

CHAPTER 15

DECONTAMINATION AND DISPOSAL

Decontamination and disposal in laboratories which utilize biohazardous materials, are closely interrelated acts in which sterilization and disinfection constitute the first phase of disposal. The goals of decontamination are the protection of personnel and the environment from exposure to biological agents. North Carolina medical waste rules (15A NCAC 13 B .1200), require that "Regulated Medical Waste", defined as "blood and body fluids in individual containers greater than 20 ml, microbiological waste, and pathological waste, must be treated before disposal in order to render the waste nonhazardous.

Sterilization is the process of treating an object or material so as to remove or kill all living organisms. Disinfection is the process of removal or inactivation of all pathogenic microorganisms. It may not remove all microorganisms and therefore, disinfection is not necessarily sterilization. Whether or not sterility is achieved depends on several factors: the number and nature of contaminating microorganisms, the presence of bacterial spores, the concentration of the germicide, the length of time of contact between the germicide and the material being disinfected, the type and condition of the material, the amount of soil present, and the temperature.

Sterilizing and disinfecting agents may attack microorganisms in several ways. Some disinfectants coagulate the cell protein so that the cell cannot function. They may injure or destroy the cell membrane altering the normal selective permeability allowing toxins to enter metabolically important components to escape, or prevent the entrance of food. Disinfectants may also react with a specific enzyme to prevent it from reacting with its natural substrate.

Microorganisms exhibit a range of resistance to inactivating agents. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

Steam Sterilization

Autoclaving, or steam sterilization, is the most dependable procedure for the destruction of all forms of microbial life. Saturated steam is employed under pressure to achieve a chamber temperature of at least 121 C (250 F) for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121 C. The critical factors in insuring the

reliability of this method other than proper temperature and time is the prevention of entrapment of air that is replaced by the steam and adequate exposure time as related to the "soil" load on contaminated items.

Gravity displacement autoclaves take advantage of the difference in density of the air relative to steam. Steam entering the upper-rear of the chamber displaces the air downward and out of the drain line that is located in the lower front of the chamber. A valve in the drain line remains open until a specific pre-set temperature is reached. After this temperature is reached, the valve closes and the steam continues to enter until the pre-set pressure and/or temperature is obtained. The concern with this type of autoclave is that air in closed or upright containers, or air trapped in closed systems (items with valves, etc.), or densely loaded chamber packages is not readily replaced. If air is not removed from an area, the temperature in that area may remain sub-lethal throughout the decontamination period. Because of this, autoclaves of this type should not be overloaded, densely packed materials should be avoided, systems should be kept open and containers should be turned on their side.

High vacuum autoclaves draw a vacuum in the chamber prior to the entrance of the steam. If the vacuum is high (greater than 27 inches Hg.), the air removal concern is alleviated. However, it should be noted that a small load should not be placed in a high-vacuum autoclave because the air remaining in the chamber can be entrained in this load.

Heavily soiled items, especially if the soil is of proteinaceous nature, should be autoclaved for longer periods of time. The reason for this is that soil may protect the microorganism from the lethal effects of the wet heat. Because of this, an exposure time of 60 minutes or greater for soiled items is not unreasonable.

Other practices to improve the effectiveness of autoclave use include removing the plug screen or strainer daily to make sure it is free of dirt, dust, or sediment that may collect in it, and cleaning the interior surfaces of residues collected from the steam or materials being sterilized. The use of spore strips (*Bacillus stearothermophilus* spores) placed at locations throughout the autoclave, can serve as a biological indicator of sterility.

NC medical waste rules state that microbiological waste must be steam sterilized in an autoclave, before disposal. Steam under pressure should be provided to maintain a minimum temperature of 250 F for 45 minutes at 15 psi of gauge pressure. The autoclave should be provided with a chart recorder which accurately records time and temperature for each cycle. Monitoring under conditions of full loading for effectiveness should be performed at least once per week through the use of biological indicators. A log of each test should be maintained, which includes the type of indicator used, date, time, and result of the test.

Criteria for autoclaving typical materials

Material	Temperature	Time
Laundry	121 C (250 F)	30 minutes
Trash	121 C (250 F)	1 hour
Glassware	121 C (250 F)	1 hour
Liquids	121 C (250 F), each gallon	1 hour
Animals	121 C (250 F)	8 hours

Dry Heat Sterilization

Dry heat is useful for the sterilization of anhydrous oils, greases, powders, etc., that can be easily permeated by steam. Dry heat sterilization is less efficient than wet heat sterilization and requires longer times and/or higher temperatures. The specific times and temperatures must be determined for each type of material being sterilized. Generous safety factors are usually added to allow for the variables that can influence the efficiency of this method of sterilization. The moisture of the sterilization environment as well as the moisture history of organisms prior to heat exposure appear to affect the efficiency of dry heat sterilization.

Sterilization can usually be accomplished at 160 - 170 C (320 - 338 F) for periods of 2 - 4 hours. High temperatures and shorter times may be used for heat resistant materials. The heat transfer properties and the spatial relationships or arrangement of articles in the load are critical in insuring effective sterilization. If items are heat sensitive and a temperature of 120 C (248 F) must be used, the exposure time necessary for decontamination is usually greater than 24 hours.

The hazards of handling hot solids and liquids are generally well known. Laboratory personnel should be cautioned that steam under pressure can be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too promptly after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers. Items being handled following dry heat sterilization can cause severe burns if protective gloves are not used.

Gas Sterilization

A variety of gases and vapors possess germicidal properties. The most useful of these are formaldehyde and ethylene oxide. When these are employed in closed systems and under controlled conditions of temperature and humidity, sterilization can be achieved. Vapor and gas

disinfectants are primarily useful in sterilizing biological safety cabinets and associated effluent air-handling systems and air filters; bulky or stationary equipment that resist penetration by liquid surface disinfectants; instruments and optics that might be damaged by other methods; and rooms and buildings and associated air-handling systems.

Ethylene oxide (ETO) gas is lethal for microorganisms including spores, viruses, molds, pathogenic fungi and highly resistant thermophilic bacteria. Some of the principal variables that determine the rate of destruction includes: temperature, concentration, humidity, and exposure time.

Temperature affects the penetration of ETO through microbial cell walls and wrapping and/or packaging materials. The activity of ethylene oxide will increase approximately 2.7 times for each 10 C (18 F) rise in temperature (between 5-37 C, concentration 884 mg/l). Normally, ethylene oxide sterilization is conducted at temperatures between 49-60 C (120-140 F).

Sterilization times may be reduced when the concentration is increased. For practical sterilization, gas concentrations of 500-1000 mg/l at approximately 49-60 C are recommended. The effect of moisture appears to be related to the moisture content of the exposed bacterial cell. A relative humidity of 30-60% is frequently employed in ethylene oxide chambers during exposure conditions.

All materials that have been sterilized with ethylene oxide must be aerated at least 24 hours before contact with human skin. Mixtures of 3-10% ethylene oxide in air are explosive. Commercially available mixtures of ethylene oxide in Freon or CO₂ are not explosive and can be safely utilized.

Formaldehyde is the chemical of choice for space disinfection of safety cabinets, incubators, refrigerators, laboratory rooms, buildings, or other enclosed spaces. Formaldehyde can be generated by vaporizing aqueous solutions of formalin or heating paraformaldehyde. Generally, the generation of formaldehyde gas from powdered or flake paraformaldehyde by heating to a temperature above 150 F is the preferred method. A concentration of 0.3 g per cubic foot of space to be treated is employed, at a temperature above 20 C and relative humidity 70% or higher, for an exposure of 8 hours or overnight. Aeration to remove excess formaldehyde should follow, with length of time related to area decontaminated.

Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas in the event of inadvertent leakage. In preparing use dilutions and when applying the, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Mutagenic potential has been attributed to ethylene oxide and formaldehyde; toxic and hypersensitivity effects are well established for formaldehyde.

Liquid Disinfection

Chemical disinfection is necessary in laboratory operations because steam under pressure is not feasible for use in large spaces, surfaces, stationary equipment, high temperatures and moisture may damage delicate instruments. There are many disinfectants available under a wide variety of trade names. In general, these disinfectants can be classified as acids or alkalis, halogens, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, and alcohols. Unfortunately, the more active the disinfectant, the more likely it will possess undesirable characteristics.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay system employed. Ineffectiveness of a decontaminant may also be due to the failure of the decontaminant to contact the microorganisms rather than the failure of the decontaminant to act. If one places an item in a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful, a decontaminant should have, most do have, incorporated surface-active agents and other detergent properties.

Selecting Chemical Decontaminants

No single chemical decontaminant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

1. What is the target microorganism(s)?
2. What decontaminants in what form are known to, or can be expected to, inactivate the target microorganism(s)?

3. What degree of inactivation is required?
4. In what menstruum is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?
5. What is the highest concentration of cells anticipated to be encountered?
6. Can the decontaminant either as an aqueous solution, a vapor, or a gas reasonably be expected to contact the microorganisms, and can effective duration of contact be maintained?
7. What restrictions apply with respect to compatibility of materials?
8. Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations of infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials presents certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acids. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Because of innate differences in the chemistry of RNA and DNA the effectiveness of a decontaminant for one cannot be extrapolated to the other. For example, RNA molecules are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2' position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

Properties of Some Common Decontaminants

1. **Alcohol** - Ethyl or isopropyl alcohol in a concentration of 70 - 85% by volume is often used. Alcohols rapidly lose their cidal activity when diluted below 50% concentration. The cidal action of ethyl alcohol is very rapid and includes all microorganisms except spores. Isopropanol is not very effective against either spores or non-lipid viruses. They are also not effective when organic soil is present. Alcohols become ineffective as soon as they evaporate. This property has the advantage of having no residue on treated surfaces, but it often makes repeated applications desirable in order to get adequate exposure.
2. **Formaldehyde** - In concentration of 8% formalin, this is an effective liquid disinfectant against vegetative bacteria, spores, and viruses. Considerable activity is lost at refrigeration temperatures. Care must be taken when using solutions in the laboratory because of its irritating odor.
3. **Phenol** - Phenol itself is not often used as a decontaminant because it is extremely toxic. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.
4. **Quaternary Ammonium Compounds or Quats** - After 30 years of testing and use, there is still a considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats have the advantages of being nontoxic, odorless, nonstaining, noncorrosive to metals, stable, and inexpensive.
5. **Chlorine** - This halogen is a universal decontaminant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from

household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

6. **Iodine** - The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The range of dilution of Wescodyne recommended by the manufacturer is 1 ounce in 5 gallons of water giving 25 ppm, of available iodine to 3 oz. in 5 gallons giving 75 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 with water or 10% ethyl alcohol (a reasonably good decontaminant itself) which will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel assigned the task of making up use-concentrations from stock solutions must be properly informed as to the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Protective face shields and goggles should be used for eye protection and long-sleeved garments and chemically resistant gloves, aprons and boots should be worn to protect from corrosive and depigmentation effects to the skin. One of the initial sources for hazard information or any given product will be the label on its container.

Disposal of Decontaminated Waste

University policy, " Decontamination and Disposal of Infectious Waste From UNC Laboratories" (Appendix 14-A) stipulates proper procedures for disposal of decontaminated laboratory waste. This policy has been developed in order to minimize the risk of exposure to Housekeeping Staff, and to those having contact with the waste outside the laboratory, and to assure the community that proper safe-guards are being undertaken to protect the public health and environment.

APPENDIX 15-A

DECONTAMINATION AND DISPOSAL OF INFECTIOUS WASTE

Definition of Infectious Waste from Laboratories

Infectious waste is defined as waste capable of producing an infectious disease. For a waste to be infectious, it must contain pathogens with sufficient virulence and quantity so that exposure to the waste by a susceptible host could result in an infectious disease. Infectious waste from laboratories includes cultures and stocks of infectious agents, including items contaminated with infectious agents, such as disposable culture dishes and devices used to transfer, inoculate, and mix cultures; human blood and blood products; animal carcasses, body parts, and bedding contaminated with infectious agents; sharp items, such as needles, syringes, broken glass, Pasteur pipets, and scalpel blades, contaminated with infectious agents.

Decontamination Methods

General. North Carolina medical waste rules (15A NCAC 13 B .1200), require that "Regulated Medical Waste", defined as "blood and body fluids in individual containers greater than 20 ml, microbiological waste, and pathological waste, must be treated before disposal in order to render the waste nonhazardous. Infectious waste is to be treated to change its biological character so as to reduce or eliminate its potential for causing disease. The most commonly used effective treatment method for the laboratory is steam sterilization (autoclaving). Steam sterilized liquid wastes may be discharged directly to the sanitary sewer (if in accordance with the University sewer disposal policy). Procedures for disposal of solid wastes following steam sterilization are given under "Steam Sterilization Procedures". Laboratories with infectious wastes not specifically addressed by this document (such as waste with multiple hazards, e.g. radioactive infectious waste) should consult with the Health and Safety Office for treatment and disposal methods.

Cultures and Stocks. Cultures and stocks of infectious agents, and items contaminated with cultures are to be steam sterilized prior to disposal in the regular trash. Liquids may be poured down the sanitary sewer after steam sterilization.

3. For effective treatment, the critical factor is the degree of steam penetration. For steam to penetrate throughout the waste load, the air must be completely displaced from the treatment chamber. To facilitate steam penetration, bags are to be opened and bottle caps and stoppers loosened before placement in the steam sterilizer.
4. N. C. medical waste rules state that autoclaves are to be provided with a chart recorder which accurately records time and temperature for each cycle. Monitoring under conditions of full loading for effectiveness should be performed at least once per week through the use of biological indicators. A log of each test should be maintained, which includes the type of indicator used, date, time, and result of the test.
5. After autoclaving, the bags are to be sealed with tape. The bags are to be labeled as having been autoclaved, by placing heat sensitive tape over the biohazard symbol prior to autoclaving. The heat sensitive tape is to be of the type where the word "autoclaved" appears after treatment. This tape is available from Scientific Supply.
6. The autoclaved wastes are then to be placed in a 44 gal. or 32 gal. white Rubbermaid Brute container(s) (with a drum dolly), lined with plastic bags, and located in the vicinity of the autoclave. These containers are to be labeled "AUTOCLAVED/ DECONTAMINATED WASTE ONLY" (found at ehs.unc.edu/labels/). Biohazard bags placed in the white Brute containers and marked with the heat sensitive tape, as indicated above, will signal to Housekeeping that the waste can be removed from the laboratory for disposal in the dumpster. Each department is responsible for providing these containers which are available through General Storeroom (966-5671). Some departments may need several containers depending on the amount of waste generated.
7. Waste bags with universal biohazard symbols are only to be used for infectious waste that will be autoclaved before disposal.
8. Housekeeping is not to remove or otherwise handle waste in biohazard bags.
9. Contaminated materials are not to be left in hallways or other public spaces prior to autoclave decontamination.

References

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