the work surface, and a portion of the air is exhausted after passing through a HEPA filter. An air barrier prevents airborne contaminants generated in the cabinet from escaping through the work opening. This air barrier is formed from the room air and downward flowing, HEPA-filtered air drawn into the front grille. The HEPA-filtered air flows downward with uniform velocity and minimum turbulence, minimizing lateral movement of aerosolized contamination within the cabinet and purging the work space.

Effective Class II biological safety cabinets have standards developed by the National Sanitation Foundation (NSF) and certified by the NIH. These cabinets may have fixed or variable vertical work openings. The Class II (Type A1, A2, B1, and B2) BSCs provide personnel, environmental, and product protection. Airflow is drawn into the front of the grille of the cabinet to provide personnel protection. The downward flow of HEPA-filtered air provides product protection by minimizing the change of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free for environmental protection. Type A1 and A2 BSCs the HEPA-filtered exhaust is recirculated to the laboratory or exhausted from the building via a anopy or “thible” connection; Type B1 and B2 BSC must be discharged directly to the outdoors via a hard connection.

HEPA filters trap particulates and thus infectious agents, but do not capture volatile chemicals or gases. Only Type A2-exhausted or Type B1 and B2 BSCs that exhaust to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited.

**Table 10: Comparison of Biosafety Cabinet characteristics**

BSC Class	Face Velocity	Airflow Pattern	Applications		
			Nonvolatile	Volatile	Toxic

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			Toxic Chemicals and Radionuclides	Chemicals and Radionuclides
<b>I</b>	75	In at front through HEPA to the outside or into the room through HEPA	Yes	When exhausted outdoors
<b>II, A1</b>	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit	Yes  (minute amounts)	No
<b>II, B1</b>	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	Yes	Yes  (minute amounts)
<b>I, B2</b>	100	No recirculation, total exhaust to the outside through a HEPA filter	Yes	Yes  (small amounts)
<b>II, A2</b>	100	Similar to II, A1, but has 100 lfm intake air velocity and plenums are under negative pressure to room; exhaust air can be ducted to the outside through a canopy unit	Yes	When exhausted outdoors (FORMALLY "B3")(minute amounts)
<b>III</b>	N/A	Supply air is HEPA filtered. HEPA filters in series and is exhausted to the outside via a hard connection	Yes	Yes  (small amounts)

All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2, 3, and 4. Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of non-volatile antineoplastic or chemotherapeutic drugs.

#### 4.9.2.2.1 Class II, Type A1 BSC

An internal fan draws sufficient room air through the front grille to maintain a minimum calculated or measured average inflow velocity of at least 75 lfm at the face opening of the cabinet. The supply air flows through a HEPA

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filter and provides particulate-free air to the work surface. Airflow provided in this manner 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 fan 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 drawn through the front and rear grilles by a fan pushed into the space between the supply and exhaust filters. 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 of the cabinet. Most Class II, Type A1 and A2 cabinets have dampers to modulate this division of airflow.

A Class II Type A1 BSC is not to be used for work involving volatile 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 exhaust the air from a Type A1 or A2 cabinet outside 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 proper method of connecting a Type A1 or A2 cabinet to the building exhaust system is through use of a canopy hood,<sup>8,10</sup> which provides a small opening or air gap (usually 1 inch) around the cabinet exhaust filter housing (Figure 4). The airflow of the building exhaust must be sufficient to maintain the flow of room air into the gap between the canopy unit and the filter housing. The canopy must be removable or be designed to allow for operational testing of the cabinet. (See Section VI.) Class II Type A1 or A2 cabinets should never be hard-ducted to the building exhaust system.<sup>8</sup> Fluctuations in air volume and pressure that are common to all building exhaust systems sometimes make it difficult to match the airflow requirements of the cabinet.

#### **4.9.2.2.2 The Class II, Type B1 BSC:**

Some biomedical research requires the use of small quantities of hazardous chemicals, such as organic solvents or carcinogens. Carcinogens used in cell culture or microbial systems require both biological and chemical containment.<sup>11</sup>

The Class II, Type B cabinet originated with the National Cancer Institute (NCI)-designed Type 212 (later called Type B) BSC (Figure 5A), and was designed for manipulations of minute quantities of hazardous chemicals with *in vitro* biological systems. The NSF International NSF/ANSI Standard 49—2007 definition of Type B1 cabinets<sup>8</sup> includes this classic NCI design Type B, and cabinets without supply HEPA filters located immediately below the work surface (Figure 5B), and/or those with exhaust/recirculation down flow splits other than exactly 70/30%.

The cabinet supply blowers draw room air (plus a portion of the cabinet's recirculated air) through the front grille and through the supply HEPA filters located immediately below the work surface. This particulate-free air flows upward through a plenum at each side of the cabinet and then downward to the work area through a backpressure plate. In some cabinets, there is an additional supply HEPA filter to remove particulates that may be generated by the blower-motor system.

Room air is drawn through the face opening of the cabinet at a minimum measured inflow velocity of 100 lfm. As with the Type A1 and A2 cabinets, there is a split in the down-flowing air stream just above the work surface. In the Type B1 cabinet, approximately 70 percent of the down flow air exits through the rear grille, passes through the exhaust HEPA filter, and is discharged from the building. The remaining 30 percent of the down flow air is drawn through the front grille. Since the air that flows to the rear grille is discharged into the exhaust system, activities that may generate hazardous chemical vapors or particulates should be conducted toward the rear of the cabinetwork area.<sup>13</sup>

Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system. As indicated earlier, fans for laboratory exhaust systems should be located at the terminal end of the ductwork to avoid pressuring the exhaust ducts. 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 and alarm should be installed to provide warning and shut off the BSC supply fan, should failure in exhaust airflow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the exhaust blower to the emergency power supply.

#### **4.9.2.2.3 The 3. Class II, Type B2 BSC:**

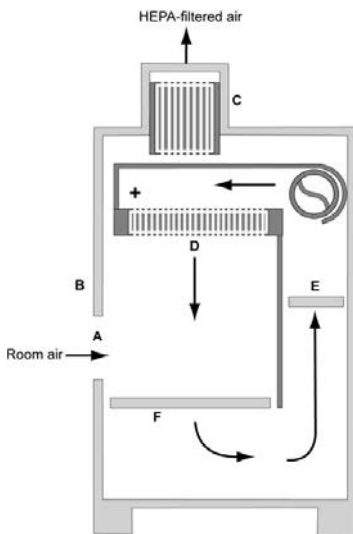
This BSC is a total-exhaust cabinet; no air is recirculated within it (Figure 6). This cabinet provides simultaneous primary biological and chemical (small quantity) containment. Consideration must be given to the chemicals used in BSCs as some chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfm. 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 if required for the work being performed) prior to discharge to the outside. This cabinet exhausts as much as 1200 cubic feet per minute of conditioned room air making this cabinet expensive to operate. The higher static air pressure required to operate this cabinet also results in additional costs associated with heavier gauge ductwork

and higher capacity exhaust fan. Therefore, the need for the Class II, Type B2 should be justified by the research to be conducted.

Should the building exhaust system 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 operating whenever the exhaust flow is insufficient; systems can be retrofitted if necessary. Exhaust air movement should be monitored by a pressure-independent device, such as a flow monitor.

#### 4.9.2.2.4 The 4. Class II, Type A2 BSC (Formerly called A/B3):

Only when this BSC is ducted to the outdoors does it meet the requirements of the former Class II Type B3.<sup>8</sup> The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfm. All positive pressure contaminated plenums within the cabinet are surrounded by a negative air pressure plenum thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment. Minute quantities of volatile toxic chemicals or radionuclides can be used in a Type A2 cabinet only if it exhausts to the outside via a properly functioning canopy connection.



**Figure 5: Model of a Class II, Type A2 BSC**

#### 4.9.2.2.5 Special Applications:5.

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. The work surface can be designed to accept a carboy, a centrifuge or other equipment that may require containment. A rigid plate with openings for the arms 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.

#### **4.9.2.2.6 The Class III BSC**

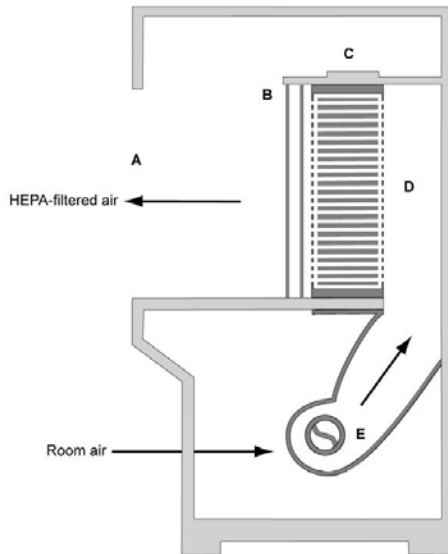
The Class III BSC (Figure 8) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than  $1 \times 10^{-7}$  cc/sec with 1% test gas at 3 inches pressure Water Gauge<sup>14</sup>) 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 (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge directly to the outdoors. Class III cabinets are not exhausted through the general laboratory exhaust system. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.)

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet to allow direct 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. Laminar airflow is not a characteristic of a Class III cabinet.

Several Class III BSCs can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom-built; the equipment installed in the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators) is generally custom-built as well.

#### **4.9.2.2.7 Horizontal Laminar Flow "Clean Bench"**

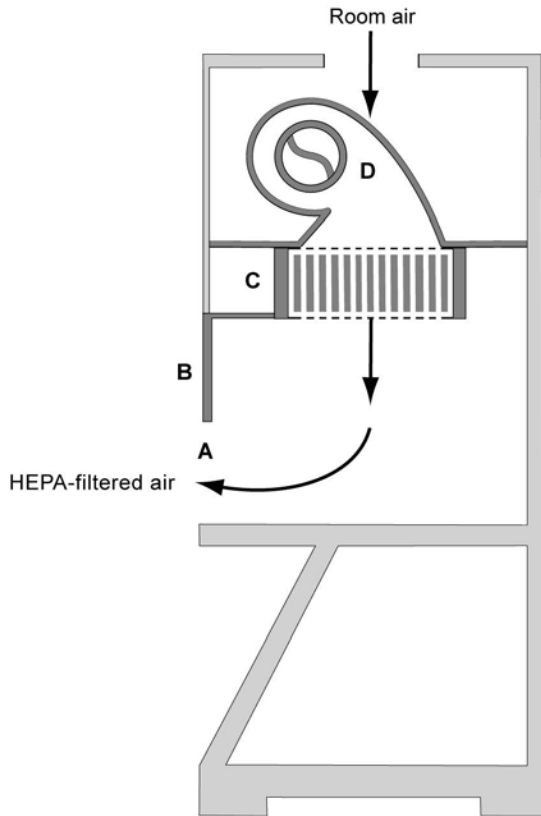
Horizontal laminar flow "clean benches" (Figure 9A) are not BSCs. These pieces of equipment discharge HEPA-filtered air from the back of the cabinet across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials, drug formulations, potentially infectious materials, or any other potentially hazardous materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled. Horizontal airflow "clean benches" must never be used as a substitute for a biological safety cabinet. Users must be aware of the differences between these two devices.



**Figure 6: Horizontal Laminar Flow "Clean Bench"**

**4.9.2.2.8 Vertical Flow "Clean Bench"**

Vertical flow clean benches (Figure 9B) also are not BSCs. They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous solutions. While these units generally have a sash, the air is usually discharged into the room under the sash, resulting in the same potential problems presented by the horizontal laminar flow clean benches. These benches should never be used for the manipulation of potentially infectious or toxic materials or for preparation of antineoplastic agents.



**Figure 7: Vertical Laminar Flow "Clean Bench"**

Remember that Class II cabinets are not absolute containment devices. Performance evaluation tests include using a nebulizer to introduce a known concentration of bacterial spore suspension at various locations inside and outside the cabinet, then scoring growth on agar plates exposed directly or collected in impingers. A protection factor is calculated from the number of spores collected outside the BSC at the face during release of a known aerosol inside the cabinet. The minimum requirement for personal protection is 105, i.e., 105 fewer spores are collected at the cabinet face than near an aerosol generated on an open bench. This protection factor, measured in a static test under ideal conditions, is not usually achieved in routine laboratory use.

The air barrier can be disturbed by an imbalance of airflows that may be caused by turbulent ventilation sources or heavy traffic, inadequate clearance above exhaust filters, mechanical failure, dirty filters, or blockage of the air-intake grilles that extend along the front and back of the work surface. The uniform, downward flow of clean air over the work surface can be disturbed by placing items on the front or rear grilles, by overcrowding the cabinet interior, by convection currents from heat sources, or rapid hand motions in and out of the cabinet. Class II cabinets are suitable for most projects, are convenient to use, and offer adequate personnel and product protection if used properly with low to moderate-risk

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oncogenic viruses, CDC classes 1 to 3 etiologic agents, and recombinant DNA materials requiring BL2 containment. Since Class II cabinets may recirculate a large fraction of the air flowing through them, they are only suitable for work with dilute concentrations of radioactive materials, toxic chemicals, or carcinogens of low volatility, provided:

#### **4.9.2.3 Biological Safety Cabinet Operations**

Good work practices are necessary to prevent compromising the protection offered by a biological safety cabinet (BSC). BSCs should be certified at least annually. All routinely used items should be left in place during the certification evaluation to reveal potential disturbances. BSCs should be left running at all times with the sashes at the eight or ten inch height. Do not override the sash alarms. By leaving the BSC running, release of cabinet air is avoided, the work area remains clean, and spills, particularly below the work surface dry rapidly reducing the potential for contaminant growth. Disruptions of the air curtain should be limited. This requires keeping front and rear grilles uncluttered and minimizing movement of hands into and from the BSC. The latter necessitates internal waste collection. The use of loose bags or mesh support stands for waste bags is discouraged because the bags can be punctured and leak when handled. Waste can be collected inside the BSC in a one cubic foot stainless steel receptacle fitted with an autoclavable biohazard bag, such that the inside of the upper portion of the bag is folded down over the outside of the container. The disturbance in the cabinet air flow induced by such a receptacle is less than the many potential disturbances caused by external waste collection. When possible use disposal pipettes. Use of non-disposable pipettes necessitates their collection in awkward liquid-filled trays inside the BSC or buckets outside the cabinet. The laminar air flow and drying atmosphere within the BSC reduces the likelihood of contamination from residues in the disposable pipettes. Use a small footprint vacuum trap bottle and hydrophobic filter instead of a vacuum flash to limit the total volume of materials in the BSC. Some users remove all items before cleaning the BSC and reintroduce the items as work recommences. Often proper decontamination of the materials is difficult and poorly performed; consequently, contaminants may be spread. It is better to keep a few dedicated items in the BSC, e.g., forceps and pipetting aids, and routinely decontaminating these items and the work surface with 70% ethanol before initiation and upon completion of work. Porous materials such as wipes or gauze sponges can not be readily decontaminated. Once placed in the cabinet, they should not be removed for routine cleaning.

Use of a flame in aseptic technique is historic, and the misconception persists that flaming enhances the 'sterility' provided by a BSC. Cabinet manufacturers, cell culture manuals, and the British Standards Institution advise against use of a burner. The upward air currents induced by the flame run counter to the clean, downward airflow. The resulting turbulence may contribute to the spread of contamination within the BSC. Plastic cultureware does not retain its integrity

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when flamed to sterility; so most users merely pass items quickly through the flame. The combination of a flame and flammable ethanol vapors or combustibles in the BSC has resulted in damaged filters and serious fires, fanned by the cabinet airflow. Consequently, burners should not be used in Class II BSCs. A gas line connection to a BSC should be permitted for only very special procedures and not for routine culture work.

Early users of microbiological cabinets needed germicidal, ultraviolet (UV) lights to improve sterility. Today some investigators use the UV light in lieu of frequent thorough cleaning with a general purpose germicidal solution, such as 70% ethanol. UV radiation is only surface effective and will not penetrate through items on the work surface or dust particles attracted to the bulb by static electricity. Intensity decreases with the square of the distance and diminishes rapidly with tube age. While UV lamps are of minimal effectiveness in improving sterility, they do present a risk to eyes and UV sensitive skin.

#### **4.9.2.4 Guidelines for Operations in a Biological Cabinet**

4. The cabinet should be left running.
5. If adjustable, the window should be lowered to 8 inches, with a 100 ft/min face velocity.
6. Keep the amount of equipment used or stored in the cabinet to a minimum.
7. Before work is started, everything needed for the procedures should be placed in the cabinet, and the air allowed to exhaust for a few minutes.
8. Nothing should be placed on or blocking the front or rear grilles.
9. Contaminated items should be segregated from clean ones and located so that they never have to be passed over clean items.
10. Avoid disrupting the air barrier in a safety cabinet by frequent and rapid arm movements and bringing the hands in and out of the cabinet.
11. Waste containers should be placed inside the cabinet to avoid breaking the air barrier and bringing contaminated items out into the room.
12. Do not use a burner (even a Touch-Omatic™ type) in a Class II biological safety cabinet because the air currents induced are counter to the normal air flow, can cause contamination of the work surface or the room, ignite ethanol and other materials in the cabinet, and damage the HEPA filter. Note: plasticware cannot withstand sterilizing temperatures.
13. Do not use a cabinet ultraviolet (UV) lamp. It only provides a minimal initial surface germicidal effect, which deteriorates rapidly with time, distance, and dust deposits, while the ocular and skin hazards from the UV light persist.
14. When working with biohazards, keep absorbent towels and decontaminating solutions, usually 70% ethanol and 10% chlorine bleach, in the cabinet and wipe down the work surface with ethanol prior to and at completion of each session, and also after any small spills. Decontaminate all equipment removed from the cabinet. Pipetting aids and tools that are used repeatedly should remain in the cabinet. Inspect, decontaminate, or

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change receivers on pipet aids regularly. Decontamination of the entire cabinet (the filters, the plenums, the work surfaces and the fan) is achieved by exposing these areas to paraformaldehyde vapor. This type of decontamination must be performed only by a certified professional.

15. A liquid trap bottle with bleach or other suitable disinfectant should be kept inside the cabinet and a small two-micron, hydrophobic filter should be placed between the trap and the vacuum spigot to protect the vacuum line.
16. Do not use a vertical or horizontal laminar flow cabinet (blow out hood) for work with biological materials.

#### **4.9.2.5 Certification and Decontamination Requirements**

Newly installed biological safety cabinets frequently fail to meet design criteria and many cabinets fail to pass routine leak tests. The performance of every safety cabinet should be tested and certified as meeting specifications after it has been purchased and installed, but before it is used, after it has been moved or serviced, and at least annually. Decontamination is required prior to moving or servicing. Do not ask maintenance personnel to service these cabinets. Certification, decontamination and service must be performed by a trained professional according to NSF Standard 49.

#### **4.9.2.6 Electricity Failure During Use of a Biological Safety Cabinet**

Should the power to the unit fail during use, stop work with biohazardous agents immediately, seal all cultures securely, and decontaminate the work area with a suitable disinfectant.

#### **4.9.3 Biological Stains**

Fixatives and stains used for the preparation of tissues and cellular materials often have toxic properties, e.g., methylene blue, trypan blue (teratogen), 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 the Department of Environmental Health and Safety.

#### **4.9.4 Incubators**

Incubators can become the inadvertent and undesired repository 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. Sodium azide is explosive when heated and is extremely toxic. In addition, the inner panels, trays,

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and the other removable parts should be autoclaved and the gaskets and non-removable parts wiped thoroughly with 70% ethanol every two months.

#### 4.9.5 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 should not be kept in freezers that are not designed for flammable storage. The use of such storage for nucleic acid precipitation appears to be contraindicated. It has been reported that centrifugation time and DNA concentration are more significant than incubation temperature for efficient recovery of DNA<sup>8</sup>. A ten-minute incubation at 0°C after addition of room temperature ethanol is more efficient than incubation at -20°C or -70°C.

Cells and virus stocks should be stored in sealed ampules and not in screw cap glass vials. Screw cap glass vials are permeable to the 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, sealed 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 liquified nitrogen may boil vigorously as warmer materials are added. Use only in a well-ventilated area. Liquified nitrogen is a cryogenic gas and expands 700-fold upon vaporization; this may result in a rapid displacement of air (see **CHEMICAL AND COMPRESSED GAS SAFETY** for more information on gases).

When thawing cells, a lab coat, face guard, thermal gloves, and closed shoes should be worn. Ampules to be thawed should be dropped into a plastic beaker containing 70% ethanol at 37°C within a styrofoam bucket and covered immediately. Confirm the identification of the sample. Open the vial in a biological safety cabinet, by nicking the ampule with a file near the neck. Wrap it in ethanol wetted material and, holding the vial upright, snap the ampule open at the nick. Add liquid slowly to dried material. Withdraw the suspension and mix in another vessel.

## **4.9.6 Spills And Decontamination**

### **4.9.6.1 Spills**

#### **4.9.6.1.1 Small or Incidental spills**

Most spills in the laboratory involve comparatively small quantities of chemicals and biohazards which can readily be cleaned up by laboratory personnel. Notify the laboratory supervisor. The spill control procedures may be performed under his supervision. Arrange for disposal of the chemicals and clean up materials with Environmental Health and Safety.

#### **4.9.6.1.2 Spills requiring assistance**

If the spill involves hazardous material(s) (i.e., radioactive, toxic, flammable, corrosive, volatile, reactive or infectious materials) additional assistance or equipment is required. Contact Environmental Health and Safety (ext. 2215); after hours, dial Wright State Police Department's Dispatch number, ext. 2111. Provide the following information:

- Name of person calling.
- Type of spill, name of material spilled and approximate quantity.
- Location: building, floor and room number.

#### **4.9.6.2 Contained spill of biological materials**

The following guide is to be followed in the event of a small contained spill of biological materials and/or until assistance from the Environmental Health and Safety office is obtained.

##### **4.9.6.2.1 Dry/Non-volatile biological materials**

If the substance is dry and/or nonvolatile, shut off hoods, close windows and doors, and vacate rooms. Label door with appropriate warning. Allow the aerosol to settle for about 30 minutes before reentering room.

##### **4.9.6.2.2 Volatile biological materials**

If the substance is volatile, leave the ventilation on and vacate room, closing door. Label the door with a warning.

- Notify your laboratory supervisor and the Environmental Health and Safety office.
- For a liquid biological spill, use absorbent pads to soak up the liquid and to act as a vapor barrier. Work from the perimeter inward.
- If an infectious agent or particulate agent is involved, close all windows and call Physical Plant at ext. 4444 (between 7:00 am and 3:30 pm, at all other times call Wright State Police Department at ext. 2111) to have them

turn off the air handling units in the building. Be sure to shut off all the fume hoods in the room of the spill. (Wait 30 minutes for the aerosol to settle before reentering the room).

If the spill occurs in public or common areas, you must notify Wright State Police Department (ext. 2111) and Environmental Health and Safety (ext. 2215) immediately.

In all cases immediately alert neighbors, laboratory supervisor; and/or department chair.

#### **4.9.6.3 Decontamination**

##### **4.9.6.3.1 Liquid cultures**

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 chemically inactivated on at least a daily schedule
- Most liquid cultures may be chemically inactivated with freshly prepared bleach (15% v/v). The bleach solution must sit for at least 20 minutes prior to drain disposal, followed by copious amounts of water.
- Do not leave untreated waste in an egress corridor or public area.

##### **4.9.6.3.2 Autoclaving biological materials**

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*Autoclaving infectious biological materials for disposal in regular trash is not approved for Wright State University.*

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##### **4.9.6.3.2.1 Autoclave guidelines**

- Biological materials 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 and should be (add at least 500 ml, if necessary) placed in each bag.

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- Materials should be autoclaved for 60 minutes at 121°C and 15 PSI.
  - Autoclave in a shallow plastic tray or other vessel suitable to contain possible leakage from the bag.
- Be sure to verify that the designated temperature was reached and maintained.
- Include spore strips routinely and ampules of *Bacillus stearothermophilus* monthly in waste bags to monitor autoclave performance in various locations of the autoclave.
- Wear loose fitting thermal gloves; remove immediately if they get wet.
- Do not remove liquids immediately following cycle as they may be superheated 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 165°C requires 5-6 hours to effectively sterilize glassware that can be sterilized by autoclaving at 121°C 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.

#### **4.9.6.3.2.2 Autoclave precautions**

- 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 materials that have been treated with chlorine bleach.
- Do not autoclave materials contaminated with radioisotopes and/or toxic chemicals. These materials may volatilize and contaminate the autoclave and expose workers.

#### **4.9.6.4 Biological safety cabinet decontamination**

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

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*Wastes that are biological, chemical and radioactive, or a combination of the above, should be inactivated first with regard to their pathogenicity and then toxicity, but ultimately must be disposed of as radioactive waste. Refer to Wright State's RADIATION SAFETY MANUAL.*

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## 4.9.7 Disinfectants

### 4.9.7.1 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 advantages of alcohols are 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 lethal action.

### 4.9.7.2 Chlorine

A very active disinfectant, chlorine is lethal 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.

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*Do not autoclave chlorine solutions.*

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### 4.9.7.3 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 iodophores vaporize at 120°F. Stable in storage if kept cool and tightly covered.

### 4.9.7.4 Ethylene Oxide

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, 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 complex apparatus and machines.

- In the event of an ethylene oxide leak, evacuate the area, and call the emergency contact number.
- Avoid all skin contact with ethylene oxide.



- 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 Environmental Health and Safety. If overexposure symptoms develop (vomiting or nausea) contact a physician. 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™.
- The room should have adequate ventilation, and the sterilizer should have dedicated ventilation.
- 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).

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*

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*Contact the Department of Environmental Health and Safety for information on emergency procedures, training, environmental monitoring.*

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#### **4.10 Infectious Waste Management**

Keeping biological waste separate from other waste streams is essential for any management program. Disposal of infectious (medical) waste, subject to federal,

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state and local laws, is becoming increasingly more regulated and costly. All biological waste that fits the infectious waste definition found in Wright State University's **Exposure Control Plan** must be disposed of by following the procedures in that manual.

Wright State University transports infectious waste to commercial treatment and disposal facility as opposed to on-site treatment of infectious waste via autoclaving, incineration, and in some cases chemical treatment. Wright State individual generators of infectious waste not permitted to autoclave or incinerate infectious waste and dispose of it as ordinary trash. Autoclaves can be used for disinfection and sterilization purposes (i.e., for glassware, equipment) and for the treatment of all other waste not meeting the Ohio Environmental Protection Agency's (OEPA) definition of infectious waste. Waste that does not meet the OEPA definition of infectious waste but requires autoclave treatment by another agency shall be autoclaved in bags not labeled with the international biohazard symbol.

On-site chemical treatment of infectious waste cultures is permitted and required. Untreated liquid or semi-liquid infectious waste consisting of blood, blood products, body fluids, and excreta may be disposed of into the sanitary sewer system without prior treatment.

Follow all of the procedures found in Wright State University's **Exposure Control Plan**. For further clarification describing the difference between biological and infectious waste, please call the Environmental Health and Safety Department.

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#### 4.13 Appendix A:

Biosafety Level	Agent Characteristics	Practices	Safety Equipment	Facilities (secondary barriers)
<b>BSL-1</b>	Not known to consistently cause disease in health adults	Standard microbiological practices	None	Open bench top sink
<b>BSL-2</b>	Associated with human disease, hazard from percutaneous injury, ingestion, mucous membrane exposure	Standard microbiological practices Limited access Biohazard warning signs Sharps precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies.	Class I or II biosafety cabinets (BSCs) or other containment devices used for all agents that cause splashes or aerosols of infectious materials  Laboratory coats and gloves  Face protection as needed	Open bench top sink  Autoclave
<b>BSL-2</b>	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	All BSL-2 practices  Controlled access  Decontamination of all waste  Decontamination	Class I or II BSCs or other physical containment devices used for all open manipulations of agents  Protective lab	Open bench top sink  Autoclave  Physical separation from access corridors  Self-closing,

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		of laboratory clothing before laundering  Baseline serum	clothing and gloves  Respiratory protection as needed	double-door access  Exhaust air not recirculated  Negative airflow in laboratory
<b>BSL-4</b>	Dangerous/exotic agents which pose high risk of life-threatening disease; aerosol-transmitted lab infections; or related agents with unknown risk of transmission	All BSL-3 practices  Clothing change before entering  Shower on exit  All material decontaminated on exit from facility	All procedures conducted in Class III BSCs, or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.	BSL-3 plus:  Separate building or isolated zone  Dedicated supply and exhaust, vacuum, and decontamination systems  Other requirements outline the text

## **5 Physical Hazards and Others**

Besides exposure to chemicals and biological agents, laboratory workers can also be exposed to a number of physical hazards.

Some of the common physical hazards that they may encounter include the following:

- ergonomic,
- ionizing radiation,
- nonionizing radiation, and
- noise hazards.

These hazards are described below in individual sections.

### **5.1 Ergonomic Hazards**

Laboratory workers are at risk for repetitive motion injuries during routine laboratory procedures such as pipetting, working at microscopes, operating microtomes, using cell counters and keyboarding at computer workstations. Repetitive motion injuries develop over time and occur when muscles and joints are stressed, tendons are inflamed, nerves are pinched and the flow of blood is restricted. Standing and working in awkward positions in front of laboratory hoods/biological safety cabinets can also present ergonomic problems. By becoming familiar with how to control laboratory ergonomics-related risk factors, employers can reduce chances for occupational injuries while improving worker comfort, productivity, and job satisfaction. In addition to the general ergonomic guidance, simple adjustments can be made at the workplace. While there is currently no specific OSHA standard relating to ergonomics in the laboratory workplace, information to laboratory workers contained in the OSHA fact sheet Laboratory Safety –Ergonomics for the Prevention of Musculoskeletal Disorders in Laboratories.

### **5.2 Ionizing Radiation**

OSHA's Ionizing Radiation standard, 29 CFR 1910.1096, sets forth the limitations on exposure to radiation from atomic particles. Ionizing radiation sources are found in a wide range of occupational settings, including laboratories. These radiation sources can pose a considerable health risk to affected workers if not properly controlled.

Any laboratory possessing or using radioactive isotopes must be licensed by the Nuclear Regulatory Commission (NRC) and/or by a state agency that has been approved by the NRC, 10 CFR 31.11 and 10 CFR 35.12. The fundamental objectives of radiation protection measures are:

- to limit entry of radionuclides into the human body (via ingestion, inhalation, absorption, or through open wounds) to quantities as low as reasonably achievable (ALARA) and always within the established limits; and
- to limit exposure to external radiation to levels that are within established dose limits and as far below these limits as is reasonably achievable.

All areas in which radioactive materials are used or stored must conspicuously display the symbol for radiation hazards and access should be restricted to authorized personnel.



**Figure 8: Radiation Symbol**

The OSHA Ionizing Radiation standard requires precautionary measures and personnel monitoring for workers who are likely to be exposed to radiation hazards. Personnel monitoring devices (film badges, thermoluminescent dosimeters (TLD), pocket dosimeters, etc.) must be supplied and used if required to measure an individual's radiation exposure from gamma, neutron, energetic beta, and X-ray sources. The standard monitoring device is a clip-on badge or ring badge bearing the individual assignee's name, date of the monitoring period and a unique identification number. The badges are provided, processed and reported through a commercial service company that meets current requirements of the National Institute of Standards and Technology's National Voluntary Laboratory Accreditation Program (NIST NVLAP).

It is a requirement for Wright State to understand and follow all applicable regulations for the use of isotopes. Wright State ensures compliance with local, state, and federal laws and regulations; to obtain licenses for official use of radioactive substances; and to designate a radiation safety officer (RSO) to oversee and ensure compliance with state and/or NRC requirements. Information on radioactive materials licenses may be obtained from the Department of Public Health from individual states or from the NRC.

The following OSHA Safety and Health Topics Page provides links to technical and regulatory information on the control of occupational hazards from ionizing radiation: [www.osha.gov/SLTC/radiationionizing/index.html](http://www.osha.gov/SLTC/radiationionizing/index.html).

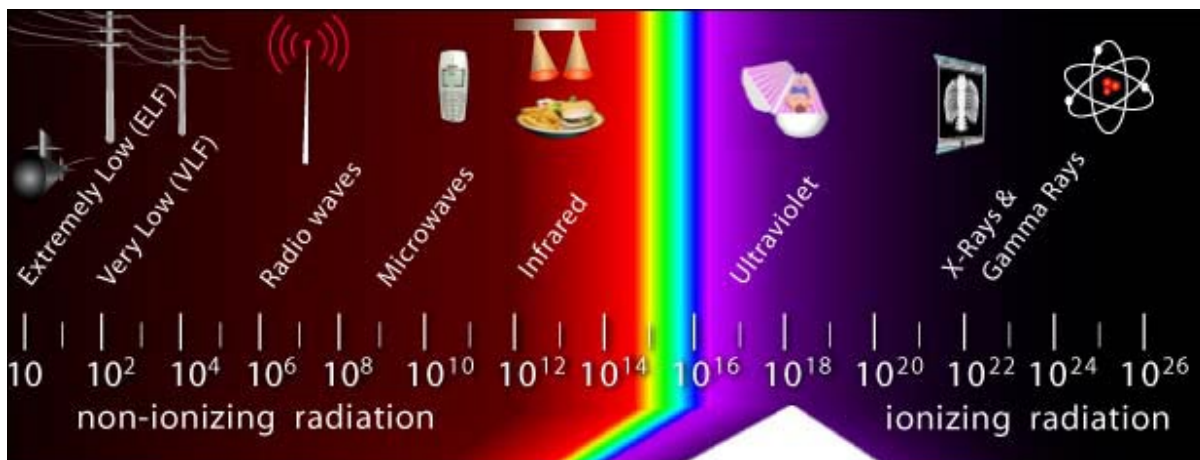


### 5.3 Non-ionizing Radiation

Non-ionizing radiation is described as a series of energy waves composed of oscillating electric and magnetic fields traveling at the speed of light. Nonionizing radiation includes the spectrum of ultraviolet (UV), visible light, infrared (IR), microwave (MW), radio frequency (RF), and extremely low frequency (ELF). Lasers commonly operate in the UV, visible, and IR frequencies. Non-ionizing radiation is found in a wide range of occupational settings and can pose a considerable health risk to potentially exposed workers if not properly controlled.

The following OSHA Safety and Health Topics Pages provide links to technical and regulatory information on the control of occupational hazards from nonionizing radiation and are available at:

[www.osha.gov/SLTC/radiation\\_nonionizing/index.html](http://www.osha.gov/SLTC/radiation_nonionizing/index.html).



**Figure 9: Electromagnetic Spectrum** (<https://www.osha.gov/SLTC/radiation/>)

#### 5.3.1 Extremely Low Frequency Radiation (ELF)

Extremely Low Frequency (ELF) radiation at 60 HZ is produced by power lines, electrical wiring, and electrical equipment. Common sources of intense exposure include ELF induction furnaces and high-voltage power lines.

#### 5.3.2 Radiofrequency and Microwave Radiation

Microwave radiation (MW) is absorbed near the skin, while radiofrequency (RF) radiation may be absorbed throughout the body. At high enough intensities both will damage tissue through heating. Sources of RF and MW radiation include radio emitters and cell phones.

#### 5.3.3 Infrared Radiation (IR)

The skin and eyes absorb infrared radiation (IR) as heat. Workers normally notice excessive exposure through heat sensation and pain. Sources of IR radiation include heat lamps and IR lasers.

#### **5.3.4 Visible Light Radiation**

The different visible frequencies of the electromagnetic (EM) spectrum are "seen" by our eyes as different colors. Good lighting is conducive to increased production, and may help prevent incidents related to poor lighting conditions. Excessive visible radiation can damage the eyes and skin.

#### **5.3.5 Ultraviolet Radiation (UV)**

Ultraviolet radiation (UV) has a high photon energy range and is particularly hazardous because there are usually no immediate symptoms of excessive exposure. Sources of UV radiation in the laboratory include black lights and UV lasers.

#### **5.3.6 Laser Hazards**

Lasers typically emit optical (UV, visible light, IR) radiations and are primarily an eye and skin hazard. Common lasers include CO<sub>2</sub> IR laser; helium - neon, neodymium YAG, and ruby visible lasers, and the Nitrogen UV laser.

LASER is an acronym which stands for Light Amplification by Stimulated Emission of Radiation. The laser produces an intense, highly directional beam of light. The most common cause of laser-induced tissue damage is thermal in nature, where the tissue proteins are denatured due to the temperature rise following absorption of laser energy.

The human body is vulnerable to the output of certain lasers, and under certain circumstances, exposure can result in damage to the eye and skin. Research relating to injury thresholds of the eye and skin has been carried out in order to understand the biological hazards of laser radiation. It is now widely accepted that the human eye is almost always more vulnerable to injury than human skin.

### **5.4 Noise**

OSHA's Occupational Noise Exposure standard, 29 CFR 1910.95, requires employers to develop and implement a hearing conservation program that includes the use of PPE (e.g., hearing protectors), if workers are exposed to a time-weighted average (TWA) of  $\geq 85$  dBA over an 8-hour work shift. In addition, when workers are exposed to noise levels  $\geq 85$  dBA, the employer must develop a monitoring program to assess noise levels. The monitoring program must include the following components:

- All continuous, intermittent, and impulsive sound levels from 80-130 dBA must be included in noise measurements, 29 CFR 1910.95(d)(2)(i);
- Instruments used to measure worker noise exposure must be calibrated to ensure measurement accuracy, 29 CFR 1910.95(d)(2)(ii); and
- Monitoring must be repeated whenever a change in production, process, equipment, or controls increases noise exposures, 29 CFR 1910.95(d)(3).

Laboratory workers are exposed to noise from a variety of sources. Operation of large analyzers (e.g., chemistry analyzer), fume hoods, biological safety cabinets, incubators, centrifuges (especially ultracentrifuges), cell washers, sonicators, and stirrer motors, all contribute to the noise level in laboratories. Further sources of noise in laboratories include fans and compressors for cryostats, refrigerators, refrigerated centrifuges, and freezers. As an example, a high-speed refrigerated centrifuge alone can generate noise levels as high as 65 dBA. To provide some further context, a whisper registers approximately 30 dBA; normal conversation about 50 to 60 dBA; a ringing phone 80 dBA and a power mower 90 dBA. If noise levels exceed 80 dBA, people must speak very loudly to be heard, while at noise levels of 85 to 90 dBA, people have to shout.

In order to determine if the noise levels in the laboratory are above the threshold level that damages hearing, the employer must conduct a noise exposure assessment using an approved sound level monitoring device, such as a dosimeter, and measuring an 8-hour TWA exposure. If the noise levels are found to exceed the threshold level, the employer must provide hearing protection at no cost to the workers and train them in the proper use of the protectors.

The potential dangers of miscommunicating instructions or laboratory results are obvious, and efforts should be made to improve the design of clinical laboratories and to evaluate new instrumentation with regard to the impact of these factors on worker noise exposure. The employer should evaluate the possibility of relocating equipment to another area or using engineering controls to reduce the noise level below an 8-hour TWA of 85 dBA in order to comply with OSHA's Occupational Noise Exposure standard.

While most laboratories' noise levels do not equal or exceed the 8-hour TWA of 85 dBA, certain accrediting agencies are implementing special emphasis programs on noise reduction in the laboratory.

Because noise is becoming more of a concern in the clinical setting, the College of American Pathologists has added evaluation of noise in the laboratory under their general checklist for accreditation (GEN.70824).

#### **5.4.1 Health Effects**

Exposure to continuous noise may lead to the following stress-related symptoms:

- Depression;
- Irritability;
- Decreased concentration in the workplace;
- Reduced efficiency and decreased productivity;
- Noise-induced hearing loss;
- Tinnitus (i.e., ringing in the ears); and
- Increased errors in laboratory work.

There are several steps that employers can take to minimize the noise in the laboratory, including:

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- Moving noise-producing equipment (e.g., freezers, refrigerators, incubators and centrifuges) from the laboratory to an equipment room;
- Locating compressors for controlled-temperature rooms remotely; and
- Providing acoustical treatment on ceilings and walls.

## 5.5 Safety Hazards

Supervisors and principle investigators must assess tasks to identify potential worksite hazards and provide and ensure that workers use appropriate personal protective equipment (PPE) as stated in the PPE standard, 29 CFR 1910.132.

Supervisors and principle investigators must require workers to use appropriate hand protection when hands are exposed to hazards such as sharp instruments and potential thermal burns. Examples of PPE which may be selected include using oven mitts when handling hot items, and steel mesh or cut-resistant gloves when handling or sorting sharp instruments as stated in the Hand Protection standard, 29 CFR 1910.138.

### 5.5.1 Autoclaves and Sterilizers

Workers should be trained to recognize the potential for exposure to burns or cuts that can occur from handling or sorting hot sterilized items or sharp instruments when removing them from autoclaves/ sterilizers or from steam lines that service the autoclaves.

In order to prevent injuries from occurring, employers must train workers to follow good work practices such as those outlined in the QuickCard™ highlighted below.

### 5.5.2 Centrifuges

Centrifuges, due to the high speed at which they operate, have great potential for injuring users if not operated properly. Unbalanced centrifuge rotors can result in injury, even death. Sample container breakage can generate aerosols that may be harmful if inhaled. The majority of all centrifuge accidents are the result of user error. In order to prevent injuries or exposure to dangerous substances, employers should train workers to follow good work practices such as those outlined in the QuickCard™ highlighted below.

Employers should instruct workers when centrifuging infectious materials that they should wait 10 minutes after the centrifuge rotor has stopped before opening the lid. Workers should also be trained to use appropriate decontamination and cleanup procedures for the materials being centrifuged if a spill occurs and to report all accidents to their supervisor immediately.

### 5.5.3 Compressed Gases

According to OSHA's Laboratory standard, a "compressed gas"

- is a gas or mixture of gases in a container having an absolute pressure exceeding 40 pounds per square inch (psi) at 70°F (21.1°C); or

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- is a gas or mixture of gases having an absolute pressure exceeding 104 psi at 130°F (54.4°C) regardless of the pressure at 70°F (21.1°C); or
- is a liquid having a vapor pressure exceeding 40 psi at 100°F (37.8°C) as determined by ASTM (American Society for Testing and Materials) D-323-72, [29 CFR 1910.1450(c)(1)-(3)].

Within laboratories, compressed gases are usually supplied either through fixed piped gas systems or individual cylinders of gases. Compressed gases can be toxic, flammable, oxidizing, corrosive, or inert. Leakage of any of these gases can be hazardous. Leaking inert gases (e.g., nitrogen) can quickly displace air in a large area creating an oxygen-deficient atmosphere; toxic gases (e.g., can create poison atmospheres; and flammable (oxygen) or reactive gases can result in fire and exploding cylinders.

In addition, there are hazards from the pressure of the gas and the physical weight of the cylinder. A gas cylinder falling over can break containers and crush feet. The gas cylinder can itself become a missile if the cylinder valve is broken off. Laboratories must include compressed gases in their inventory of chemicals in their Chemical Hygiene Plan.

Compressed gases contained in cylinders vary in chemical properties, ranging from inert and harmless to toxic and explosive. The high pressure of the gases constitutes a serious hazard in the event that gas cylinders sustain physical damage and/or are exposed to high temperatures.

Store, handle, and use compressed gases in accord with OSHA's Compressed Gases standard (29 CFR 1910.101) and Pamphlet P-1-1965 from the Compressed Gas Association.

- All cylinders whether empty or full must be stored upright.
- Secure cylinders of compressed gases. Cylinders should never be dropped or allowed to strike each other with force.
- Transport compressed gas cylinders with protective caps in place and do not roll or drag the cylinders.

#### **5.5.4 Cryogenics and Dry Ice**

Cryogenics, substances used to produce very low temperatures [below -153°C (-243°F)], such as liquid nitrogen (LN<sub>2</sub>) which has a boiling point of -196°C (-321°F), are commonly used in laboratories.

Although not a cryogen, solid carbon dioxide or dry ice which converts directly to carbon dioxide gas at -78°C (-109°F) is also often used in laboratories.

Shipments packed with dry ice, samples preserved with liquid nitrogen, and in some cases, techniques that use cryogenic liquids, such as cryogenic grinding of samples, present potential hazards in the laboratory.

#### **5.5.4.1 Overview of Cryogenic Safety Hazards**

The safety hazards associated with the use of cryogenic liquids are categorized as follows:

##### **5.5.4.1.1 Cold contact burns**

Liquid or low-temperature gas from any cryogenic substance will produce effects on the skin similar to a burn.

##### **5.5.4.1.2 Asphyxiation**

Degrees of asphyxia will occur when the oxygen content of the working environment is less than 20.9% by volume. This decrease in oxygen content can be caused by a failure/leak of a cryogenic vessel or transfer line and subsequent vaporization of the cryogen. Effects from oxygen deficiency become noticeable at levels below approximately 18% and sudden death may occur at approximately 6% oxygen content by volume.

##### **5.5.4.1.3 Explosion - Pressure**

Heat flux into the cryogen from the environment will vaporize the liquid and potentially cause pressure buildup in cryogenic containment vessels and transfer lines. Adequate pressure relief should be provided to all parts of a system to permit this routine outgassing and prevent explosion.

##### **5.5.4.1.4 Explosion - Chemical**

Cryogenic fluids with a boiling point below that of liquid oxygen are able to condense oxygen from the atmosphere. Repeated replenishment of the system can thereby cause oxygen to accumulate as an unwanted contaminant. Similar oxygen enrichment may occur where condensed air accumulates on the exterior of cryogenic piping. Violent reactions, e.g., rapid combustion or explosion, may occur if the materials which make contact with the oxygen are combustible.

#### **5.5.4.2 Employer Responsibility**

It is the responsibility of Wright State, specifically the supervisor in charge of an apparatus, to ensure that the cryogenic safety hazards are minimized. This will entail

- a safety analysis and review for all cryogenic facilities,
- cryogenic safety and operational training for relevant workers,
- appropriate maintenance of cryogenic systems in their original working order, i.e., the condition in which the system was approved for use, and
- upkeep of inspection schedules and records.

Employers must train workers to use the appropriate personal protective equipment (PPE) Whenever handling or transfer of cryogenic fluids might result in exposure to the cold liquid, boil-off gas, or surface, protective clothing must be worn.

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This includes:

- face shield or safety goggles;
- safety gloves; and
- long-sleeved shirts, lab coats, aprons.

Eye protection is required at all times when working with cryogenic fluids. When pouring a cryogen, working with a wide-mouth Dewar flask or around the exhaust of cold boil-off gas, use of a full face shield is recommended.

Hand protection is required to guard against the hazard of touching cold surfaces. It is recommended that Cryogen Safety Gloves be used by the worker.

#### **5.5.5 Electrical**

In the laboratory, there is the potential for workers to be exposed to electrical hazards including electric shock, electrocutions, fires and explosions.

Damaged electrical cords can lead to possible shocks or electrocutions. A flexible electrical cord may be damaged by door or window edges, by staples and fastenings, by equipment rolling over it, or simply by aging.

The potential for possible electrocution or electric shock or contact with electrical hazards can result from a number of factors, including the following:

- Faulty electrical equipment/instrumentation or wiring;
- Damaged receptacles and connectors; and
- Unsafe work practices.

#### **5.5.5.1 Employers are responsible for complying with OSHA's standard 1910 Subpart S Electrical**

Subpart S is comprehensive and addresses electrical safety requirements for the practical safeguarding of workers in their workplaces. This Subpart includes, but is not limited to, these requirements:

- Electrical equipment must be free from recognized hazards, 29 CFR 1910.303(b)(1);
- Listed or labeled equipment must be used or installed in accord with any instructions included in the listing or labeling, 29 CFR 1910.303(b)(2);
- Sufficient access and working space must be provided and maintained around all electrical equipment operating at  $\leq 600$  volts to permit ready and safe operation and maintenance of such equipment, 29 CFR 1910.303(g)(1);
- Ensure that all electrical service near sources of water is properly grounded.
- Tag out and remove from service all damaged receptacles and portable electrical equipment, 29 CFR 1910.334(a)(2)(ii);
- Repair all damaged receptacles and portable electrical equipment before placing them back into service, 29 CFR 1910.334(a)(2)(ii);

- Ensure that workers are trained not to plug or unplug energized equipment when their hands are wet, 29 CFR 1910.334(a)(5)(i);
- Select and use appropriate work practices, 29 CFR 1910.333; and
- Follow requirements for Hazardous Classified Locations, 29 CFR 1910.307. This section covers the requirements for electric equipment and wiring in locations that are classified based on the properties of the flammable vapors, liquids or gases, or combustible dusts or fibers that may be present therein and the likelihood that a flammable or combustible concentration or quantity is present.

Notes:

- Only “Qualified Persons,” as defined by OSHA in 29 CFR 1910.399, are to work on electrical circuits/systems.
- Workers must be trained to know the locations of circuit breaker panels that serve their lab area.

### 5.5.6 Fire

Fire is the most common serious hazard that one faces in a typical laboratory. While proper procedures and training can minimize the chances of an accidental fire, laboratory workers should still be prepared to deal with a fire emergency should it occur. In dealing with a laboratory fire, all containers of infectious materials should be placed into autoclaves, incubators, refrigerators, or freezers for containment.

Small bench-top fires in laboratory spaces are not uncommon. Large laboratory fires are rare. However, the risk of severe injury or death is significant because fuel load and hazard levels in labs are typically very high. Laboratories, especially those using solvents in any quantity, have the potential for flash fires, explosion, rapid spread of fire, and high toxicity of products of combustion (heat, smoke, and flame).

#### 5.5.6.1 Training for Fire Prevention

- Plan work. Have a written emergency plan for your space and/or operation.
- Minimize materials. Have present in the immediate work area and use only the minimum quantities necessary for work in progress. Not only does this minimize fire risk, it reduces costs and waste.
- Observe proper housekeeping. Keep work areas uncluttered, and clean frequently. Put unneeded materials back in storage promptly. Keep aisles, doors, and access to emergency equipment unobstructed at all times.
- Observe restrictions on equipment (i.e., keeping solvents only in an explosion-proof refrigerator).
- Keep barriers in place (shields, hood doors, lab doors).
- Wear proper clothing and personal protective equipment.
- Avoid working alone.
- Store solvents properly in approved flammable liquid storage cabinets.



- Shut door behind you when evacuating.
- Limit open flames use to under fume hoods and only when constantly attended.
- Keep combustibles away from open flames.
- Do not heat solvents using hot plates.
- Remember the “RACE” rule in case of a fire.

R= Rescue/remove all occupants

A= Activate the alarm system

C= Confine the fire by closing doors

E= Evacuate/Extinguish

### 5.5.6.2 Training for Emergency Procedures

Workers should be trained in the following emergency procedures:

- Know what to do. You tend to do under stress what you have practiced or pre-planned. Therefore, planning, practice and drills are essential.
- Know where things are: The nearest fire extinguisher, fire alarm box, exit(s), telephone, emergency shower/eyewash, and first-aid kit, etc.
- Be aware that emergencies are rarely “clean” and will often involve more than one type of problem. For example, an explosion may generate medical, fire, and contamination emergencies simultaneously.
- Train workers and exercise the emergency plan.
- Learn to use the emergency equipment provided.

OSHA’s Portable Fire Extinguishers standard, 29 CFR 1910.157 requires that workers need to be trained and to be aware of the different fire extinguisher types and how to use them. OSHA’s Portable Fire Extinguishers standard, 29 CFR 1910.157, applies to the placement, use, maintenance, and testing of portable fire extinguishers provided for the use of workers. This standard requires that a fire extinguisher be placed within 75 feet for Class A fire risk (ordinary combustibles; usually fuels that burn and leave “ash”) and within 50 feet for high-risk Class B fire risk (flammable liquids and gases; in the laboratory many organic solvents and compressed gases are fire hazards).

The two most common types of extinguishers in the chemistry laboratory are pressurized dry chemical (Type BC or ABC) and carbon dioxide. In addition, you may also have a specialized Class D dry powder extinguisher for use on flammable metal fires.

Water-filled extinguishers are not acceptable for laboratory use.

### 5.5.6.3 “PASS” Rule for Fire Extinguisher

Laboratory workers need to remember the “PASS” rule for fire extinguishers

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PASS summarizes the operation of a fire extinguisher.

P – Pull the pin

A – Aim extinguisher nozzle at the base of the fire

S – Squeeze the trigger while holding the extinguisher upright

S – Sweep the extinguisher from side to side; cover the fire with the spray

#### 5.5.6.4 Procedures for Clothing Fire

Laboratory workers need to know appropriate procedures in the event of a clothing fire.

- If the floor is not on fire, STOP, DROP and ROLL to extinguish the flames or use a fire blanket or a safety shower if not contraindicated (i.e., there are no chemicals or electricity involved).
- If a coworker's clothing catches fire and he/she runs down the hallway in panic, tackle him/her and smother the flames as quickly as possible, using appropriate means that are available (e.g., fire blanket, fire extinguisher).

#### 5.5.7 Lockout/Tagout

Workers performing service or maintenance on equipment may be exposed to injuries from the unexpected energization, startup of the equipment, or release or stored energy in the equipment. OSHA's Control of Hazardous Energy standard, 29 CFR 1910.147, commonly referred to as the "Lockout/Tagout" standard, requires the adoption and implementation of practices and procedures to shut down equipment, isolate it from its energy source(s), and prevent the release of potentially hazardous energy while maintenance and servicing activities are being performed. It contains minimum performance requirements, and definitive criteria for establishing an effective program for the control of hazardous energy. However, employers, including Wright State, have the flexibility to develop Lockout/Tagout programs that are suitable for their respective facilities. Wright State has a Lockout/Tagout program.

This standard establishes basic requirements involved in locking and/or tagging equipment while installation, maintenance, testing, repair or construction operations are in progress. The primary purpose is to prevent hazardous exposure to personnel and possible equipment damage. The procedures apply to the shutdown of all potential energy sources associated with the equipment. These could include pressures, flows of fluids and gases, electrical power, and radiation.

This standard covers the servicing and maintenance of machines and equipment in which the "**unexpected**" energization or startup of the machines or equipment, or release of stored energy could cause injury to workers.

Under the standard, the term “**unexpected**” also covers situations in which the servicing and/or maintenance is performed during ongoing normal production operations if:

- A worker is required to remove or bypass machine guards or other safety devices, 29 CFR 1910.147(a)(2)(ii)(A) or
- A worker is required to place any part of his or her body into a point of operation or into an area on a machine or piece of equipment where work is performed, or into the danger zone associated with the machine’s operation, 29 CFR 1910.147(a) (2)(ii)(B).

The Lockout/Tagout standard establishes minimum performance requirements for the control of such hazardous energy.

Maintenance activities can be performed with or without energy present. A probable, underlying cause of many accidents resulting in injury during maintenance is that work is performed without the knowledge that the system, whether energized or not, can produce hazardous energy. Unexpected and unrestricted release of hazardous energy can occur if:

- all energy sources are not identified;
- provisions are not made for safe work practices with energy present; or
- deactivated energy sources are reactivated, mistakenly, intentionally, or accidentally, without the maintenance worker’s knowledge.

Problems involving control of hazardous energy require procedural solutions. Procedural solutions must be adopted for controlling hazards to ensure worker safety during maintenance. However, such procedures are effective only if strictly enforced.

Wright State is committed to strict implementation of such procedures.

#### **5.5.7.1 Trips, Slips and Falls**

Worker exposure to wet floors or spills and clutter can lead to slips/trips/falls and other possible injuries. In order to keep workers safe, employers are referred to OSHA standard 29 CFR 1910 Subpart D – Walking-Working Surfaces, Subpart E – Means of Egress, and Subpart J - General environmental controls which states the following:

- Keep floors clean and dry, 29 CFR 1910.22(a)(2). In addition to being a slip hazard, continually wet surfaces promote the growth of mold, fungi, and bacteria that can cause infections.
- Provide warning (caution) signs for wet floor areas, 29 CFR 1910.145(c)(2).
- Where wet processes are used, maintain drainage and provide false floors, platforms, mats, or other dry standing places where practicable, or provide appropriate waterproof footwear, 29 CFR 1910.141(a)(3)(ii).

- The Walking/Working Surfaces standard requires that all employers keep all places of employment clean and orderly and in a sanitary condition, 29 CFR 1910.22(a)(1).
- Keep aisles and passageways clear and in good repair, with no obstruction across or in aisles that could create a hazard, 29 CFR 1910.22(b)(1).
- Provide floor plugs for equipment, so that power cords need not run across pathways.
- Keep exits free from obstruction. Access to exits must remain clear of obstructions at all times, 29 CFR 1910.37(a)(3).
- Ensure that spills are reported and cleaned up immediately.
- Eliminate cluttered or obstructed work areas.
- Use prudent housekeeping procedures such as using caution signs, cleaning only one side of a passageway at a time, and provide good lighting for all halls and stairwells to help reduce accidents, especially during the night hours.
- Instruct workers to use the handrail on stairs, to avoid undue speed, and to maintain an unobstructed view of the stairs ahead of them even if that means requesting help to manage a bulky load.
- Eliminate uneven floor surfaces.
- Promote safe work practices, even in cramped working spaces.
- Avoid awkward positions, and use equipment that makes lifting easier.

## **6 Fume Hood Inspections and Operating Procedures**

Wright State University's Department of Environmental Health and Safety and all laboratories subjected to the provisions of the OSHA Laboratory Standard will follow the procedures contained within as it relates to the inspection operations of chemical fume hoods.

### **6.1 Responsibilities**

#### **6.1.1 Environmental Health and Safety:**

Inspections – All chemical fume hoods will be inspected annually. The following hood features will be checked (refer to Section I, General Laboratory Safety).

17. Check adjustment of the back baffle slots (see Figure 1).

- 1) Slot C should be fixed (not adjustable) and set at 2.0 to 2.5 inches.
- 2) Slot B should be fixed (not adjustable) and set at 1.5 inches and should be approximately 14 inches above the work surface.

- 3) Slot A should be fixed (not adjustable) and set at 0.5 inches and located approximately 1.0 inch forward of the exhaust duct.
- b. Sash Operations – The following physical conditions should be met relating to sash operations:
- 1) The sash moves up and down easily.
  - 2) The sash does not bind at any place in the track.
  - 3) The safety glass is intact and clear, allowing for an unobstructed view of the inside of the cabinet.
  - 4) The sash heights, in the full raised position, should be between 29 and 31 inches. The preferred position is 31 inches.
  - 5) Check for leakage at the top where the vertical sash goes past the upper structure of the hood (See Figure 2).

If: A = 72 inc.

then: B = approximately 5 in. less than A.

C = 29 to 30 in.

D = 37 to 38 in.

Air Foil – All fume hoods must be equipped with an air foil. Ideally, air foil design should meet the following criteria (See Figure 3).

- 6) A should be 1.0 to 1.25 inches.
  - 7) B should be 2 inches.]
  - 8)  $\theta$  should be sloped down at an angle between 20 to 30°.
- c. Fume Hood usage: Check the following for compliance:
- 1) Work is being conducted six (6) inches back into the hood.
  - 2) The hood is not being used for storage.

- 3) There is not excessive lab apparatus in the fume hood and the present lab apparatus is not interfering with the desired air flow pattern.
  - 4) The fume hood itself (inside) is being kept clean.
2. Air Flow Measurements - Quarterly inspections will be made of all chemical fume hoods to verify proper hood operations. Annual fume hood surveys will be made relative to capture and face velocities.
- a. Smoke Velocity – Utilizing smoke tubes, a check is to be made in front of the fume hood to verify the in-flow of air into the hood and to verify the absence of serious turbulence which would throw contaminated air back into the workplace and into the staff person’s breathing zone.
  - b. Face Velocity – All hood should be equipped with a magnehelic gauge which provides for a quick and easy means of verifying average face velocity. In the absence of a magnehelic gauge or other flow-indicating device, an actual hood survey will need to be accomplished using a thermoanemometer.

#### **6.1.2 Laboratory Supervisor:**

The laboratory supervisor or his/her designee is responsible for the following daily checks:

- Verify that the fume hood is working satisfactory. This can be accomplished by the reading of the magnehelic gauge, where available. In the absence of a magnehelic gauge, a “flag” device such as a piece of tissue can be attached to the hood sash. This will not give a quantitative reading but will indicate that the exhaust fan is working. The angle that the tissue is pulled into the hood can provide a non-quantitative indication that the exhaust fan is operating satisfactory.
- Verify that the sash is working properly and used when there is potential for a reaction or fire/explosion in the hood.
- Verify that all work is conducted within six (6) inches inside the hood.
- Maintain a satisfactory level of housekeeping within the hood and ensure that the hood is not being used for storage purposes.
- Ensure that the air foil is available and installed properly.
- Keep source of air movement in front of the hood to a minimum.
- Ensure that lights and all utilities inside the hood are operational. Keep the light fixture within the hood clean.



## 7 Training and Information

Personnel training at all levels within the university is essential. Responsibility and accountability throughout the organization are key elements in a strong safety and health program.

Laboratory workers must be provided with information and training relevant to the hazards of the chemicals present in their laboratory (29 CFR 1910.1450(f)). The training must be provided at the time of initial assignment to a laboratory and prior to assignments involving new exposure situations.

At a minimum, laboratory personnel should be trained on their facility's specific CHP, methods and observations that may be used to detect the presence or release of a hazardous chemical (such as monitoring conducted by the employer, continuous monitoring devices, visual appearance or odor of hazardous chemicals when being released), the physical and health hazards of chemicals in the work area and means to protect themselves from these hazards. Trained laboratory personnel must know shut-off procedures in case of an emergency. All SDSs must be made available to the employees.

Training required by 29 CFR 1910.1450 which is applicable to laboratory operations under the OSHA Occupational Exposure to Hazardous Chemicals in Laboratories Standard will be provided either by qualified staff of the Department of Environmental Health and Safety and/or the laboratory supervisor.

Summary of training requirements:

Chemical Hygiene Plan – At the time of implementation, Environmental Health and Safety will provide training to laboratory supervisors and staff. Subsequent training will be the responsibility of the laboratory supervisor.

Chemical Inventory Procedures – At the time of implementation, Environmental Health and Safety will provide training to laboratory supervisors and staff. Subsequent training will be the responsibility of the laboratory supervisor.

Hazard Communication Plan – Environmental Health and Safety will provide the initial and refresher training.

Bloodborne Pathogen Plan – Environmental Health and Safety will provide the initial and refresher training.

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*Reminder: Bloodborne Pathogen Training is required for new employees working with blood and/or other body fluids within ten (10) days of hire.*

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Maintenance/Custodial Personnel – Environmental Health and Safety will provide the initial and annual refresher training on the Do's and Don'ts of working safely before and during tasks in the laboratory.

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Specific Needs – Other training needs that are identified by Environmental Health and Safety and/or the laboratory supervisor, which are within the expertise of the Environmental Health and Safety staff. Examples of this training include fire extinguisher training, emergency egress, use of protective equipment, etc.

Laboratory Supervisors: Laboratory supervisors are responsible for the following training within their work areas. They are also responsible for ensuring that the staff know and follow the chemical hygiene rules, and that protective equipment is available and in working order.

- Chemical Hygiene Plan – Following the initial training given by Environmental Health and Safety, the laboratory supervisor is responsible for providing training on the chemical hygiene plan to all new employees. The training and education should be a regular, continuing activity, not simply an annual refresher training as required under 29 CFR 1910.1450.
- Chemical Inventory Procedures – Following the initial training given by Environmental Health and Safety, the laboratory supervisor is responsible for training the laboratory staff to conduct the annual chemical inventory within his/her assigned laboratories.
- Job Specific Training – The laboratory supervisor is responsible for identifying specific training needs for their staff and possibly the University maintenance and custodial staff to ensure that all operations are conducted in a manner conducive to their health and well-being. The laboratory supervisor should seek the advice and assistance of the Environmental Health and Safety Department, as needed.

Information and Training Program – The training should include, but not be limited to, the following items:

## **7.1 Information and Training**

### **7.1.1 Information**

Laboratory workers must be informed about the following:

- The content of the OSHA Laboratory standard and its appendices (the full text must be made available);
- The location and availability of the Chemical Hygiene Plan;
- Permissible exposure limits (PELs) for OSHA regulated substances, or recommended exposure levels for other hazardous chemicals where there is no applicable standard;
- Signs and symptoms associated with exposure to hazardous chemicals in the laboratory; and
- The location and availability of reference materials on the hazards, safe handling, storage and disposal of hazardous chemicals in the laboratory, including, but not limited to, Safety Data Sheets (SDSs).

### 7.1.2 Training Requirements

The training must include the following:

- Methods and observations used to detect the presence or release of a hazardous chemical. These may include employer monitoring, continuous monitoring devices, and familiarity with the appearance and odor of the chemicals;
- The physical and health hazards of chemicals in the laboratory work area;
- The measures that workers can take to protect themselves from these hazards, including protective equipment, appropriate work practices, and emergency procedures;
- Applicable details of the employer's written Chemical Hygiene Plan;
- Retraining, if necessary.

## 8 Laboratory Procedures Requiring Prior Approval

It is not the intention of the Department of Environmental Health and Safety to curtail educational and research laboratory activities. It is, however, our responsibility to ensure that activities conducted in the university laboratories are done so in a manner that

1. does not effect the health and well-being of employees, students and visitors,
2. does not offer the potential for property damage as a result of fire and/or explosions,
3. does not release materials to the atmosphere or the storm and sanitary sewers which could have adverse effects on the university and/or adjacent communities, and
4. permits the disposal of waste products (biological, chemical and/or radiological) in a manner of prudent practice and in compliance with federal and state environmental rules and regulations.

To accomplish this, the Department of Environmental Health and Safety (EHS) needs to know when highly toxic and/or hazardous materials are planned for use in university laboratories. Identification of the specific agent(s), quantities of use, duration of experiment and how they will be used also needs to be known. Once this information is provided to EHS, an evaluation will be conducted to determine if the EHS staff/facilities are adequately trained/prepared in the handling, storage and disposal procedures. This evaluation will include verifying that emergency response procedures are available and acceptable, laboratory equipment is in good repair (i.e. fume hoods) and adequate for the proposed study, and all other health and safety considerations are properly addressed. Following our satisfaction that all conditions are met and that other interested parties, such as Fairborn Fire Department, Wright State Police Department, Physical Plant, etc. have also been notified, approval to proceed will be given.

The Department of Environmental Health and Safety will exercise approval authority over the following biological and chemical agents. Prior approval authority is always vested for the use of radioactive materials, radiation-producing equipment and laser systems.

- **Chemicals**: All chemicals listed under Group 1 (“carcinogenic to humans”) in the current edition of IARC’s (International Agency for Research on Cancer) Monographs.
- **Biologicals**: All **Risk Group 3** Biological agents.
- **Toxins**: All reproductive toxins and substances which have a high degree of acute toxicity to man.
- The Environmental Health and Safety Chemical Hygiene Officer (CHO) will work with the department safety representative and the applicable faculty/staff member in the approval process. The involvement of the applicable Institutional Safety Committee will be sought when deemed necessary.

## **9 Medical Consultation and Examinations**

Wright State provides provisions for medical consultation and examination when exposure to a hazardous chemical has or may have taken place for employees covered under 29 CFR 1910.1450.

The Department of Environmental Health and Safety will investigate all incidents of chemical exposures and will make recommendations for medical consultation when deemed necessary or as required by the Laboratory Standard. All costs associated with medical consultation will be paid for by Wright State University.

### **9.1 Requirements Under 29 CFR 1910.1450, Laboratory Standard**

Wright State University shall provide all employees who work with hazardous chemicals an opportunity to receive medical attention, including any follow-up examinations which the examining physician deems necessary under the following circumstances:

- Provide all exposed workers with an opportunity to receive medical attention by a licensed physician, including any follow-up examinations which the examining physician determines to be necessary.
- Provide an opportunity for a medical consultation by a licensed physician whenever a spill, leak, explosion or other occurrence results in the likelihood that a laboratory worker experienced a hazardous exposure in order to determine whether a medical examination is needed.
- Provide an opportunity for a medical examination by a licensed physician whenever a worker develops signs or symptoms associated with a hazardous chemical to which he or she may have been exposed in the laboratory.
- Establish medical surveillance for a worker as required by the particular standard when exposure monitoring reveals exposure levels routinely exceeding the OSHA action level or, in the absence of an action level, the PEL for an OSHA regulated substance.
- Provide the examining physician with the identity of the hazardous chemical(s) to which the individual may have been exposed, and the conditions under which the exposure may have occurred, including quantitative data, where available, and a description of the signs and symptoms of exposure the worker may be experiencing.
- Provide all medical examinations and consultations without cost to the worker,
- without loss of pay, and at a reasonable time and place.

### **9.2 Written Opinion**

All medical examinations and consultations will be performed by or under the direct supervision of a licensed physician.

The examining physician must complete a written opinion that includes the following information:

- Recommendations for further medical follow-up.

- The results of the medical examination and any associated tests.
- Any medical condition revealed in the course of the examination that may place the individual at increased risk as a result of exposure to a hazardous chemical in the workplace.
- A statement that the worker has been informed of the results of the consultation or medical examination and any medical condition that may require further examination or treatment. However, the written opinion must not reveal specific findings of diagnoses unrelated to occupational exposure.

The identity of the hazardous chemical, a description of the incident, and any signs and symptoms that the employee may experience will be relayed to the physician.

The requirements of providing information to the examining physician and the physician's written as addressed in 1910.1450 (g) (3) and (4) will be the responsibility of the Environmental Health and Safety Department.

### **9.2.1 Recordkeeping**

Wright State's Department of Environmental Health and Safety maintains an accurate record of exposure monitoring activities and exposure measurements as well as medical consultations and examinations, including medical tests and written opinions.

Employers generally must maintain worker exposure records for 30 years and medical records for the duration of the worker's employment plus 30 years, unless one of the exemptions listed in 29 CFR 1910.1020(d)(1)(i)(A)-(C) applies. Such records must be maintained, transferred, and made available, in accord with 29 CFR 1910.1020, to an individual's physician or made available to the worker or his/her designated representative upon request.

All accident, fatality, illness, injury, and medical records and exposure monitoring records will be retained by Wright State in accordance with the requirement of state and federal regulations (29 CFR part 1904 and 29 CFR 1910.1450(j)).

Any exposure monitoring results will be provided to affected laboratory staff within 15 working days after receipt of the results (29 CFR 1910.1450(d)(4)).

### **9.3 Requirements Under CFR 1910.20, Access To Records:**

Whenever an employee requests access to exposure or medical records, Wright State University will assure that access is provided in a reasonable time, place and manner (within 15 days) at no cost to the employee.

The medical records will be maintained for the duration of the employee's employment plus thirty (30) years. All exposure records will be maintained for at least thirty (30) years.

## 10 Particularly Hazardous Substances

Additional worker protection is required for work with particularly hazardous substances.

Particularly hazardous substances include:

- “select carcinogens”,
- reproductive toxins and
- substances which have a high degree of acute toxicity and/or hazardous properties

which in view of its intended use could present an extraordinary potential for a fire/explosion or an intense chemical reaction.

Laboratory supervisors are responsible for notifying the Environmental Health and Safety Department when work is anticipated to include *particularly hazardous substances*.

Please refer to Section 8: Laboratory Procedures Requiring Prior Approval.

The Environmental Health and Safety Department, in a cooperative effort with the researcher will ensure that a thorough review of the proposed activity is performed and that all measures necessary to protect life and property are taken before the activity commences. Measures could include, but not necessarily be limited to:

1. The establishment of a designated area for the proposed activity.
2. The use of a containment device such as a fume hood, glove box or other type of ventilated enclosure.
3. Use of explosive-proof equipment, remote operations, blast shields, etc.
4. The development of decontamination procedures following daily operations and in the event of a spill or other accident.
5. Development of emergency response procedures beyond those already established, if deemed necessary.
6. Restriction of non-laboratory personnel to the area during the duration of the research activity, if necessary.
7. Special training and medical surveillance of involved employees, if deemed necessary.
8. The posting of the laboratory and the designated area.
9. Procedures for the safe removal and disposal of contaminated waste.

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*The need for special protective measures should be identified when particularly hazardous substances are identified.*

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It is absolutely essential that Environmental Health and Safety be notified at the earliest date possible when research involving extremely toxic and/or hazardous substances is planned for use.