Sterilization

Sterilization can be defined as any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and prions) from a surface, equipment, foods, medications, or biological culture medium. In practice sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time. Various agents used as steriliants are: *elevated temperature, ionizing radiation, chemical liquids or gases etc.* The success of the process depends upon the choice of the method adopted for sterilization.

- With terminal methods of sterilization of a parenteral product, particularly steam under pressure, a probability of no more than one nonsterile unit in a million (10^{-6}) is readily achievable. Even greater levels of assurance can be achieved with current technology.
- The term aseptic indicates a controlled process or condition in which the level of microbial contamination is reduced to the degree that microorganisms can be excluded from a product during processing. It describes an "apparently" sterile state.
- Microorganisms exhibit varying resistance to sterilization procedures. The degree of resistance varies with the specific organism. In addition، spores, the form that preserves certain organisms during adverse conditions, are more resistant than vegetative forms of the organism. Therefore, the conditions required for a sterilization process must be planned to be lethal to the most resistant spores of microorganisms normally encountered, with additional treatment designed to provide a margin of safety against a sterilization failure.

Pharmaceutical Importance of Sterilization

- Moist heat sterilization is the most efficient biocidal agent. In the pharmaceutical industry it is used for: Surgical dressings, Sheets, Surgical and diagnostic equipment, Containers, Closures, Aqueous injections, Ophthalmic preparations and Irrigation fluids etc.
- Dry heat sterilization can only be used for thermostable, moisture sensitive or moisture impermeable pharmaceutical and medicinal. These include products like; Dry powdered drugs, Suspensions of drug in nonaqueous solvents, Oils, fats, waxes, soft hard paraffin, silicone, Oily injections, implants, ophthalmic ointments and ointment bases etc.

- Gaseous sterilization is used for sterilizing thermolabile substances like; hormones, proteins, various heat sensitive drugs etc.
- U.V light is perhaps the most lethal component in ordinary sunlight used in sanitation of garments or utensils.
- Gamma-rays from Cobalt 60 are used to sterilize antibiotic, hormones, sutures, plastics and catheters etc.
- Filtration sterilizations are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products, air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing.

Variables that affect sterilization include:

1. The dryness of devices to be processed
2. The temperature and humidity of the processing area
3. Whether or not the devices were properly prepared and loaded into the sterilizer
4. Whether or not the sterilizing agent is properly delivered into the system
5. The sterilizer's condition and maintenance protocol
6. Whether or not the correct sterilization method and cycle were used

Validation of Sterilization Processes

All sterilization processes (thermal, chemical, radiation, and filtration) are designed to destroy or eliminate microbiologic contaminants present in a product. The official test for sterility of the product is a destructive test on a selected sample; thus, the task of proving that all units of a product are sterile must involve the employment of probability statistics. The statistics of probability depend on such parameters as the length or degree of exposure to the steriliants, the type and number of microorganisms present, the desired level of microbial destruction or elimination, and the resistance of the microorganism(s) presented to the sterilization process.

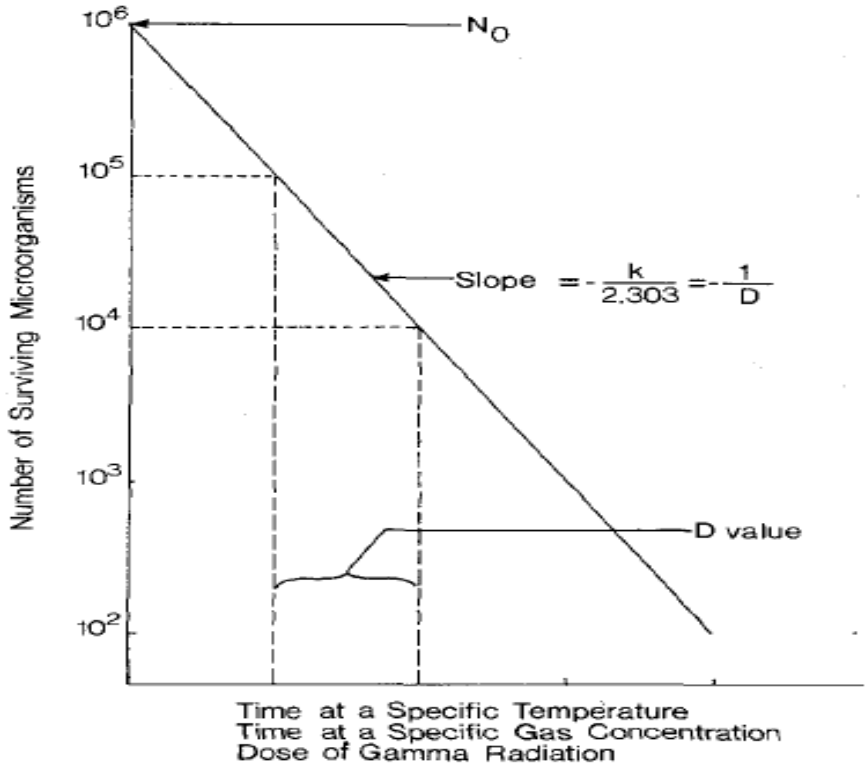
Microbial Death Kinetic Terms

An important term in expressing microbial death kinetics for heat, chemical, and radiation sterilization is the D *value.* The D value is the time (for heat or chemical exposure) or the dose (for radiation exposure)

required for the microbial population to decline by one decimal point (a 90%, or one logarithmic unit, reduction). The D value may be estimated graphically, or mathematically, as shown by equation

$$D = \frac{U}{\log N_0 - \log N_u}$$

where U is the exposure time or exposure dose, under specific conditions, N_0 is the initial microbial population (product bioburden) and Nu is the microbial population after receiving U time



or dose units of sterilant exposure. For example, after 5 min of product exposure to a temperature of 121°C, the microbial population was reduced from 2 x 10^5 to 6 x 10^3 . Then, the D value at 121 c is:

$$D_{121} = \frac{5 \text{ min}}{\log(2 \times 10^5) - \log(6 \times 10^3)} = 3.28 \text{ min}$$

Thus, at 121 C^o, the microbial population is decreased by 90% every 3.28 min. D values have been defined precisely for various microorganisms contained in certain environments (liquids and solid surfaces) at specific temperatures for heat sterilization, and at direct exposure to cobalt-60 irradiation. D values can't be defined precisely for microorganisms exposed to such gases as ethylene oxide because of the complex interaction of heat, concentration of gas, and relative humidity. D values are estimated for gas sterilization when it is possible to keep heat and humidity values constant, varying only the concentration of gas.

D value is important in the validation of sterilization process for several reasons.

1. It is specific for each microorganism in environment subjected to specific sterilizing agent or condition.
2. The knowledge of D value at different temperature in heat sterilization is necessary for the calculation of Z value.
3. The D value is used in the calculation of biological factor F.
4. Extra-polation of D value predicts number of log reduction of microbial population.

D value is affected by several parameters which are as follows.

1. The type of microorganism used as biological indicator
2. The formulation component and characteristics
3. The surface on which the microorganism is expose
4. The temperature and radiation dose

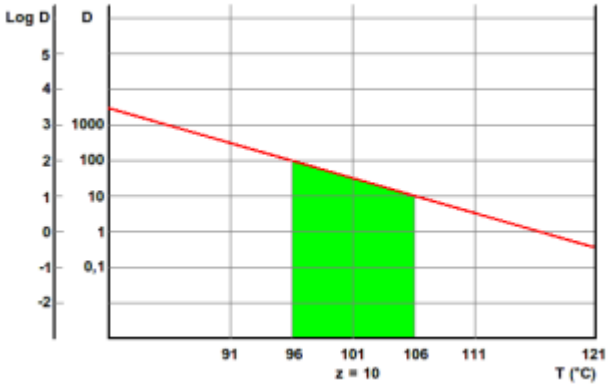
D value is determined by

1. Survival curve method: The survival curve method is based on plotting the log number of the surviving organism verses independent variable such as time, gas concentration or radiation dose
2. Fraction negative method: In this method, sample containing similar spore population are treated in an identical environment and the number of sample still showing microbial growth after treatment and incubation are determined.

The ***z-value*** of an organism in a particular medium is the temperature change required for the D-value to change by a factor of ten, or put another way, the temperature required for the thermal destruction curve to move one log cycle. It is the reciprocal of the slope resulting from the plot of the logarithm of the D-value versus the temperature at which the D-value was obtained. While the D-value gives the time needed at a certain temperature to kill 90% of the organisms, the z-value relates the resistance of an organism to differing temperatures. The z-value allows calculation of the equivalency of two thermal processes, if the D-value and the z-value are known.

Example: if it takes an increase of 10°F to move the curve one log, then our z-value is 10. Given a D-value of 4.5 minutes at 150°F, the D-value can be calculated for 160°F by reducing the time by 1 log. The new D-value for 160°F given the z-value is 0.45 minutes. This means that each 10°F increase in temperature will reduce our D-value by 1 log. Conversely, a 10°F decrease in temperature will increase our D-value by 1 log. So, the D-value for a temperature of 140°F would be 45 minutes.

$$z = \frac{T_1 - T_2}{\log D_2 - \log D_1}$$



F0-Value : F0-Value at a particular temperature other than 121ºC, is the time in minutes required to provide the lethality equivalent to that provided at 121ºC for a stated time.

The F_0 value is a term widely used in sterilization cycle design and validation. Its current application is limited to steam sterilization although an F value can be computed for any thermal method of sterilization. The F_0 value can be defined by the following two equations

$$F_0 = \Delta t \ \Sigma \ 10^{\frac{T-121}{10}} \qquad (2)$$

where Δt is the time interval between product temperature measurements T.

$$F_0 = D_{121} \ (\log N_0 - \log N_u) \qquad (3)$$

where N_0 and N_u are those terms defined previously.

At least three factors affect the F 0 value. They are (1) the container characteristics: size, geometry, and heat transfer coefficient, (2) the product volume and viscosity, and (3) the size and configuration of the batch load in the sterilizer.

Question 1

(a) Estimate the D value for the microorganism when given the following thermal resistance

data for a spore suspension:

Time (min)	Number of survivors
0	10^6
15	2.9×10^5
30	8.4×10^4
45	2.4×10^4
60	6.9×10^3

(b) Construct the survivor curve on regular and semilogarithmic coordinates.

Question 2

The results of a thermal resistance experiment gave a D value of 7.5 minutes at 110oC. If there were 4.9 x 104 survivors at 10 minutes, calculate the No and ratio (N/No) for 5, 15, and 20 minutes.

Question 3

Calculate the z value for a microorganism that has the following decimal reduction times: D110 = 6 minutes,D116 = 1.5 minutes, D121 = 0.35 minutes, and D127 = 0.09 minutes.

Question 4

If the z value of a microorganism is 16.5o C and D121 is 0.35 minutes, Calculate the D110 ?

Methods of Sterilization

The various methods of sterilization are:

1. Physical Method
   A. Thermal (Heat) methods
   B. Radiation method
   C. Filtration method
2. Chemical Method
   A. Gaseous method

Thermal Methods

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160-180 C) sterilization, and for moisture-resistant materials for which moist heat (121-134 C) sterilization is used.

The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature time exposures are required to kill

microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

1. ***Dry Heat Sterilization:*** Examples of Dry heat sterilization are:
   A. Incineration
   B. Red heat
   C. Flaming
   D. Hot air oven

It employs higher temperatures in the range of 160-180^{0}C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glasswares and metal surgical instruments. It is also used for sterilizing non-aqueous thermostable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Incineration

If the total destruction is required. By using incineration everything is destroyed, not only microorganisms. Incineration is applicable to materials used only once or when the contamination level is so high, that for safety reasons it must be destroyed. Radioactive materials not included, of course. It turns everything into ashes therefore reducing load volume by 90%.

Flaming: Sterilization in an Open Flame

The oldest method around is flaming, holding an instrument in an open flame. This is exactly what is done when you burn a tip of a needle to remove a thorn.

Hot-air oven

Dry heat sterilization is usually carried out in a hot air oven, which consists of the following:

A. An insulated chamber surrounded by an outer case containing electric heaters.
B. A fan
C. Shelves
D. Thermocouples
E. Temperature sensor
F. Door locking controls.

Operation

A. Articles to be sterilized are first wrapped or enclosed in containers of cardboard, paper or aluminum.
B. Then, the materials are arranged to ensure uninterrupted air flow.
C. Oven may be pre-heated for materials with poor heat conductivity.
D. The temperature is allowed to fall to 40^0C, prior to removal of sterilized material.

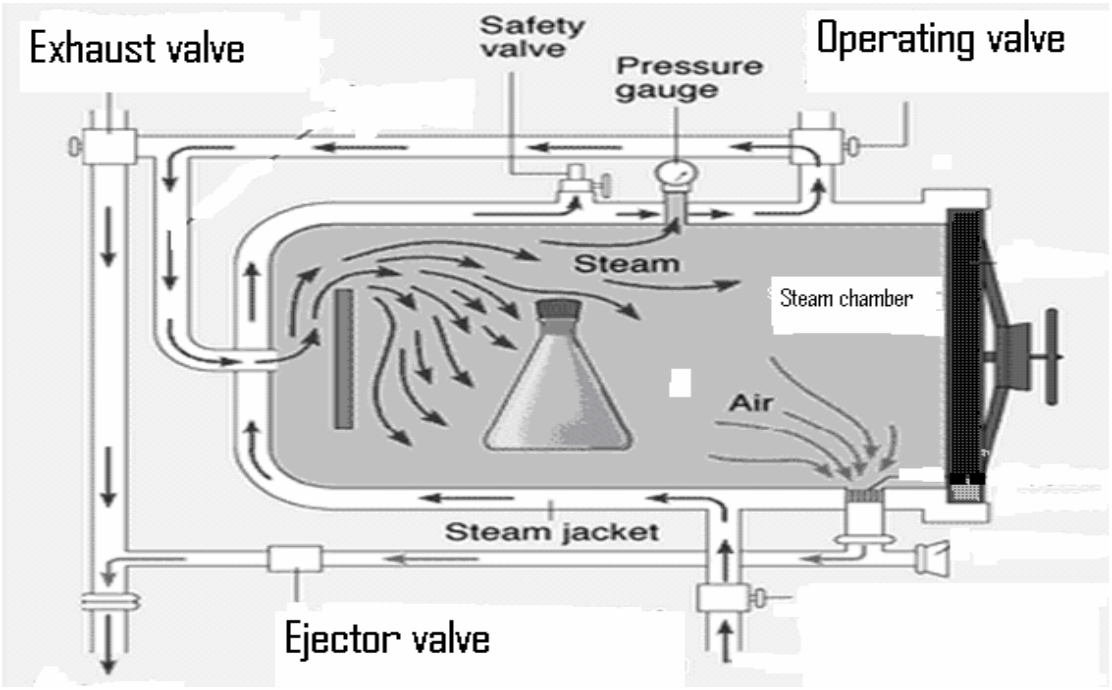
2. ***Moist Heat Sterilization:*** Moist heat may be used in three forms to achieve microbial inactivation

A. Dry saturated steam – Autoclaving
B. Boiling water/ steam at atmospheric pressure
C. Hot water below boiling point

Moist heat sterilization involves the use of steam in the range of 121-134^0C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam (steam in thermal equilibrium with water from which it is derived) acts as an effective sterilizing agent. Steam for

sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation. Autoclaves should be tested periodically with biological indicators like cultures of *Bacillus stearothermophilus* to ensure proper function. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures.



Autoclaves, or steam sterilizers essentially consist of following:

A. A cylindrical or rectangular chamber, with capacities ranging from 400 to 800 liters.
B. Water heating system or steam generating system
C. Steam outlet and inlet valves
D. Single or double doors with locking mechanism.
E. Thermometer or temperature gauge

F. Pressure gauges

Operation

For porous loads (dressings) sterilizers are generally operated at a minimum temperature of 134^0C, and for bottled fluid, sterilizers employing a minimum temperature of 121^0C are used. Ensure that there should be sufficient water in the autoclave to produce the steam. The stages of operation of autoclaves include air removal, steam admission and sterilization cycle (includes heating up, holding/exposure, and cooling stages).

Gaseous Sterilization method

The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide $(CH_2)_2O$ possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas.

The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids. The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 70-75°C respectively.

Both of these gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa.

A. Ethylene oxide sterilizer: An ethylene oxide sterilizer consists of a chamber of 100-300-Litre capacity and surrounded by a water jacket. Air is removed from sterilizer by evacuation, humidification and conditioning of the load is done by passing sub-atmospheric pressure steam, then evacuation is done again and preheated vaporized ethylene oxide is passed. After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system. Ethylene oxide gas has been used widely to process heat-sensitive devices, but the aeration times needed at the end of the cycle to eliminate the gas made this method slow.

B. Low temperature steam formaldehyde (LTSF) sterilizer: An LTSF sterilizer operates with sub atmospheric pressure steam. At first, air is removed by evacuation and steam is admitted to the chamber.

Liquid Sterilization

A. Peracetic Acid liquid sterilization: Peracetic acid was found to be sporicidal at low concentrations. It was also found to be water soluble, and left no residue after rinsing. It was also shown to have no harmful health or environmental effects. It disrupts bonds in proteins and enzymes and may also interfere with cell membrane transportation through the rupture of cell walls and may oxidize essential enzymes and impair vital biochemical pathways.

In a low-temperature liquid chemical sterile processing system, several steps must be followed for effective sterilization

1. Pre-cleaning of the devices is necessary because many devices have small connected lumens.
2. Leak testing is done to ensure there are no leaks that could allow fluid to enter/leak the ampoules/vials and cause damage.
3. The appropriate tray/container must then be selected, and if the device has lumens, the appropriate connector attached.
4. The steriliants concentrate is provided in a sealed single- use cup and requires no pre-mixing or dilution.

The disadvantages of this method of sterilization are that the devices must be immersible, must fit in the appropriate tray, and must be able to withstand the 55°C temperature the process uses.

B. Hydrogen Peroxide Sterilization: This method disperses a hydrogen peroxide solution in a vacuum chamber, creating a plasma cloud. This agent sterilizes by oxidizing key cellular components, which inactivates the microorganisms. The plasma cloud exists only while the energy source is turned on. When the energy source is turned off, water vapor and oxygen are formed, resulting in no toxic residues and harmful emissions. The temperature of this sterilization method is maintained in the 40-50°C range, which makes it particularly well-suited for use with heat-sensitive and moisture-sensitive medical devices. The instruments are wrapped prior to sterilization, and can either be stored or used immediately.

There are five phases of the hydrogen peroxide processing cycle:

1. A vacuum phase creates a vacuum in the chamber and the pressure drops to less than one pound per square inch. This phase lasts about 20 minutes.
2. In the injection phase, the aqueous hydrogen peroxide is introduced into the vacuum chamber and is vaporized into a gas, which creates a rise in pressure due to the increase of molecules.
3. During the diffusion phase the hydrogen peroxide vapor spreads throughout the chamber and the increased pressure drives the sterilant into the packs, exposing the instrument surfaces to the sterilant and killing the microorganisms.
4. During the plasma phase the radio frequency energy is applied, stripping the electrons from some of the molecules and producing a low-temperature plasma cloud. Following this reaction, the activated compounds lose their high energy and recombine to form oxygen and water.
5. The purpose of the venting phase is to introduce filtered air into the chamber and return the chamber to atmospheric pressure so that the door can be opened. It lasts about one minute.