Appendix G: Sterilization and Disinfection

Sterilization is the process of treating an object or material to remove or kill all living organisms.

Disinfection is the process of killing pathogens agents by chemical or physical means directly applied. Disinfection does not mean the destruction or removal of all organisms. Therefore, this may not necessarily create sterile conditions.

Decontamination is defined as the reduction of microorganisms to an acceptable level. The process of decontamination can be achieved by either disinfection or sterilization.

Whether or not sterility is achieved depends on several factors: 1) Types and number of microorganisms; 2) Concentration of the agent; 3) Length of contact time with the agent; 4) Presence of organic matter and dirt; 5) Temperature; 6) Condition and nature of the surface(s). Sterilizing and disinfecting agents attack microorganisms in various ways. Some disinfectants will coagulate or denature the protein rendering the cell nonfunctional. They may injure the cell membrane, altering the normal selective permeability, allowing metabolically important components to escape, or prevent the entrance of food. Also, they may react with a specific enzyme to prevent it from reacting with its natural substrate.

There is a wide range of reaction from microorganism to inactivating agents. Most vegetative bacteria, fungi, and lipid containing viruses are relatively susceptible to chemical decontamination. The non-lipid containing viruses and bacteria with a waxy coat occupy a midrange of resistance. Spore forms are the most resistant.

Methods of Sterilization

Steam Sterilization

Autoclaving provides heat and moisture as the damage factors to destroy organisms. Most organisms can be destroyed in the presence of steam under pressure at 121^{o}C for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121^{o} C. The major problem to insuring the reliability of this method, other than time and temperature, is the prevention of air entrapment. Air must be replaced by the steam and adequate exposure time as related to the soil load on contaminated items.

Some type of autoclaves have downward or gravity displacement which takes advantage of the difference in air density relative to steam. The displaced air is driven out through the drain line located in the lower front of the chamber. A valve in the drain line remains open until a specific preset temperature is reached. When this temperature is reached, the valve closes and steam continues to enter until the pre- set pressure or temperature is achieved. A concern with this type of autoclave is that air trapped in closed or upright containers placed in the chamber, or reduced loads in the chamber are not completely displaced. If the air is not displaced, the temperature will remain to low in that area throughout the sterilization process thereby not being effective. Therefore, autoclaves of this type should not be over loaded; tightly packed and open containers should be turned on their sides.

High vacuum autoclaves draw a vacuum in the chamber prior to entrance of steam. If the vacuum is greater than 27 inches Hg, the air removal concern is alleviated.

The principal advantages of the high vacuum sterilizers are their fast cycle time and the fact that a much larger volume of material can be processed per day than with downward displacement. Another advantage is the minimized damage to materials because of the shortened overall exposure to heat. Heavily soiled items, especially if the soil is of a proteinaceous nature, should be autoclaved longer because soil may protect the microorganism from the lethal effects of wet heat.

Other practices that improve the efficacy of autoclaving include removing and cleaning the equipment plug screen or strainer daily to make sure it is free of dirt, dust, or sediment; cleaning the interior surfaces of residue collected from the steam or materials being sterilized. Spore strips or other satisfactory performance testing materials can be placed at various locations within the autoclave as indicators of sterility.

NOTE: Autoclave tape does not assure sterility; the tape indicates only that the proper temperature has been achieved and is not dependent on time.

Dry Heat

Dry heat is used for the sterilization of anhydrous oils, greases, powders, etc., that cannot be easily permeated by steam. Dry heat is less efficient than wet-heat sterilization and requires longer times or higher temperatures; specific time and temperature must be determined for each type of material being sterilized.

Sterilization can usually be accomplished at 160-170^{o} C for periods of 2-4 hours. Higher temperatures and shorter times may be used for heat resistant materials. The heat transfer properties and arrangement of articles in the load are critical to insuring effective sterilization.

Gas Sterilization

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

Ethylene oxide gas is lethal for microorganisms including spores, viruses, fungi, and highly resistant thermophilic bacteria. The affects of time, temperature, concentration, and humidity upon the rate of sterilization of ethylene oxide are directly related. Doubling the concentration will achieve sterilization in about half the time. The affect of temperature is that with each 10^{o}C temperature increase, the sterilization activity is doubled. At a relative humidity (RH) of 30%, sterilization is most rapid, becoming progressively slower as the relative humidity increases to 100%.

All materials sterilized with ethylene oxide must be aerated at least 24 hours before contact with the skin. Mixtures of 3-10% ethylene oxide in air are explosive. Commercially available mixtures of ethylene oxide in Freon or $C0_2$ are not explosive and can be used safely.

Other Factors Associated with Sterilization

Other factors that are associated with sterilization are: 1) Number of organisms on the material and their resistance to the sterilizing agent; 2) Protection afforded organisms by extraneous matter – direct steam must establish direct contact on all surfaces; 3) Exponential death rate. The numbers of organism dying per unit of time are proportional to numbers present at start to time interval; 4) Functional efficiency of sterilizer and reliability of mechanical components; 5) Human error in operation of equipment.

Standardization of Sterilization (Integrated factors)

Integrated factors on standardizing sterilization are: 1) Proper preliminary cleaning, assembling, and packaging of supplies to insure direct steam contact; 2) Proper loading of the sterilizer; 3) Approved sterilizer with demonstrated reliability; 4) Adequate exposure period that will provide for complete penetration of the load with a liberal margin of error.

Steam Sterilization

Advantages

1. Destruction of most resistant bacterial spores with relatively brief exposure.
2. Easy control of lethality for various materials and supplies.
3. Not toxic residue and materials following sterilization process
4. Most economical method.

Disadvantages

1. Incomplete air elimination from sterilizer depresses temperature and prevents sterilization. Air is a stubborn opponent to the diffusion and expansion of steam.
2. Possible superheated steam with diminished microbial power if sterilizer is used incorrectly.
3. Unsuitable method for sterilization of anhydrous oils, greases, and powders.

Ethylene Oxide Sterilization

Advantages

1. Not deleterious to heat-labile materials.
2. Terminal sterilization of packaged items.
3. Egress of gaseous residues.
4. Penetrability.
5. Not readily inactivated by organic matter.
6. Simple equipment can be used.

Disadvantages

1. Special handling because of flammability, toxicity.
2. Long sterilization and decontamination time.
3. Potential health hazard; fumes must be monitored.
4. Decreased effectiveness when improperly processed.
5. More costly than heat.

Chemical Disinfectants

Chemical disinfectants are effective alternatives since steam sterilization is not feasible for use in large spaces, surfaces, and stationary equipment, high temperatures and moisture also may damage delicate instruments. There are many trade names for the wide variety of disinfectants. Basically, the chemical disinfectants fall into the following categories: acids/alkalis; alcohols; chlorides; formaldehyde; glutaraldehyde; iodine; mercurical; phenolics, and quaternaries.
The relative resistance to chemical disinfectants can be substantially altered by such factors as:

1. Contact time
2. Human error
3. Concentration
4. Presence of organic matter and dirt
5. Temperature
6. Humidity
7. Types and numbers of microorganisms
8. Condition and nature of the surfaces.

The degree of success achieved with chemical decontaminants may range from minimal inactivation of the target microorganism to sterility, depending upon how these factors are manipulated.

Selecting Chemical Decontaminants

No single chemical disinfectant or method will be effective or practical for all decontamination situations. Therefore, consider when selecting chemical disinfectants and procedures, the purpose for decontamination and the interacting factors must be considered. The following questions will to help in choosing which chemical disinfectant is best:

1. What is the target microorganism?
2. What disinfectants are known to inactivate the target microorganism(s)?
3. What degree of inactivation is required?

4. How is the microorganism suspended (i.e. simple or complex, on solid or porous surfaces, airborne)?
5. What is the highest concentration of cells anticipated to be encountered?
6. Can the disinfectant be expected to contact the microorganisms and can effective contact duration be maintained?
7. Is it compatible with the material to be contaminated?
8. What is the product stability?
9. Will there be an absence of residues?
10. Is the disinfectant nontoxic, non-allergenic, non-carcinogenic, non-irritating, and have no noxious odors?

Agar, proteinaceous nutrients, and cellular materials can be very effective in physically retarding or chemically binding active moieties of chemical disinfectants. These interferences will dictate the use of disinfectant concentrations and contact items in excess of those shown to be effective in the tube test.

Monitoring Sterilization

All sterilizers should have time-temperature recorders to provide evidence of adequate exposure for each load. Evidence that a sterilizing temperature has been held for an adequate time, however, does not insure sterilization. This is because the temperature is measured at the outlet valve. Therefore, it does not indicate whether adequate sterilization occurred within dense volumes of liquid or large, dense, fabric-wrapped packs. Residual air or super heating may also result in incomplete sterilization. The use of chemical monitors, i.e. test-tapes, within the autoclave provides only an indication that a sterilizing temperature may have been reached. However, such monitors do not show whether there was adequate exposure. The best means of insuring sterility is to use a biologic spore monitor.

Microorganisms chosen for spore strips are more resistant to sterilization than are most naturally occurring contaminants. The test organisms are in high concentrations to insure a margin of safety. The spores will be in either impregnated filter-paper strips or in solution in glass ampoules. For steam and hot-air sterilization, the thermophile, *Bacillus sterathermophilus*, is used. *Bacillis globigii* is used for ethylene oxide.

Most spore strip preparations are provided in envelopes that contain one or two strips and a control strip. The test strips are packaged in separate envelopes that are removed and sterilized at the time other material is processed. Subsequently, the test strips and control strips are cultured by placing the strips in a tube of tryptic-digest, casein-soy broth. These are incubated at 37^{o}C for gas sterilization and 56^{o}C for steam sterilization. Other types of spore preparations are commercially available. The manufacturer's directions should be followed closely.

Steam and hot air sterilizers should be tested once a week. Every load of material sterilized with ethylene oxide that is to be placed in contact with deep tissues should be tested.

Place the test strips in the center of the test specimen. Never place the strips on an open shelf in the autoclave. Place an ampoule containing a spore solution in the largest vessel to test fluid sterilization.

Handling the spore strips in the laboratory requires considerable care to prevent secondary contamination. Make the transfer with sterile forceps and scissors. Take care not to cross-contaminate the sterilized spore strips with the control strip.

Perform gram staining and sub culturing to prevent false-positive reports that could result from secondary contamination of these cultures.

Whenever positive results are obtained, retest the sterilizers immediately with careful examination of thermometer and pressure-gauge readings as well as review of recent time and temperature records. If any deficiency is observed, or if the repeated sterility test still results in growth, engineering personnel should be consulted promptly.