Office of Research Safety
Laboratory Safety Manual
307 Biosafety Practices

Introduction

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

Procedures for Biohazard Control

Each laboratory supervisor must develop or adopt safety and operational procedures to identify the hazards that will, or may likely, be encountered. They must also specify practices and procedures designed to minimize or eliminate identified risks, as well as the procedures to be used in the event of an accidental exposure. Personnel must be required to read and follow the established practices and procedures and must be advised of any special hazards present in the laboratory.

OSHA Bloodborne Pathogens Standard

Any laboratory which works with potentially infectious material (i.e. blood, tissue, viruses, etc.) must comply with the Occupational Safety and Health Administration's Bloodborne Pathogens Standard. Under this law, the employer is required to ensure that the laboratory employee is trained on appropriate methods to control accidental exposure to potentially infectious agents. Employers must communicate the potential hazards, provide employees with appropriate personal protective equipment (gloves, eye protection, lab coats etc.) and ensure these are used whenever the potential exists for accidental exposure.

Routes of Transmission

Exposure and potential infection from biological material can occur by one or multiple forms of direct contact between the laboratory worker and an organism. For these reasons, workers must always be on guard against the following types of exposure and must take adequate measures to reduce the risk to the following types of contact.

Respiratory Route Infection

A variety of agents infect by the respiratory route. Aerosol generation and dissemination can be reduced by the following:

Properly operating laminar-flow biological safety cabinets for protection against immediately generated aerosols.

Thorough decontamination of work surfaces before and after work following spills of biohazardous material. This method is particularly effective in preventing secondary aerosols generated by agents resistant to drying.

Use of absorbent materials on immediate work surfaces, to contain splashes and drips.

Infection by Ingestion

A variety of organisms used in the laboratory are enteric pathogens which use ingestion as the primary route of infection (intestinal parasites, Salmonella, agents of infectious hepatitis, polio virus, and enteropathogenic E. coli strains). Infection by these organisms generally occurs in the following ways:

Direct ingestion of the culture by mouth pipetting.

"Hand to mouth" infection whereby infectious materials are indirectly transferred by the hand to the oral cavity. Activities such as smoking, eating, and drinking are therefore prohibited in laboratories. Frequent handwashing with germicidal soap between activities is strongly recommended.

Needlesticks, Punctures, Contact with Non-intact Skin

Contact can be avoided by limiting the use of needles and syringes and by using nonbreakable containers whenever possible.

Workers' hands must never come into direct contact with infectious agents. Therefore, gloves should be worn and discarded appropriately before handling other equipment or objects.

Exposure to Mucous Membranes

All manipulations capable of generating a splash or spray must be conducted within a biological safety cabinet with the sash and seat properly adjusted to afford protection of the eyes.

Manipulations which may create splashes and that cannot be conducted within a biological safety cabinet (e.g. disposal of disinfected liquid waste to the sanitary sewer) must be performed while wearing eye protection and a mask (to protect the nose and mouth) or a full face shield. Work can also be conducted behind an acrylic splash shield; however, this must be adequately sized to provide ample protection against eye, nose and mouth exposure.

Operations and Equipment

Handwashing

One of the most effective methods of protection against accidental exposure to potentially infectious agents is executed every time an individual washes his/her hands. Hand contamination with transient microbes (some of which can be pathogenic) can easily occur during manipulation of specimens, equipment and supplies as well as from contact with work surfaces. For these reasons, it is important that all laboratory personnel wash their hands:

a. whenever they come on duty

b. when leaving the laboratory for whatever reason

c. when hands are obviously soiled

d. before and after completion of a task in a biological safety cabinet, even if gloves are worn

e. upon completion of tasks

Standard handwashing protocol:

Turn on the faucets and wet the hands using warm water.

Dispense antiseptic soap compound into a cupped hand.

Spread the soap around both hands and between the fingers. If needed, add a small amount of water to facilitate spreading and lathering.

Wash the hands for approximately 10 seconds. Vigorously rub both sides of the hands beginning a few inches above the wrists and moving downward between the fingers, around and under the fingernails.

Rinse thoroughly under warm water beginning with the area above the wrists and continuing downward past the fingers.

If the sinks are foot, knee or elbow operated, turn off the water. If not, leave the water running, dry the hands with paper towels and then use the towel (as a barrier to your clean hand) to turn off the faucet.

Pipetting

Mouth pipetting is prohibited in all situations. Use one of the mechanical aids that are commercially available. Delivery must be accomplished with the tip of the pipette resting against the container, allowing the fluid to flow down the surface thereby minimizing aerosols. In addition, the following practices must be observed:

No infectious mixture should be prepared by bubbling air through the liquid with the pipette.

No infectious material should be forcibly discharged from a pipette.

Placing a disinfectant soaked towel over the immediate work surface is useful in minimizing aerosolization from accidental splashing.

Use of Syringes and Needles

To reduce the risk of accidental injection, aerosol production or spills, the following practices must be observed:

Restrict the use of needles and syringes for practices in which no alternative is available, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles.

Do not use a syringe and needle as a substitute for a pipette in making dilution's of hazardous or infectious fluids. Syringe-type pipettes with blunt-ended delivery are preferable.

Reusable or disposable syringes used with biohazardous materials should be of the LEUR-LOK or equivalent type to assure that the needle cannot separate during use.

Used disposable needles must not be bent, sheared, broken, recapped, or removed from disposable syringes.

Disposable needles and syringes must be disposed as a single unit into puncture-resistant leak-proof "sharps" containers. Full containers must be sealed and placed into the red bag waste stream for incineration.

Syringes not associated with needles or which have not come into contact with biohazardous material must also be placed in puncture-resistant leak-proof containers before being placed into the red bag waste stream for incineration. Syringes and needles must never be discarded into the regular waste stream.

Never discard syringes and needles into pans containing pipettes or other glassware which must be separated from syringes and needles.

Use of Centrifuges and Shakers

To reduce the opportunity for aerosol production of biological material when using centrifuges and shakers, the following practices must be observed.

All tubes must be capped.

Biohazardous agents must be centrifuged in an enclosed centrifuge with sealed rotor. Safety cups and rotors with covers and O-rings are both effective at minimizing aerosol production.

Decanting from centrifuge tubes must be performed in a biological safety cabinet.

When mixing broth cultures utilizing a Vortex or similar mixer, avoid wetting the plug or cap.

As an additional safety measure, centrifuges and shakers are not permitted in corridor areas and must be housed within laboratory or common equipment spaces.

Note: Items for centrifugation should always be balanced to avoid vibration, which can result in failure of the unit as well as considerable aerosolization.

Opening Culture Plates, Tubes, Bottles, and Ampules

Aerosols are produced when contaminated plugs or screw caps are removed from tubes and bottles. Employing good, sterile technique when opening tubes, bottles and culture plates will minimize the potential for aerosolizing the culture.

Opening ampules is also potentially hazardous after the seal has been broken because air rushing in causes the dry contents to be dispersed.

After scoring the ampule with a file, wrap it in cotton that has been wet with disinfectant. Wear gloves.

If a disinfectant may damage the culture, use a biological safety cabinet and the following procedure:

After scoring the ampule with a file, apply a hot, glass rod to the mark. The glass will crack, allowing air to enter the ampule and equalize the pressure. After a few seconds, wrap the ampule in a few layers of tissue, and break it along the crack. The tissues and ampule neck must be discarded appropriately.

Employing good, sterile techniques when opening tubes, bottles and culture plates will minimize the potential for aerosolizing the culture. Also, it is recommended that a culture plate be open so that the lid is between you and the culture medium.

Blenders, Ultrasonic Disintegrators, Grinders, Mortars and Pestles, and Homogenizers

Blenders, disintegrators, grinders and homogenizers release considerable aerosols during their operation.

Blending, grinding, and homogenizing must be performed within a biological safety cabinet.

Disinfectant-soaked absorbent material can be placed over the blender during operation to further reduce the production of aerosols.

Water Baths and Warburg Baths

It is recommended that water baths and Warburg baths used to inactivate, incubate or test biohazardous materials, contain a disinfectant such as Clorox (2.9 ml/3.8 L of water) or a phenolic detergent (29 ml/3.8 L of water). Water should be changed at frequent intervals.

Laboratory vacuum lines

When a laboratory vacuum is used to manipulate biohazardous materials, a trap containing a suitable disinfectant must be employed to ensure that the building vacuum lines do not become contaminated. Clorox, added such that the final concentration will equal 10%, is a suitable agent. An inline filter must also be present between the trap and vacuum line. Empty all traps frequently and whenever more than 3/4 full.

Contaminated Glassware (flasks, beakers, reusable pipettes, etc.)

Contaminated glassware and similar materials which will be used again must be disinfected before washing.

Labeling

Storage vessels containing biohazardous agents must be labeled to provide identification of their contents. Equipment used for the manipulation or storage of biohazardous material must be labeled with a biohazard sticker and a description of contents (e.g. human cell lines).

Contaminated Materials

Contaminated materials that are transferred from work sites to decontamination and disposal staging areas shall be properly labeled with the individual's name and transported in a manner that prevents accidental spills.

Containers

Nonbreakable impermeable closed containers must be used during transport of biohazardous material through a building corridor or between buildings.

Personal Protective Equipment (PPE)

Gloves and adequate protective clothing such as a fully fastened laboratory coat must be worn as a minimal form of protection against exposure to biological agents. When additional risks are present, other types of PPE may be necessary (such as faceshields to protect against splashing, etc.). PPE must not be worn outside the laboratory nor to public eating areas.

Refrigerators, Deep Freezers and Dry Ice Chests Used to Store Biological Material

Refrigerators, deep freezers, and dry ice chests must be checked, defrosted and disinfected periodically. Remove any samples which may have broken during storage.

Equipment containing potentially biohazardous material must be locked at all times when stored outside of the laboratory in a hallway or common equipment area. Such equipment must also be labeled with the name and telephone number of a contact individual, as well as the laboratory room number, in the event of an equipment failure.

Class II Biological Safety Cabinets

General Information

The Class II cabinet, also known as the biological safety cabinet, provides protection of personnel as well as the product. The cabinet has an open front with inward air flow for personal protection. Air flowing downward over the working surface is filtered by a high efficiency particulate air (HEPA) filter for product protection. The cabinet exhaust air is also filtered through a HEPA filter.

Cabinet Usage

The Class II Type A biological safety cabinet is used when working with infectious agents requiring Biosafety Level 2 or 3 containment. It is not for use with volatile or toxic chemicals and radionuclides, since the HEPA filtered cabinet exhaust is discharged into the workspace.

The Class II Type B biological safety cabinet differs from Type A in that it is hard ducted to the exhaust system and has an increased face velocity. These features allow for work with small amounts of toxic chemicals and radionuclides in addition to infectious agents.

Effectiveness

The effectiveness of Class II cabinets in controlling contamination depends on:

The integrity of the filter

Filter housing

The uniformity of air flow

Proper decontamination methods

Certification/Decontamination

All biological safety cabinets must be certified (to be working correctly) at least once each year or whenever the equipment is relocated.

Decontamination of a biological safety cabinet must be performed prior to moving the equipment. It is also recommended whenever the use of the cabinet changes.

CAUTION: Formaldehyde decontamination procedures should not be attempted by research personnel unless they have received proper instruction

Posting of Biological Hazard Signs

Purpose

The necessity for establishing policies and procedures for proper identification of hazardous biological agents within the University laboratories is to alert support personnel who may enter the area to take precautionary measures and to restrict traffic to potentially hazardous areas.

Responsibility

It is the primary responsibility of the Principal Investigator (PI) to properly identify biohazards. Upon determination that a potential biohazard exists, the PI should notify Laboratory Safety Officer, ext 4996.

Biohazard Warning Sign

To ensure proper identification, a standardized, easily recognized sign is essential.

For the purpose of issuance, the term "biohazard" includes only those infectious agents presenting a risk or potential risk to the well-being of a human.

The warning sign shall be prominently placed so that it can be easily seen and shall be displayed ONLY for the purposes of signifying the presence of actual or potential biohazardous agents.

Recommended Biosafety Levels for Infectious Agents and Infected Animals

The selection of an appropriate biosafety level or work with a particular agent or animal study is dependent upon a number of factors. The most important of these include the:

Virulence, pathogenicity, biological stability, and communicability of the agent

Nature or function of the laboratory

Quantity or concentration of the agent

Endemicity of the agent

Availability of effective vaccines or therapeutic measures

Documented or suspected route of transmission of the agent

In general, the biosafety level used for activities involving infectious agents or infected animals must be commensurate with that required for the agent of highest virulence known, or likely to be encountered in the course of the contemplated work. For example, all material of human origin, including cell lines, tissue, and blood, must be considered potentially infectious for hepatitis and HIV and handled under Universal Precautions, which reasonably preclude cutaneous, oral, and parenteral exposure to personnel.

If, in the course of diagnostic or other laboratory examination, there is evidence that the materials being studied contain an agent of higher or lower risk than expected, the biosafety level can be raised or lowered accordingly.

Occasions will arise when it will be necessary to assign a biosafety higher than that recommended in these guidelines. For example, a higher biosafety level may be indicated by the unique nature of the proposed activity (e.g., the need for special containment for experimentally generated aerosols for inhalation studies).

It is the responsibility of the Principal Investigator to inform the Biosafety Committee when he or she begins to work or ceases to work with any agents.

Summary of Laboratory Practices for Each Biosafety Level*

Biosafety Level 1 (Risk Group 1)

This level is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

Biosafety Level 2 (Risk Group 2)

This level is similar to Level 1 and suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by scientists competent in this biosafety level, (2) access to the laboratory is limited when work is being conducted and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

Biosafety Level 3 (Risk Group 3)

This level is applicable to clinical, diagnostic teaching, research, or production facilities in which

work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. No work is performed with agents requiring Biosafety Level 3 containment at KSU.

Biosafety Level 4 (Risk Group 4)

This level is reserved for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease. No work is performed with agents requiring Biosafety Level 4 containment at KSU

The former classification according to Biological Safety Level (BL), has been replaced with the World Health Organization classification according to Risk Group (RG). The terms are equivalent.

Summary of Classification of Biological Agents According to Risk

(Modified from Biosafety in Microbiological and BioMedical Laboratories, Centers for Disease Control and the National Institutes of Health, 3rd Ed., 1993.)

This list is not all-inclusive. For information relating to additional agents not listed below, contact the Department of Environmental Health and Safety at extension 3-6260.

Biosafety Level 2: Bacterial Agents†

Bacillus anthracis
Bordetella pertussis
Campylobacter - all species
Chlamydia psittaci, C. Pneumoniae, C. trachomatis
Clostridium botulinum, C. tetani
Corynebacterium diptheriae
Leptospira interrogans- all serovars
Legionella pneumophila; other Legionella-like agents
Mycobacteria except M. bovis or M. leprae
Neisseria gonorrhoeae, N. Meningitidis
Pseudomonas psudomallei
Salmonella - all serotypes
Shigella - all species and all serotypes
Vibrionic enteritis (Vibrio cholerae, V. parahaemolyticus)

† Additional primary containment and precautions, such as those described for work at the BL-3

level are recommended for activities with high potential for droplet or aerosol production, for work with antibiotic - resistant strains and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 2: Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Sporothrix schenckii

Pathogenic members of the genera Epidermophyton, Microsporum and Trichophyton

Miscellaneous molds

Cladosporium (Xylohypha) trichoides
Cladosporium bantianum
Penicillium marnefii
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Dactylaria gallopava (Ochroconis gallopavum)

Biosafety Level 2: Parasitic Agents

Nematode parasites of humans

Ascaris spp.
Stronglyoides spp.
Hookworms
Enterobius spp.

Protozoal Parasites of Humans

Toxoplasma spp. Plasmodium spp.
Trypanosoma spp. Entamoeba spp.
Coccidia spp.
Giardia spp.
Leishmania spp.
Sarcocystis spp.
Cryptosporidia spp.

Trematode Parasites of Humans

Schistosoma spp.
Fasciola spp.
Cestode Parasites of Humans
Echinococcus granulosus
Taenia solium (cysticercus cellulosae)
Hymenolepsis nana.

Biosafety Level 2: Viral Agents ††

Hepatitis A, B, C, D, and E virus
Herpes viruses - except Herpesvirus simiae (Monkey B virus) which is BL-4
Influenza virus
Polioviruses
Poxviruses - all types except variola which is restricted
Rabies virus

†† Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 3: Bacterial Agents

Brucella - all species
Francisella tularensis
Mycobacterium bovis; M. tuberculosis
Yersinia pestis

Biosafety Level 3: Fungal Agents

Coccidioides immitis
Histoplasma capsulatum

Biosafety Level 3: Viral and Rickettsial Agents

Lymphocytic choriomeningitis virus (LCM)
Rickettsiae - all species when used for transmission or animal inoculation experiments
Vesicular Stomatitis Virus
Retroviruses, including Human and Simian Immunodeficiency viruses (HIV and SIV)
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru and related agents)

Arboviruses and Arenaviruses

A complete listing of all arboviruses and arenaviruses assigned to Biosafety Levels 2 and 3 is available from the Department of Environmental Health and Safety at extension 3-6260.

Respiratory Protection Program for M. tuberculosis

Appropriate respiratory protection (filtration masks approved by the National Institute of Occupational Safety and Health [NIOSH] for TB protection) will be worn by persons potentially exposed to M. tuberculosis in settings where administrative and engineering controls may not provide adequate protection. Such settings include TB isolation rooms and areas in which patients who may have infectious TB are undergoing cough-inducing or aerosol generating procedures. Appropriate respiratory protection must also be made available to individuals working with TB positive diagnostic samples in clinical settings, for the purpose of emergency cleanup procedures.

A representative of the Department of Environmental Health and Safety will supervise the selection and use of respirators based on the type of hazard, the conditions for potential exposure and the severity of the potential exposure.

Cell Culture Systems

Cultured cells are a routine source material in many research laboratories. Most cultured cells are known to harbor viruses either adventitiously (in many cases of detectable C-type particles) or deliberately (as in the cases of SV40 transformed rodent and human cell lines or human lymphoid cell lines, which are transformed by Epstein-Barr virus).

Long term culture of cells may enhance the risk of rescuing an oncogenic agent, whereas an autonomous infectious virus is more likely to be released upon short-term manipulation (two to three weeks) of freshly isolated cells.

It is therefore prudent to adopt Universal Precautions for the handling of cultured cells. All cell manipulations should be performed in a biological safety cabinet using BL-2 practices and procedures, including the use of personal protective equipment such as a buttoned lab coat and gloves.

Shipment of Diagnostic Specimens and Infectious Substances*

Adopted with modification from the International Air Transport Association (IATA) publication "Dangerous Goods Regulations, 37th Edition", Effective January 1996.

Definitions:

Infectious Substances: Substances containing viable microorganisms including a bacterium, virus, rickettsia, parasite, fungus or a recombinant, hybrid or mutant that are known or reasonably believed to cause disease in humans.

Diagnostic Specimens: Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, being shipped for purposes of diagnosis, but excluding live infected animals.

Shipper's Responsibility

Investigators must be aware that all biological products, both diagnostic and infectious, are subject to specific shipping regulations. The Shipper must determine whether a substance is infectious and therefore classified as dangerous goods, or whether it can be classified as a diagnostic specimen. The Shipper is responsible for properly packaging, labeling and documenting all shipments. The Shipper's responsibility does not end when the Carrier accepts the package. The Shipper's responsibility ends when the package arrives at its destination in good condition.

Emergency Response

Biological Spills Inside a Biological Safety Cabinet

Leave the cabinet operating in order to contain aerosols.

Initiate cleanup as soon as possible with a suitable disinfectant such as 10% Clorox.

Items within the cabinet should be wiped carefully, with disinfectant.

Allow the cabinet to run at least 10 minute after cleanup before activity is resumed.

Biohazard Spills Outside of a Biological Safety Cabinet

The following procedure should be followed in the event of a spill of a Biosafety level 1 or 2 agent outside of a biological safety cabinet:

Notify others of the spill.

Remove any contaminated clothing and wash any affected body parts with a disinfectant soap.

Wearing personal protective equipment, cover the spill with paper towels, and add a suitable disinfectant such as 10% Clorox. Allow at least twenty minutes of contact time.

Remove toweling, and wipe entire area with 10% Clorox.

Dispose of all cleanup materials as biohazard waste

Biohazardous Waste

The following categories are considered potentially infectious and must be packaged as red bag waste items. Red bags must be of adequate strength to resist punctures. These bags containing the waste material must then be placed into an appropriately labeled box (which is resistant to moisture) prior to transport off site for incineration.

Biohazardous Waste Definitions

Waste material which meets any of the following definitions must be disposed in a responsible safe manner.

Human Waste

All human blood, tissues, body fluids, secretions, excretions, organs, and cadavers. All items contaminated with human waste are also considered to be infectious biohazard waste, even when the source patient is not known to have a communicable disease or to be on Isolation Precautions.

Sharps Waste

All needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, culture dishes, suture needles, slides, cover slips and other broken or unbroken glass or plasticware.

Microbiological Waste

All materials containing or in contact with cultures of microbiological organisms and all patient specimens sent for microbiological culture or items contaminated by patient specimens.

Biological Materials Waste

All discarded vaccines, immunoglobulins, plasma, albumin, blood or tissue fractionation products, enzyme preparations, etc.

Animal Waste

All animal carcasses, tissues, blood, body fluids, and all items grossly contaminated with these items.

Animal Pathogen Contaminated Waste

All bedding and other materials contaminated with blood, excreta or secretions of animals infected with transmissible human or animal pathogens.

Decontamination, Sterilization, Disinfection

All biohazardous waste must be rendered noninfectious prior to final disposal. While Biosafety Level-3 and the higher risk BL-2 agents must be autoclaved prior to further processing, most infectious material can be disposed safely through proper handling and packaging directly for incineration. The guidelines listed in Appendix D are intended to ensure that all biohazardous waste is processed in a safe and timely manner, as well as in accordance with all applicable regulations.

Each generator of biohazardous waste has an obligation to handle and dispose their material in a manner which affords protection from leakage and injury or exposure to service personnel handling their waste material.

Each individual working with biohazardous material or contaminated items is responsible for their decontamination, disinfection, and appropriate preparation prior to disposal or reuse.

All laboratories, in which work with biohazardous materials is carried out, must have labeled, leak-proof, covered containers for temporary holding of infectious materials awaiting disinfection or disposal.

When autoclaving:

test tape or another suitable indicator must be used on each load placed in the autoclave. This will aid in determining which items have been sterilized.

waste bags must be marked with the room number from which the waste originated.

bagged waste must be placed into a containment pan prior to autoclaving. The purpose of the containment pan is to prevent release of material in the event that the bag loses its integrity during the cycle. This prevents waste from building up within drainage pipes, allowing for proper function of the autoclave.

only approved autoclavable bags are to be used. Red bags are reserved for incineration, and are not acceptable for autoclaving.

autoclaves may only be operated by trained individuals. Operators must never attempt to open an autoclave door while the chamber contains any pressure. Doing so may result in severe burns, forceful release of the autoclave door and injury, as well as damage to the unit.

log sheets must be available at each autoclave to record the name of the user, time of run, and amount being autoclaved.

All floors, laboratory benches, and other surfaces in areas where biohazardous materials are handled must be disinfected upon completion of operations involving plating, pipetting, centrifugation and similar procedures.

Floors should be mopped with disinfectant. Avoidance of dry sweeping and dusting will reduce the formation of aerosols. If sweeping is necessary, a push broom and floor sweeping compounds should be used. Waxing and buffing should be done only after mopping.

Floor drains must be flooded with water periodically in order to fill traps and prevent the backflow of sewer gases.

Specific Disinfection and Sterilization Methods

Wet Heat

1. The destruction of all forms of microorganisms is most readily accomplished by wet heat or autoclaving (saturated steam under pressure).

Higher pressures give higher internal temperatures.

Appropriate biological indicators should be used in containers or between densely packed materials to determine the effectiveness of the decontamination cycle.

Other critical factors which ensure the effectiveness of the autoclaving (besides saturated steam and proper temperature) are the removal of air from the chamber and its contents and adequate exposure time as related to the "soil" load on contaminated items.

Heavily "soiled" items, especially if the "soil" is of proteinaceous nature, should not be flash autoclaved because that "soil" may briefly protect the microorganism from the lethal effects of the wet heat.

Autoclave times are directly proportional to the volume of materials to be autoclaved. Twenty min. at 121oC is adequate for the smallest loads. When volumes in excess of 500 ml are autoclaved, times must be increased. Consult the autoclave manufacturers' handbook for your unit.

It should also be noted that overloading or underloading of an autoclave also reduces the efficiency of decontamination.

Suggested Temperatures and Exposure Times from NIH Biohazards Guidelines

Laundry - 121oC (250oF), 30 min.
Trash - 121oC (250oF), 1 hr.
Glassware - 121oC (250oF), 1 hr. or 160o 320oF) dry heat, 4 hr.
Liquids - 121oC (250oF), each gallon, 1 hr.
Small Animals - 121oC (250oF), 8 hr.

Never autoclave hazardous chemicals! Doing so can create hazardous conditions. Very few chemicals are considered acceptable to autoclave. If you have questions about autoclaving chemicals, contact the Environmental Health Officer at extension 4-4996.

Dry heat

1. The use of dry heat for the disinfection or sterilization of biohazardous materials and contaminated items is less efficient than autoclaving and requires a longer exposure time with higher temperatures.

a) It may be possible to disinfect "soiled" materials by exposing them to 160oC (320oF) for four hours.

b) If items are heat sensitive, a temperature of 120oC (248oF) must be used, and exposure time necessary for disinfection or sterilization is usually greater than 24 hours.

c) The use of biological indicators (Bacillus subtilis spores) is also necessary with dry heat to determine the effectiveness of the sterilization cycle, and to determine the most effective temperature and/or exposure time for sterilization of materials or equipment.

CAUTION !

Dry heat at high temperatures and for long durations should be used to sterilize oils and anhydrous materials such as powders.

Ethylene Oxide

Ethylene oxide (EtO) gas is lethal for all known microorganisms. This is true whether EtO is used undiluted or with CO2, or other dilutents. Some of the process variables which affect the microbiocidal rate are as follows:

Temperature affects the penetration of EtO through microbial cellular components and wrapping and/or packaging materials. The microbiocidal activity of EtO increases with the increase in temperature. Generally, temperatures between 38oC and 54oC (100oF and 130oF) are employed in the EtO sterilization process.

Microbiocidal activity is increased as the concentration of EtO is increased, up to about 1,000 micrograms per liter of EtO. For practical sterilization, gas concentrations of 500 to 1,000 micrograms per liter at approximately 49oC to 60oC to (120oF to 140oF) are recommended.

Moisture is required for the microbiocidal activity of EtO and appears to be related to the moisture content of the exposed microorganism. This is especially true for the moisture content of the bacterial cell wall. A relative humidity of 30 to 60% is frequently employed in EtO chambers to ensure the proper moisture conditions.

The exposure time depends on the above noted variables. Since these variables will not be the same in different commercially available EtO chambers, exposure times recommended by the manufacturers should be followed.

PRECAUTIONS FOR USE OF ETHYLENE OXIDE

The use of EtO to sterilize heavily "soiled" items has not been adequately documented. Thus, if "soiled", heat sensitive items are sterilized with EtO, subsequent treatment with a chemical disinfectant is recommended.

All items except those made of glass and metal should be aerated prior to handling or contact with human skin because EtO which has been absorbed by PVC, rubber, etc., can cause burns or skin irritation unless first removed by aeration. The elimination of harmful EtO residues from the most challenging materials (PVC) can be achieved as follows:

Storage at room temperature for seven days.

Mechanical aeration at elevated temperature (60oC) in an aeration cabinet for eight hours.

Mixtures of EtO and air are explosive. However, commercially available mixtures of EtO and CO_2 are not explosive.

Use the manufacturer's recommended exposure time for EtO.