

Appendix E

Guidelines for Disinfection or Sterilization – Infection Control in Biosafety Laboratories

Cleaning, Decontamination, Disinfection, Sanitization and Sterilization

The Purpose of Decontamination, Disinfection and Sterilization

Good housekeeping in a research laboratory should be a principle of operation at all times. Failure to clean up after a procedure, to properly clean used glassware, to clean up after spills or even to have routine cleaning of the laboratory floors by custodial personnel on a scheduled basis can all contribute to the incidental inoculation of research material and of the unsuspecting laboratory worker. General housekeeping of the facility can reduce the chances of accidental exposures, however a more thorough cleaning of equipment and work surfaces is needed to eliminate the potential for accidental contamination.

The cleaning of laboratory equipment, work surfaces and work areas by decontamination, disinfection and sterilization methods is essential to eliminate or inhibit the ability of a contaminate microorganism from being spread throughout the work area or inoculating research material.

Selecting the most effective decontamination procedure or disinfectant will be dependent upon the physical limitations of the material being cleaned, how thorough the cleaning needs to be and the other potential contaminants that may be present.

Physical sterilization processes; i.e., heat and gas sterilization, are used on laboratory equipment and labware that are capable of withstanding the exposure to the process. Chemical disinfectants are used on those items or materials that are not designed to withstand heat or gas sterilization or may involve contact with living tissue.

By the general nature of the product, gas sterilants and chemical disinfectants are toxic and are to be handled according to the manufacturer's directions. Appropriate personnel protective equipment is to be worn when these materials are in use.

Definitions

Antiseptics: Chemical disinfectants that are designed to be used on living tissue surfaces. Antiseptics are less toxic than disinfectants used on inanimate objects. Due to the lower toxicity, antiseptics can be less active in the destruction of normal and any pathogenic flora present.

Autoclave: An apparatus used for sterilizing using superheated steam under high pressure. To sterilize using superheated steam under high pressure. (See Steam Sterilization)

- Chemical Sterilant: A germicide that can destroy all forms of microbial life when adequate exposure conditions are realized. Chemical sterilants are often used as high-level disinfectants when shorter contact times are utilized.
- Decontamination: The killing of organisms or removal of contamination after use, with no quantitative implication, generally referring to procedures for making items safe before disposal.
- Disinfectant: A germicide that inactivates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms. May not be effective against bacterial spores.
- Disinfection: The elimination or destruction of all pathogenic microorganisms. The term has been extensively misused and generally applies to the destruction of any pathogenic vegetative bacteria.
- High-level: The elimination or destruction of all microorganisms with the exception of high numbers of bacterial spores.
- Intermediate-level: The elimination or destruction of all vegetative bacteria including the *Mycobacteria*, most viruses, and most fungi but does not necessarily kill bacterial spores.
- Low-level: The elimination or destruction of pathogenic vegetative bacteria, some viruses, and some fungi but not *Mycobacteria* or bacterial spores.
- Germicide: An agent that destroys microorganisms, particularly pathogenic microorganisms.
- Sanitization: The process of reducing microbial contamination to an acceptable “safe” level. The process of cleaning objects without necessarily going through sterilization.
- Steam Sterilization: Autoclave, the process of sterilization by the use of heated steam under pressure to kill vegetative microorganisms and directly exposed spores. Common temperature and pressure for being effective is 121°C (250°F) at 15 psi (pounds per square inch) over pressure for 15 minutes. Special cases may require a variation of the steam temperature and pressure used.
- Sterilization: The complete elimination or destruction of all forms of life by a chemical or physical means. An absolute not a relative term.

Sterilization

Steam Sterilization

The use of steam under pressure is perhaps the most efficient means of sterilization and is widely used in laboratory and medical facilities to sterilize equipment, glassware, and contaminated materials. All pathogenic bacteria, both vegetative and spore forms, are destroyed within twelve minutes of exposure and direct contact to pure steam heat of 121°C (250°F). Most are destroyed within seconds of exposure. Pure steam at a pressure of 15 psi (pounds per square inch), one atmosphere over pressure, corresponds to the temperature of 121°C. Adequate time must be permitted to attain the 121°C for an exposure of at least 12 minutes for all portions of the articles that are being steam autoclaved. Because of the necessity to allow for adequate exposure for all portions of the materials that are being autoclaved it is necessary to increase the minimum exposure time to 15 minutes. The duration of time needed to adequately heat sterilize material will be dependent upon the quantity and type of material being sterilized at one time, the larger the load the longer the time needed to achieve the needed temperatures deep within the load.

The effectiveness of a routine steam sterilizing cycle can be determined by using the appropriate biological indicator (**Appendix C**), ampoules or test strips containing *Bacillus stearothermophilus* spores or a spore enzyme (α-D-glucosidase) based rapid readout test. There are also several chemical indicators that can also provide reliable information. The standard biological indicator that is used in monitoring the effectiveness of steam sterilization are the *Bacillus stearothermophilus* spores because the spores are highly resistant to high temperatures. The use of the spore enzyme test is increasing in popularity because of its ability to provide results within 3 hours of exposure. The use of temperature sensitive autoclave tape can be misleading since the tape is only capable of indicating that a general temperature was reached. It does not indicate how long the material was exposed to the high temperature.

Autoclaved biological indicator samples should be examined for growth following an exposure to an actual autoclave cycle. The presence of growth in a *Bacillus stearothermophilus* sample or the presence of a color or of a fluorescing color change in other indicators after being steam autoclaved indicates that the exposure cycle was not adequate and must be repeated. In addition to the use of a biological indicator for determining the effectiveness of an autoclave cycle it is important that the researcher be aware of any special handling requirements that may be needed to effectively neutralize their cultured microbial agent or contaminated laboratory equipment. The researcher must understand and handle potentially infectious materials accordingly to reduce the potential for exposure.

The types of materials that may be steam sterilized in an autoclave can be varied in form; by shape and size, solid or liquid in composition or a combination of all, and the autoclave must be capable of accommodating for the type of load. The type of load to be autoclaved will determine the type of steam sterilizing cycle to be used; a liquid load requires a slow depressurization to prevent the liquid from boiling over once the autoclave pressure is reduced.

There are a number of different manufactures and different model designs of steam autoclaves. Before using any steam autoclave, the operation instructions for proper use and timing

requirements must be reviewed. Operators of a steam autoclave must remember that a steam autoclave is operated under pressure and at elevated steam temperatures. Failure to review the operational directions can result in improper sterilizing cycle being used, damage to the materials being exposed to the steam heat, damage to the autoclave and potentially serious or fatal injuries of the operator. Personal injuries can result from steam burns and from not allowing the autoclave to depressurize properly. If a steam autoclave is not working properly do not use the unit until it is repaired, contact the responsible person for the unit and inform them of the problem and label the unit "Out of Service".

In accordance with Wright State University policies dealing with the handling of infectious waste materials, infectious waste materials are to be disposed of according to university procedures defined in **Appendix D, *Infectious Waste Management Guide*** of this manual.

Not all materials are capable of being exposed to steam sterilization in an autoclave. For those items that can not be steam sterilized there are other alternatives in the form of gas sterilization or chemical disinfectants that can be used given proper consideration to practicality, the desired level of disinfection and potential hazards associated with handling of the item and the disinfectant.

Gas Sterilization

Ethylene oxide and formaldehyde gases are generally used for gas disinfection as fumigants under controlled conditions. Ethylene oxide and formaldehyde require special handling procedures to minimize potential personal exposure. Both materials are considered to be suspect carcinogens according to OSHA and an occupational carcinogens according to NIOSH.

Ethylene Oxide (CAS #75-21-8)

Ethylene Oxide (ETO) is used primarily as a means of sterilizing materials that are not designed to be exposed to steam sterilization. The use of ethylene oxide on sensitive plastics, medical and biological preparations and other heat sensitive equipment has contributed to revolutionizing developments in the medical field. Early testing found that ethylene oxide was very effective as a killing agent of bacteria, spores, molds and viruses.

Studies that were conducted to identify the method of activation involved in the destruction of exposed microorganisms found that ethylene oxide caused the replacement of a labile hydrogen with an alkyl group on hydroxyl, carboxyl, sulfhydryl, amino and phenolic groups. The alkylation of these compounds in organisms affects cellular function and structure which leads ultimately to inactivation of cellular function and ultimately death.

As effective as ethylene oxide is as a gas sterilizer, it has some major drawbacks that are potentially hazardous that limit its use in a general laboratory environment. Ethylene oxide is a highly flammable and potentially explosive gas. The gas has an explosive concentration range of 3 to 100 percent, and it is listed as a suspect human mutagen and carcinogen. Because of the potential health risks and flammability potentials there are special handling and ventilation requirements that must be used when handling ethylene oxide. Due to the hazards associated with potential exposures OSHA has listed an

exposure limit of 1 ppm for the duration of a work day. Ethylene oxide is a gas at room temperature and is not to be used in the open environment of the laboratory due to its volatility and health affects.

Ethylene oxide sterilizers are specifically designed to either use a mixture of ethylene oxide and carbon dioxide (10:90) or to use 100 percent ethylene oxide. Before an ethylene oxide sterilizer is to be used the unit should be checked for integrity and the operator must be familiar with operational procedures. The exposure time for a sterilization cycle is usually 4 to 6 hours in duration followed by a period of ventilation to allow for thorough dissipation of absorbed gas. The venting of the sterilizer following use is necessary, exposure to the residual material can be damaging to skin and may present a potential fire hazard.

To test for proper operation of an ethylene oxide sterilizer the biological indicator *Bacillus subtilis* var. niger is used. The spores from *B. subtilis* were found to be highly resistant to the effects of exposure to ethylene oxide

If ethylene oxide is being used in the laboratory it is the laboratory supervisor's responsibility to review all relevant safety information in the safe use, handling and disposal of this material and to be certain that others working in the laboratory receive appropriate training and warnings. Contact the Department of Environmental Health and Safety for assistance in assessing the potential for personal exposures and evaluation of laboratory handling procedures.

Formaldehyde (CAS#50-0-0)

Formaldehyde gas is most frequently used in the process of performing space fumigation of a room or of a piece of laboratory equipment that operated with a controlled environment. At the present time the only accepted method available for decontaminating a biological safety cabinet is to use formaldehyde gas. Formaldehyde gas for decontamination of a biological safety cabinet is generated by heating flaked or powdered paraformaldehyde in the presence of an elevated humidity of nearly 65 percent.

Paraformaldehyde generates formaldehyde gas when it is depolymerized by heating to 232 to 246°C (450 to 475°F); the depolymerized material reacts with the moisture in the air to form formaldehyde gas.

Using a balanced amount of ammonium bicarbonate neutralizes the formaldehyde gas within the biological safety cabinet. Only individuals that have specific training are permitted to decontaminate biological safety cabinets.

In areas where formaldehyde may be used for fumigation it is important to be aware of potential contacts with incompatible materials that could cause the formation of dangerous reaction products. Clear all materials out of an area where formaldehyde may be used to minimize the chance of a possible reaction with incompatible chemicals. Formaldehyde can react violently or explosively when exposed to incompatibles; in the presence of strong oxidizers there is a chance of fire and explosion or when exposed to hydrogen peroxide there is a violent reaction. Most notable however, formaldehyde may combine with hydrochloric acid or hydrogen chloride to form *bis*(chloromethyl) ether (BCME), a carcinogenic compound.

OSHA, NIOSH and IARC recognize formaldehyde as a suspect carcinogen. OSHA has established an exposure limit of 0.75 ppm during a workday. The Department of Environmental Health and Safety can

evaluate work tasks and perform monitoring tests to determine the potential for an occupational exposure.

Chemical Disinfectants

Choosing a Chemical Disinfectant

A variety of concerns must be addressed when choosing a disinfectant for use in a biohazard area. No one disinfectant is universally ideal and the decision as to the optimum disinfectant involves the consideration of factors such as:

- Organism susceptibility
- Material or surface to be disinfected
- Organic load of the material being disinfected
- Potential health risks to laboratory personnel
- Hazardous properties of the disinfectant (i.e., flammable, corrosive, toxic)
- Stability of the disinfectant
- pH, temperature and presence of other contaminants in media and water for dilution
- Required contact time for effective disinfection
- Requirements for disposal of the disinfectant
- Cost

Choosing a disinfectant is, therefore, a decision that requires a fairly detailed knowledge of the target organism, a basic knowledge of disinfectants, and careful consideration of the above factors as they apply to the unique potential conditions in which your laboratory will employ the disinfectant. Always consult the product information, the material safety data sheet (MSDS), on a disinfectant before using the material. Appropriate personnel protective equipment is required to be worn when materials are being mixed and used.

For the chosen chemical disinfectant to be effective when used it must be able to make direct contact with the target organism. Environmental factors such as air bubbles, grease, dirt, a dense concentration of microorganisms and the presence of other chemicals (i.e., soaps) can reduce the effectiveness of the disinfectant.

The Halogens

Chlorine

Chlorine is one of the least expensive and most effective disinfectants. The recommended concentration of sodium hypochlorite for "clean surface" disinfection is 200 ppm, representing approximately a 1:250 dilution of household bleach. The CDC recommends a 1:10 dilution of household bleach as the disinfectant of choice for blood spills while many laboratory safety texts recommend the use of undiluted household bleach for biohazard spill containment. These varying recommendations occur primarily because of chlorine's easy inactivation by organic material (serum, blood, proteins, etc.) and the fact that chlorine's disinfectant activity, unlike many of the other disinfectants, increases as the concentration increases.

Of all the disinfectants, chlorine has one of the most extensive ranges of organisms that are susceptible to destruction under ideal circumstances. All of the vegetative bacteria that have been tested are susceptible to chlorine destruction, including the acid-fast bacteria. Bacterial spores are also susceptible although longer exposure times are generally required. Both enveloped and non-enveloped viruses are susceptible to chlorine inactivation.

One of the main disadvantages of chlorine as a disinfectant is the ease with which it is inactivated by organic material. Materials to be disinfected should be first cleaned to remove the organic material or the concentration of the chlorine must be increased to compensate for the organic material inactivation. Chlorine is also easily inactivated by a variety of metals including copper, zinc, nickel, iron, etc. and the use of chlorine as a disinfectant on these materials requires increased concentrations of chlorine, often resulting in damage to the substrate materials being disinfected.

Chlorine disinfectant solutions are also extremely sensitive to pH and the sensitivity has dramatic implications on the effectiveness of these solutions. Chlorine solutions are most active under slightly acid conditions (pH 6 to pH 7), the activity level decreases rapidly under conditions where the pH goes from a pH 7 to pH 8.5. As the pH of chlorine solutions increases the disinfectant activity levels decrease.

The limited pH range in which chlorine is effective, slightly acid to slightly basic, is also a limiting factor necessitating the use of nonionic detergents or precleaning followed by thorough rinsing.

A number of alternative forms of chlorine exist to use in the form of household bleach (sodium hypochlorite). Chlorine dioxide compounds are high level disinfectants/sterilants that offer somewhat increased activity and resistance to organic inactivation in comparison to household bleach. Chloramine-T and other organic chlorine compounds also offer increased resistance to organic inactivation but at the cost of decreased activity. While these compounds offer specific advantages, household bleach remains one of the best disinfectants available.

Important Information when Considering to Use Hypochlorite Solutions:

Three situations exist where the uses of hypochlorite solutions pose a potential risk to personnel using the compound. First, the addition of acid to hypochlorite solutions will produce a rapid production of toxic chlorine gas. Second, the contact of chlorine solutions with formaldehyde produces the carcinogen bis-chloromethyl ether. Lastly, the heating of chlorine solutions produces the carcinogen trihalomethane. Chlorine solutions, therefore, must never be autoclaved.

Iodine

Iodine-based disinfectants share the same properties as the chlorine-based disinfectants but are somewhat less reactive with substrates and microorganisms. Like chlorine disinfectants, the iodines are effective against vegetative bacteria, acid-fast bacteria, bacterial spores, and both enveloped and non-enveloped viruses although longer contact times are generally required under similar conditions. Most of the iodine-based disinfectants utilized in laboratory and medical situations are combinations of elemental iodine or triiodide with a neutral polymer carrier molecule. These compounds are collectively referred to as iodophors. Iodophors are excellent disinfectants and antiseptics and are extensively used for surgical scrub solutions, hand-washing compounds, and disinfectants for small laboratory objects.

Unlike the elemental chlorine and iodine, however, iodophors are extremely sensitive to concentration and are quite expensive.

Alcohols

Ethyl and Isopropyl Alcohol

Ethanol and Isopropyl alcohol are both excellent disinfectants whose germicidal properties are generally underestimated. Both are rapidly bacteriocidal against vegetative bacterial forms, tuberculocidal, fungicidal, and virucidal. Neither inactivates bacterial spores and isopropyl alcohol fails to inactivate hydrophilic viruses. Both ethanol and isopropyl alcohol should be considered as intermediate-level disinfectants.

One of the most critical factors in the use of alcohols as disinfectants is concentration. The disinfectant properties of both ethanol and isopropyl alcohol rapidly drop at concentrations below fifty percent (50%) and above ninety percent (90%). Peak disinfectant activity occurs at approximately sixty-seven percent (67%) concentration. The recommended concentration for use is sixty - ninety percent (60 - 90%) by volume.

Both ethanol and isopropyl alcohol are volatile and flammable compounds and must only be used with adequate ventilation. Alcohols, in general, are destructive to rubber compounds and to most of the cement and glues used in instruments, especially optics.

Phenolic Compounds

Phenol

Ever since the adoption of carbolic acid by Lister as the first germicide, phenols have been extensively used. Numerous studies, beginning with a study by Kronig and Paul in 1897, have explored the various chemical substitutions and their effect upon germicidal properties. Today, the only phenolic derivatives found in extensive use, as disinfectants are *o*-phenylphenol, *o*-benzyl-*p*-chlorophenol, and *p*-tert-amylphenol. The mode of action of phenolic compounds appears to be a generalized cytoplasmic poisoning at higher concentrations and an inactivation of enzyme systems and cell wall integrity at lower concentrations.

Overall the phenolic derivatives are all characterized by a broad-spectrum of activity against gram-positive and gram-negative bacteria, fungicidal, tuberculocidal, and virucidal activity against lipophilic viruses (enveloped viruses). Phenols have a high tolerance to both organic load and hard water. Their use also results a residual activity on surfaces. Overall, phenolic derivatives are best classified as low- to intermediate- level disinfectants appropriate for general use in noncritical or semicritical areas. They lack sporicidal activity and are ineffective against nonenveloped viruses. Phenol should never be used for sterilization purposes.

Phenolic compounds may exhibit dramatic toxic effects. Phenol compounds rapidly penetrate porous compounds and tend to accumulate in the body fat of exposed animals. Reports of phenolic disinfectant induced skin depigmentation, nerve demyelination and skin contact dermatitis that requires personnel using phenolic disinfectant be provided with appropriate protective clothing and equipment.

Two halogenated phenolic derivatives; parachlorometaxyleneol (PCMX) and 2,4,4'-trichloro-2-hydroxydiphenol (Triclosan, Irgasan), are commonly used as antibacterial agents in soaps and scrubs as well as preservatives in a number of products. PCMX has become the most widely used antiseptic scrub in surgery and is used as a preservative in products ranging from printing inks to cosmetics to shoe polishes. Triclosan is now commonly used in antibacterial soaps and deodorants as well as being incorporated into plastics as a "permanent" (but questionable) antibacterial.

Chlorhexidine

Discovered during a search for potential anti-malarial drugs, chlorhexidine proved to have a high level of antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes, all of which are desirable characteristics for an antiseptic. Chlorhexidine compounds are generally active against gram-positive and gram-negative vegetative bacteria and lipophilic viruses. Many fungi are sensitive to chlorhexidine and acid-fast bacteria are generally inhibited but not killed (bacteriostatic). Bacterial spores are not killed but germination is inhibited while in contact with chlorhexidine.

Chlorhexidine's activity at relatively low concentrations involves a series of related cytologic and physiologic changes culminating in ion leakage from the cytoplasmic membrane and cytoplasmic precipitation. Chlorhexidine's primary advantage over other disinfectants and antiseptic agents involves both its rapid rate of bacteriocidal activity and its strong binding to skin and mucous membranes.

Chlorhexidine is best classified as a low- to intermediate- level disinfectant appropriate for noncritical and semicritical area disinfectant. As an antiseptic, the lack of direct tissue toxicity and the rapidity of action makes chlorhexidine an excellent bacteriocidal skin cleanser and wound cleaning agent.

Quaternary Ammonium Compounds

Quaternary ammonium disinfectants (quats) first appeared in the late 1930's. Since the original introduction, there has been the addition of numerous compounds, blends, different adjunctive agents, etc., making the entire group of quaternary ammonium disinfectants a rather broad group with a variety of activities, advantages, and disadvantages. The major advantages that are common to the group are an inherent surfactant activity, allowing them to also serve as cleansing agents, and a relatively low level of mammalian toxicity. Common disadvantages include a lack of sporicidal activity and a lack of activity against acid-fast bacteria (except for some of the latest generation quats).

The first generation of quaternary ammonium compounds were the standard benzalkonium chloride compounds developed in the 1930's. Substitution of the aromatic ring hydrogen with chlorine, methyl, and ethyl groups resulted in increased activity and the generation of the second generation of quaternary ammonium compounds. The third generation of quaternary ammonium compounds, or the dual quats, were developed in 1955 and represented compounds with superior microbiological activity. Presently, the quaternary ammonium compounds, now polymeric and polysubstituted quaternary ammonium compounds, are in the seventh generation of development. The newest generation of quats possess a wide spectrum of activity with minimal mammalian host damage and are used in pharmaceuticals, ophthalmic solutions, and contact lens solutions, etc.

The antimicrobial activity of quaternary ammonium compounds appears to be by inactivation of critical enzyme systems. Inactivating substances vary dramatically between the generations of quats with the later generations generally much less susceptible to inactivation by extraneous material such as organic load or hard water.

As far as choosing a quaternary ammonium disinfectant, it is critical to read the label directions on the bottle. Organism susceptibilities differ dramatically between different generations of quats and different formulations.