

Several infectious agents are known to affect embryonic development. Anyone who may become pregnant or who lives with someone who is pregnant or may become pregnant should be aware of the risks associated with these agents. The following is a partial list of infectious organisms that may have an adverse effect on human embryo and fetal development.

- Rubella virus
- Herpes simplex virus
- Varicella virus
- Toxoplasma
- HIV
- Influenza virus
- Mumps virus
- Parainfluenza type 2

This is not an all-inclusive list. Anyone wishing to become pregnant should inform her obstetrician and gynecologist of any infectious agents and chemicals encountered in her work.

Other medical restrictions or recommendations may be made on an individual basis after discussion with either an occupational medicine practitioner or personal physician.

Examples of some conditions that might warrant special precautions are HIV infection, immunosuppressive conditions, or drug therapy that suppresses the immune system. Anyone affected by these or other conditions should discuss exposure control options prior to beginning work that may expose him/her to infectious agents.

3.10 LABORATORY PRACTICES

3.10.1 Personal Protective Equipment

PPE is an essential element laboratory safety. PPE includes, but is not limited to:

- Gloves
- Laboratory coats (impervious)

- Face shields/masks
- Safety glasses/Prescription safety glasses
- Goggles
- Hoods
- Shoe covers
- Respiratory protection
- Other site-specific personal protective equipment

At a minimum, laboratory personnel shall wear gloves and a laboratory coat whenever handling biological agents, cells and tissues. Safety glasses with side shields, goggles, or face shield shall be worn when these materials could potentially be splashed in the face. Laboratory personnel should wear other personal protective equipment (apron, face shield, mask, etc.) as needed or required to prevent potentially infectious materials from reaching their clothes, skin, eyes, mouth, or other mucous membranes. PPE must be removed prior to leaving the work area and placed in designated areas. PPE must be treated as medical waste when discarded. If PPE is not disposable, PPE shall be cleaned with disinfectant before and after use.

3.10.2 Biological Safety Cabinets

Biological safety cabinets (BSCs) provide a primary level of containment for working safely with potentially hazardous biological materials. When combined with standard microbiological practices, BSCs can protect both laboratory personnel and the environment. Although some may think that the purpose of BSCs is to protect cells and cultures from contamination by bacteria and fungi, **their primary purpose is to protect the laboratory workers from exposures to potentially infectious agents.**

BSCs are designated as Class I, II, or III based on specific airflow patterns within the BSC and on the locations of high efficiency particulate air (HEPA) filters within the unit. HEPA filters are usually composed of a pleated sheet of borosilicate fiber material that has been treated with a wet-strength water-repellant binder. These filters are specifically designed to remove particles equal and greater than 0.3 microns with an efficiency of 99.97%. This filtration level will capture a majority of bacteria, spores, and viruses from the filtered air.

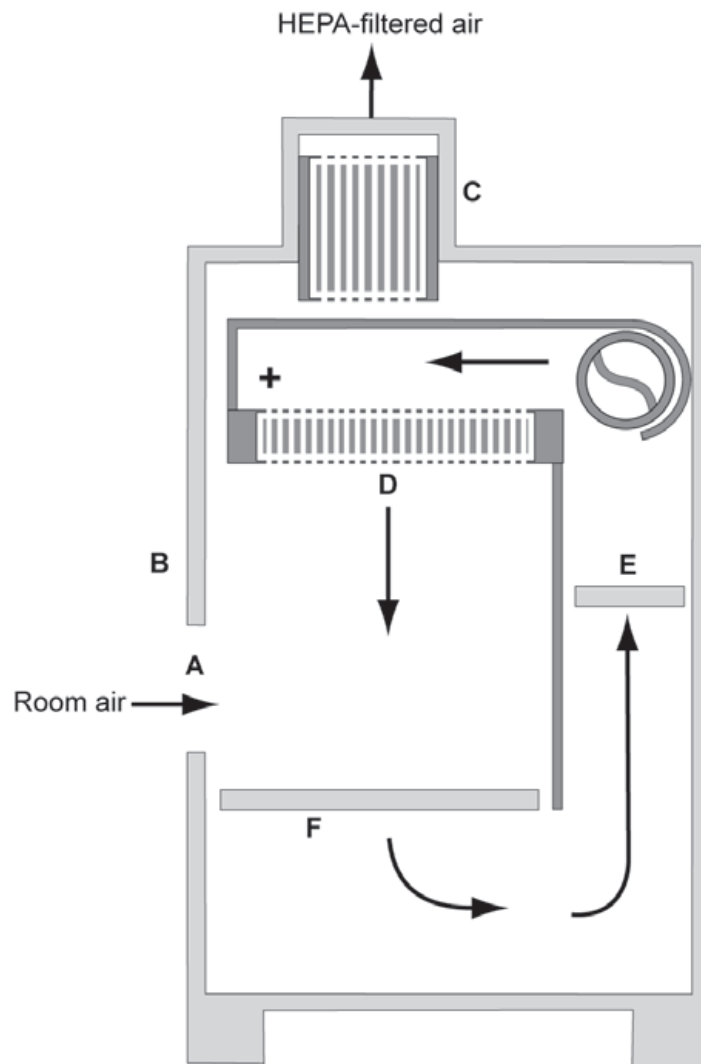


Figure 3.1 Tabletop Model of a Class II, Type A2 Biosafety Cabinet

(A) front opening; (B) sash; (C) exhaust HEPA filter; (D) supply HEPA filter; (E) positive pressure common plenum; (F) negative pressure plenum. The Class II Type A2 BSC is not equivalent to what was formerly called a Class II Type B3 unless it is connected to the laboratory exhaust system. Note: The A2 BSC should be canopy connected to the exhaust system. (Figure taken from *Biosafety in Microbiological and Biomedical Laboratories*, Fifth Edition, 2009.)

Implementation of the following procedures will ensure optimal operation of a BSC:

- Front and rear grills should be free of clutter to allow proper air intake.
- Sash should not be raised above the specified level.
- Bunsen burner use will cause airflow disruptions and damage to the HEPA filter, and should be avoided
- Certification must be performed annually.

BSCs are required to be tested and certified annually by technicians accredited by the National Sanitation Foundation (NSF International). Additionally, BSCs will be certified when they are first installed and whenever they are moved, even to a nearby laboratory, because the HEPA filters may be dislodged from their proper fitting during these moves.

3.10.3 Biological Waste Procedures

Biological waste may be disposed of in three ways: designated biological waste box, chemical disinfection, and steam sterilization/autoclave. Appropriate disinfection procedures will be chosen and utilized in accordance with both the PI and the BSO in order to ensure adequate decontamination of biological wastes.

Liquid biological waste must be rendered non-infectious by steam sterilization or chemical disinfection prior to sink disposal. If chemical disinfection is selected, full-strength household chlorine bleach may be added to the waste container, such as an aspiration flask, so that the **final** solution concentration of bleach will be 10%. Contact time should be at least 30 minutes prior to sink disposal for bleach.

NOTE: If bleach is not an adequate disinfectant for the biological agent in use, an U.S. Environmental Protection Agency (EPA) approved disinfectant must be used. Ensure the proper contact time prior to disposal.

Before disposing of the treated solution down the sink, check the pH to ensure it is within the permissible pH **range** (5.5 – 12.0 standard units). If it is within the permissible range, then disposal of the treated solution in the sink should be done with running tap water to minimize possible plumbing damage due to the corrosive effects of the disinfectants. Autoclaving solutions containing bleach is **not permitted** due to the potential for production of toxic chlorine gas.

3.10.4 Sharps Management

Some of the most serious accidents in biological laboratories are those caused by puncture wounds through skin (percutaneous exposures). All objects that can puncture skin are designated as sharps and require special disposal treatment. Examples of sharps include hypodermic needles, glass Pasteur pipettes, razor blades, broken glass, and

suture needles. Sharps must be disposed of separately from all other waste streams and sharps containers cannot be disposed of with other biological waste.

Federal regulations concerning sharps primarily focus on work with human bodily fluids. Research work conducted with animals only is not required to utilize engineered sharps; however, it is recommended that engineered devices be used whenever practical. Because the majority of laboratory biohazard injuries are due to hypodermic needles, special attention has focused on their use and disposal. Some guidelines include:

- Minimize use of needles and syringes.
- Do not bend, shear, or break needles.
- Do not recap needles.
- Do not remove needles from syringes.
- Throw away the entire syringe-needle combination.
- Be careful during cleanup; some sharp items may be hidden in the waste materials.
- If a needle stick occurs, encourage the wound to bleed for a few minutes, wash the area, and then get medical attention immediately.

In 2001, in response to the [*Needlestick Safety and Prevention Act*](#), OSHA revised the BBP Standard 29 CFR [1910.1030](#). The revised standard clarifies the need for employers to select safer needle devices and to involve employees in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps. Further information can be found at <http://www.osha.gov/SLTC/bloodborne pathogens/index.html>.

To prevent injury from sharps, place all needles, Pasteur pipettes, syringes, suture needles, scalpels, and razor blades into standard sharps containers. Large volumetric/serological pipettes, or other items that can puncture the biological waste red bags should be disposed of in Sharps Boxes. Sharps containers must be leakproof, rigid, puncture-resistant, shatterproof containers that are marked prominently with the universal biohazard warning symbol and the word “Biohazard” in a contrasting color. Place sharps containers in convenient locations near work areas so they will be used. **Do not overfill the sharps containers.** Containers should be sealed when they are three-quarters (3/4) full.

3.10.5 Disinfection, Decontamination, and Sterilization Methods

Disinfection and decontamination are terms that are often used interchangeably, but they each have specific definitions. Disinfection is a chemical or physical treatment that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects. Decontamination refers to a chemical or physical treatment that removes pathogenic microorganisms from objects so they are safe to handle, use, or discard. A number of disinfectants are commonly used in laboratory settings, particularly to wipe down surfaces to remove infectious agents. Types of disinfectants and their uses are summarized in Table 4.2.

Table 3.2 Summary of Disinfectants and Their Uses			
Disinfectant	Final Concentration	Effective On	Ineffective On
Sodium Hypochlorite Bleaches: e.g., Clorox™*	1:10	Bacteria, some spores, viruses, TB†, HIV	Some spores
Chlorine Dioxide: e.g., Clidox®-S*	1:5:1 or 1:18:1	Bacteria, spores, viruses, TB	
Alcohols (Ethanol, Isopropanol)	70%	Bacteria, most viruses	Spores, TB
Quaternary Ammonium Compounds: e.g., Quatricide®*	Ready to use	Bacteria, spores, viruses, TB	
TB tuberculosis HIV human immunodeficiency virus * The use of brand names does not imply a recommendation. † Use 1/5 dilution			

Sterilization is a chemical or physical treatment that destroys or neutralizes all forms of microbial life. The most common method of sterilization in a laboratory setting is autoclaving. Autoclaves work by denaturing biological molecules with superheated steam; dry heat is not nearly as effective. For example, it takes 12 minutes to kill most spores with steam at 121 degrees Celsius (°C), while 6 hours are required with dry heat at the same temperature. It is the steam that kills.

As a result, anything that does not come in contact with steam inside the autoclave may not be adequately decontaminated. The potential for inadequate decontamination

becomes a greater concern when sealed biohazard bags are placed in an autoclave. There are two simple solutions: 1) cut open the bag, or 2) place about 200 milliliters of water in the bag before sealing.

Typically, bags (24" x 36") of solid plastic waste take from 45 minutes to 1 hour to reach sterilizing temperatures throughout its contents.

Autoclaves should be tested routinely and validated to insure that they are operating properly and killing the biological organisms in each autoclave load. The preferred method for autoclave validation is to test it with a commercial spore test system. This system contains a color indicator and a thermophilic bacterial species, such as *Bacillus stearothermophilus*, that is tolerant to high temperatures. The system is autoclaved under realistic conditions, such as in the middle of a bag of waste, and then incubated. If the spores grow, a color change will occur indicating inadequate sterilization in the autoclave. If there is no growth, no color change occurs and the autoclaving procedure is adequate.

Using an established autoclave test procedure, quarterly checks with a biological indicator are usually adequate to assure proper autoclave function and to detect gradual deterioration of operation. It is important to note that autoclave tape indicates only that a critical temperature was reached; it **does not** indicate the length of time at the desired temperature or whether steam was present.

In the research laboratory setting, the target organisms to be killed are usually known and are usually heat sensitive. In practice, the same autoclave is used for sterilizing laboratory materials and waste. If sterilized materials are subsequently determined to be contaminated, it is an indication that the autoclave is not working properly.

The following tips will help prevent injury and property damage when using the autoclave.

- Do not overfill containers. Leave the top third as empty expansion space.
- Use only vented closures.
- Place contaminated materials in autoclave bags. Place bags inside plastic or metal trays when autoclaving.
- Use only containers designed for sterilization. Use plastic or metal trays.

Bottles should be cool to the touch before attempting to remove them. Do not place hot bottles directly on a room temperature or cool surface. Tighten screw caps when the liquid is completely cooled.

3.10.6 Spill Response

The following procedures are recommended for the management of small spills of blood, body fluids, or other potentially infectious materials in the laboratory or in a biosafety cabinet.

- Put on protective clothing (laboratory coat, gloves, face and eye protection, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, and paper towels).
- If the spill has occurred in a biosafety cabinet, keep the cabinet turned on.
- Spray the affected area with a disinfectant, such as a fresh 10% bleach solution.
- Pick up any broken glass with forceps and dispose it in a sharps container.
- Let disinfectant sit for 30 minutes.
- Soak up the disinfectant and spill with paper towels.
- Discard all clean-up materials in a biological waste box. Autoclave any reusable items, such as laboratory coats.
- Remove PPE and place disposable PPE into a biological waste box. Reusable PPE should be cleaned with the proper disinfectant.
- Wash hands and exposed skin areas thoroughly with soap and water.

The following procedures are recommended for a large volume biological spill in the laboratory area, in a BSC, or if equipment malfunctions while processing biological materials:

- If the spill occurs in a BSC, close the sash and leave the BSC running.
- Keep people out of the area to prevent spread of the contamination. Put up a warning sign indicating that there was a spill in the BSC, the steps taken to treat/contain the spill, and contact information for a responsibly party.
- Remove any contaminated clothing and put it into a biohazard bag for decontamination later.

- Wash hands and exposed skin thoroughly with soap and water.
- Call BSO at (202) 806-9710 to report the size, location, and composition of the spill.

3.11 SHIPPING OF HAZARDOUS MATERIALS

Import, export, and interstate transport of hazardous materials are subject to requirements and laws from several regulatory agencies. The U.S. Public Health Service (PHS), U.S. Department of Transportation (DOT), U.S. Department of Agriculture (USDA), and U.S. Postal Service, regulate transport of hazardous materials by rail, air, vessel, and public highway. The guidelines and regulations of the International Air Transport Association (IATA) and International Civil Aviation Organization also apply when shipping substances by air. Import/Export Permit requirements are regulated by the Bureau of Customs; the Department of Commerce, CDC, and USDA require permits for certain materials. Materials considered hazardous and regulated for shipping purposes include hazardous chemicals, wastes, etiologic agents, infectious substances, diagnostic specimens, and dry ice.

The PHS defines etiologic agents as viable microorganisms that cause disease in humans; infectious substances are those substances that contain etiologic agents. This terminology is used by the DOT and IATA. Diagnostic specimens are anything that the shipper reasonably believes to contain an infectious substance. Diagnostic and infectious specimens are regulated by the USDA, U.S. Food and Drug Administration (FDA), PHS, and IATA. Biological product means a product prepared in accordance with regulations that govern the manufacture of vaccines, reagents, or all viruses, serums, toxins, etc. intended for use in the diagnosis, treatment, or prevention of diseases in humans or animals. Biological products are regulated by the USDA, FDA, PHS, DOT, and IATA.

Laboratory staff may receive awareness training from EH&S office for the shipment of hazardous materials. Individuals packaging specimens/hazardous materials for shipment must receive function-specific training. The training is required every two years or when there is change in the regulations. For assistance regarding training and other requirements necessary for the legal shipping of hazardous materials, please contact EH&S office at (202) 806-1033.