Unit 2
Sterilization

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Contents

- Study of Principle,
- Procedure, merits, demerits and applications of
  - Physical,
  - Chemical
  - Gaseous,
  - Radiation and
  - Mechanical Method Of Sterilization.
- Evaluation of the efficiency of sterilization methods.
- Equipments employed in large scale sterilization.
- Sterility indicators.

Control of Micro-organisms

In order to prevent the

- transmission of diseases,
- infection,
- stop decomposition and spoilage,
- prevent unwanted microbial contamination and
- To product sterile products

Control of Micro-organisms

- Microorganisms can be controlled by
  - physical means and
  - chemical means
- Physical means include methods as high or low temperature, desiccation, osmotic pressure, radiation, and filtration.
- Chemical means refers to the use of disinfectants, antiseptics, antibiotics, and chemotherapeutic antimicrobial chemicals.

Definitions

Disinfection

- It is a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.
- It is a process which reduces the number of viable microorganisms to an acceptable level but may not inactive some viruses and bacterial spores.
- Process that eliminates defined pathogens and not all microbial forms
- Categorized into 3 levels:
  - High,
  - Intermediate
  - Low

Disinfectants

- A disinfectant is a chemical agent, which destroys or inhibits growth of pathogenic microorganisms in the non-sporing or vegetative state.
- Disinfectants do not necessarily kill all organisms but reduce them to a level, which does not harm health or the quality of perishable goods.
- Disinfectants are applied to inanimate objects and materials such as instruments and surfaces to control and prevent infection.
- They may also be used to disinfect skin and other tissues prior to surgery.

Antiseptics

- An antiseptic is a type of disinfectant, which destroys or inhibits growth of micro-organisms on living tissues without causing injurious effects when applied to surfaces of the body or to exposed tissues.
- Some antiseptics are applied to the unbroken skin or mucous membranes, to burns and to open wounds to prevent sepsis by removing or excluding microbes from these areas.
- Iodine has been modified for use as an antiseptic. The iodophore, polyvidone-iodine , is effective against bacteria, fungi, viruses, protozoa, cysts and spores and significantly reduces surgical wound infections.
- The solution of polyvidone-iodine releases iodine on contact with the skin.
- Chlorhexidine has a wide spectrum of bactericidal and bacteriostatic activity and is effective against both Grampositive and Gram-negative bacteria although it is less effective against some species of Pseudomonas and Proteus and relatively inactive against mycobacteria.

- CLEANING - It is a process which removes visible contamination but does not necessarily destroy micro organisms. It is necessary prerequisite for effective disinfection or sterilization.
- ASEPSIS -Term used to describe methods which prevent contamination of wounds and other sites, by ensuring that only sterile object and fluids come into contact with them.
- ANTISEPSIS - It is the procedure or application of an antiseptic solution or an agent which inhibits the growth of microorganisms, while remaining in the contact with them.
- Sanitizing - process that reduces microbial population on object to a safe level.
- Decontamination - process that removes pathogenic microorganisms from an object to make it safe to handle.

- Sterilization is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.
- Disinfection is the elimination of microorganisms, but not necessarily endospores, from inanimate objects or surfaces.
- Decontamination is the treatment of an object or inanimate surface to make it safe to handle.
- Disinfectant is an agents used to disinfect inanimate objects but generally to toxic to use on human tissues.
- Antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.
- Sanitizer is an agent that reduces microbial numbers to a safe level.
- Antibiotic is a metabolic product produced by one microorganism that inhibits or kills other microorganisms.
- Chemotherapeutic synthetic drugs are synthesized chemicals that can be used therapeutically.
- Cidal agent that shows cidal action and kill the microorganisms and viruses.
- Static agent that is static in action and inhibit the growth of microorganisms.

Sterilization

- Sterilization refers to any process that removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic Plasmodium, etc.)
- Sterilization destroys all microorganisms on the surface of an article or in a fluid to prevent disease transmission associated with the use of that item.
- the destruction of all living microorganisms, as pathogenic or saprophytic bacteria, vegetative forms, and spores.

Sterilization concept

- Sterilization is essential concept in the preparation of sterile pharmaceutical products .
- Sterilization –a process that by which all viable M.O are removed or destroyed , based on a probability function .

Method of Sterilization

1. Physical
   - Moist heat (Thermal)
   - Dry heat (Thermal)
   - Irradiation
   - Sterilization by filtration(mechanical)
2. **Gaseous or** Chemical
   a. Gaseous
   b. Liquid sterilant

Thermal sterilization

- Involves the use of either moist or dry heat
- Moist –heat sterilization is the most widely used and reliable sterilization method.
- Dry – heat sterilization is appropriate for materials that cannot withstand moist – heat sterilization

1-Moist –heat sterilization

- Microorganism are destroyed by cellular protein coagulation .
- An autoclave is commonly used for moist – heat sterilization .
- Because it does not require as high a temperature, moist – heat sterilization cause less product and equipment damage compared to dry – heat sterilization .

Moist heat

1. Pasteurization :

The temperature employed is either 63^0C for 30mins (Holder method) or 72^0C for 15-20 seconds (Flash method) followed by cooling quickly to 13^0C. Method is used for heat sensitive liquid and pharmaceutical products.

2. Tyndallisation :

Exposure of 100^0C for 20 min for 3 successive day. Principle: 1st exposure kills all vegetative bacteria & spores, since they are in a favorable medium, will germinate and be killed on subsequent occasions.

3. Autoclaving(Steam Sterilization):

The objects to be sterilized are exposed to saturated steam under 15 lb(1 atmosphere pressure) at a minimum temperature of 121°*C* for at least 20-60 minutes.

Autoclaves(Steam Sterilizer)

- Is a device to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C or more, typically for 15-20 minutes
- It is a machine that killing bacteria, viruses, and even spores present in the material by putting inside of the vessel using steam under pressure.
- Steam is the effective means of sterilization, because of its
  - High penetrating capacity.
  - It gives of large amount of heat to surface with which it comes in contact.

Autoclaves: Principle

- When pressure is increased in a closed vessel the temperature increases proportionately. i.e. for about 15 pounds of pressure per square inch (Psi) the temperature rises to 121°C.
- This pressure and temperature is kept constant for 20 minutes during autoclaving.
- It is sufficient to kill all the vegetative forms and spores of the organism.

Pressure – Temperature Relations in Autoclave
(Figure based on complete replacement of air by steam)

Pressure in (PSI)	Temperature °C	Temperature °F
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267
30	135	275

https://www.pharmaguideline.com/2018/09/principle-and-working-of-autoclave.html

Autoclaves types

- Mode of Action: destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins.
- Portable autoclave (small Sterilizer)
- Horizontal Bench autoclave (large steriliser)
  - For porous loads e.g., dressing
  - For bottled fluids

Portable autoclave (small Sterilizer): Construction

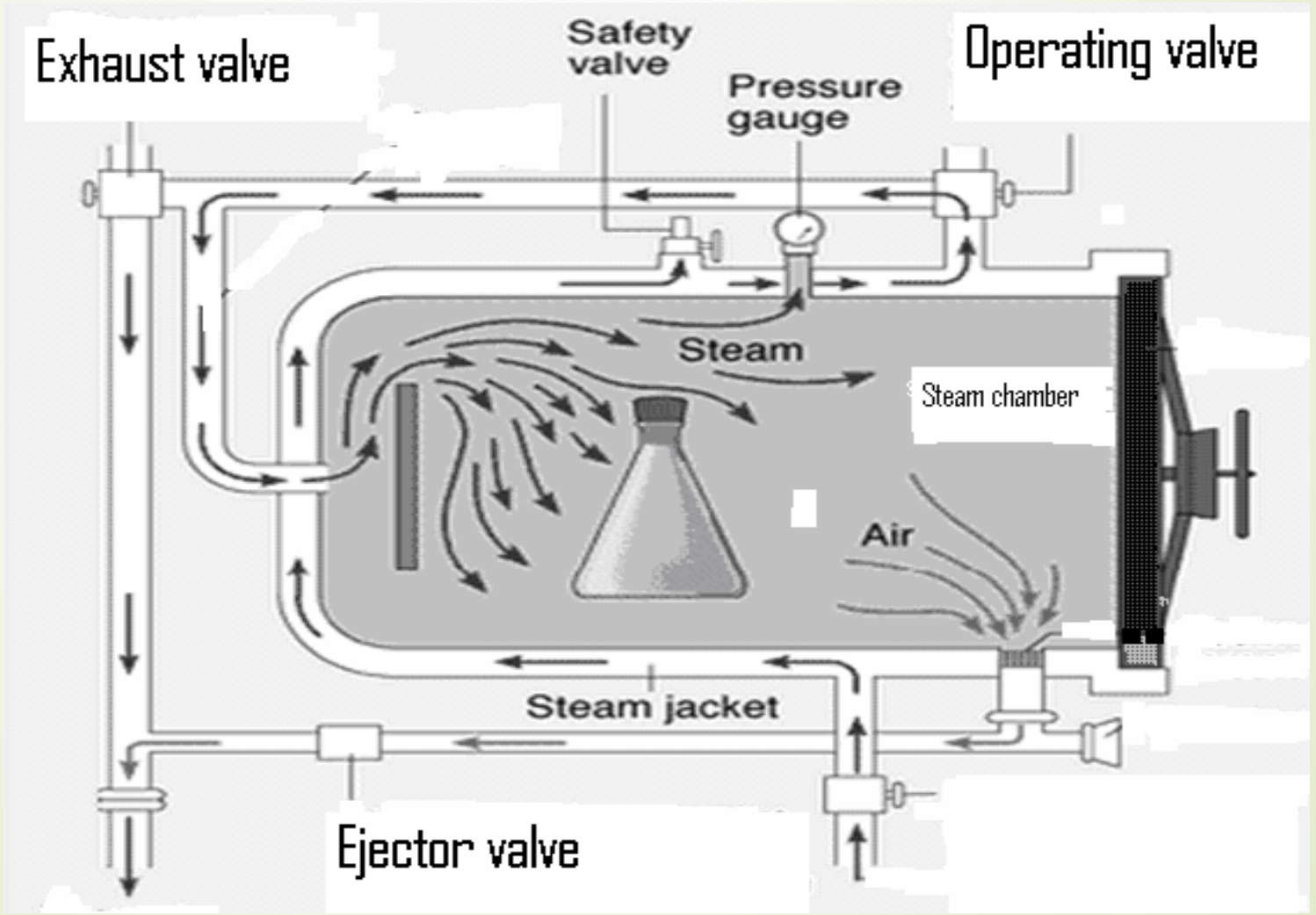
- A cylindrical vessel made of gun metal.
- Controller with time and temperature programmable by user.
- A backlit alphanumeric two line 32 character LCD display.
- Low water level, sensor open/short alarms and cut off.
- Lid is fitted with pressure gauge, safety valve, safety fusible plug manual exhaust valve, vacuum breaker.
- Lid ensure an air tight closure in the autoclave.
- A perforated plate, which is used for keeping the material to be sterilized.
- Drain valve for easy draining and cleaning.
- Moulded Rubber Gasket and
- Stainless Steel carrier along with heater cover stand.

https://pharmawiki.in/autoclave-sterilization-principle-working-pdf-ppt-autoclave-validation-autoclave-diagram/autoclave-sterilization-principle-working-diagram/

Portable autoclave: Main Components

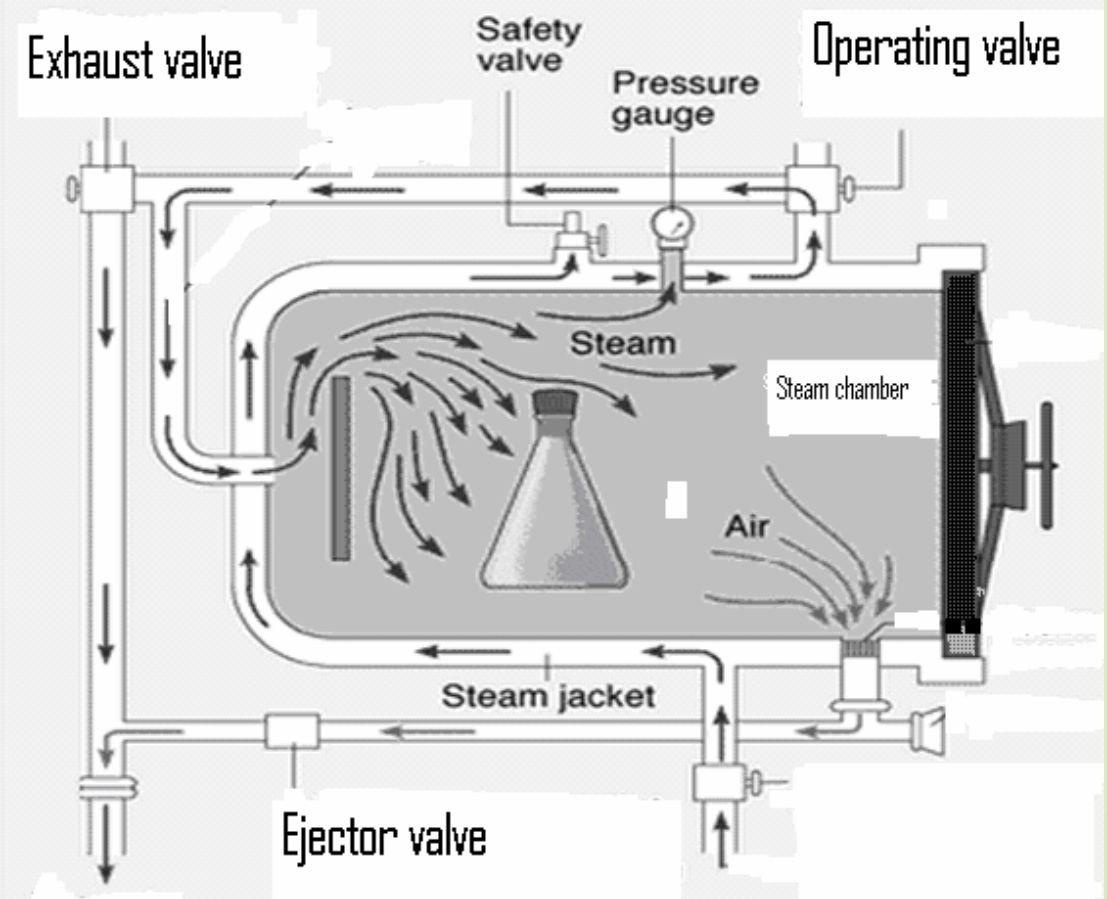
- 1. Heating Elements
- 2. Temperature Controller
- 3. Pressure Sensor
- 4. Chamber
- 5. Door gasket
- 6. Solenoid valve
- 7. Water level Sensor

Horizontal Bench autoclave (large steriliser)

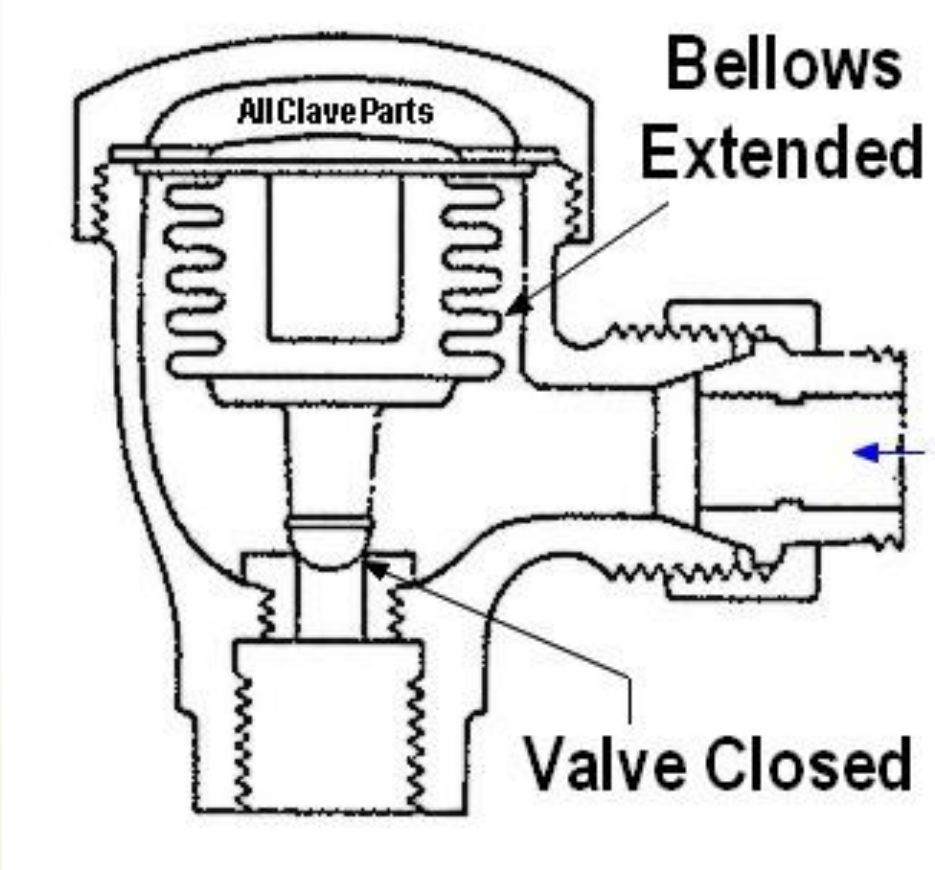


Horizontal Bench autoclave (large steriliser)

Autoclaves, or steam sterilizers essentially consist of following:
i) A cylindrical or rectangular chamber, with capacities ranging from 400 to 800 liters.
ii) Water heating system or steam generating system
iii) Steam outlet and inlet valves
iv) Single or double doors with locking mechanism.
v) Thermometer or temperature gauge
vi) Pressure gauges
To achieve sterility, a holding time of at least 15 minutes at 121 °C (250 °F) or 3 minutes at 134 °C (273 °F) at 15 psi (100 kPa) above atmospheric pressure is required.



Stem Trap



Horizontal Bench autoclave: Working

Most autoclaves contain - a sterilizing chamber to place articles - a steam jacket where steam is maintained.

- Steam flows from the steam jacket into the sterilizing chamber
- Cool air is forced out
- A special valve increases the pressure to 15 pounds/square inch above normal atmospheric pressure.
- The temperature rises to 121.5^{o}C, and
- The superheated water molecules rapidly conduct heat into microorganisms.
- The time is reduced to 15 minutes to kill bacterial spore
- For denser objects, up to 30 minutes of exposure may be required.

Horizontal Bench Autoclave: Operation

- Mode of operation depend on **type of material** like porous material
  - Vacuum should be applied

1. Loading- in which the objects or items are packaged and loaded in the sterilizer.
2. Removal of Air-
   - Downward displacement
   - High Pre vacuum Process(20mmHg)
3. Heat-up Time(depend upon volume)
4. Sterilization Period
5. Drainage of Air & Condensate

Some causes of failure to produce a sterile load are:

Faults in the autoclave and the way it is operated

It maybe:

- Poor quality steam
- Failure to remove air and condensate
- Faulty gauges and timings
- Leaking door seals

Other Methods of Autoclaving

- Fractional Sterilization(Tyndallisation)
- Heating with Bactericide
- Pasteurization
- Low Pressure Method

Dry Heat Sterilization

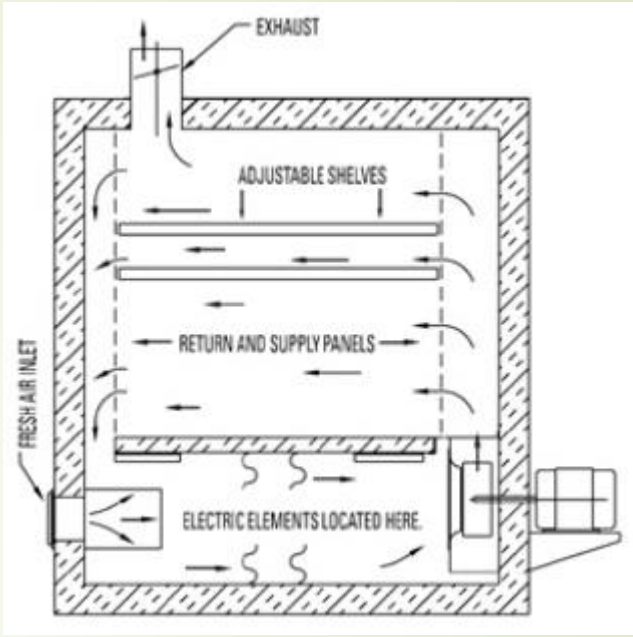
- Require High temperature (160^0C)
- More Time of Exposure(1 hr)
- Thermostatically Controlled Hot Air oven
- Mode of Action -Oxidation
- Heat transfer: conduction, convection and radiation
- Fan or Turboblower

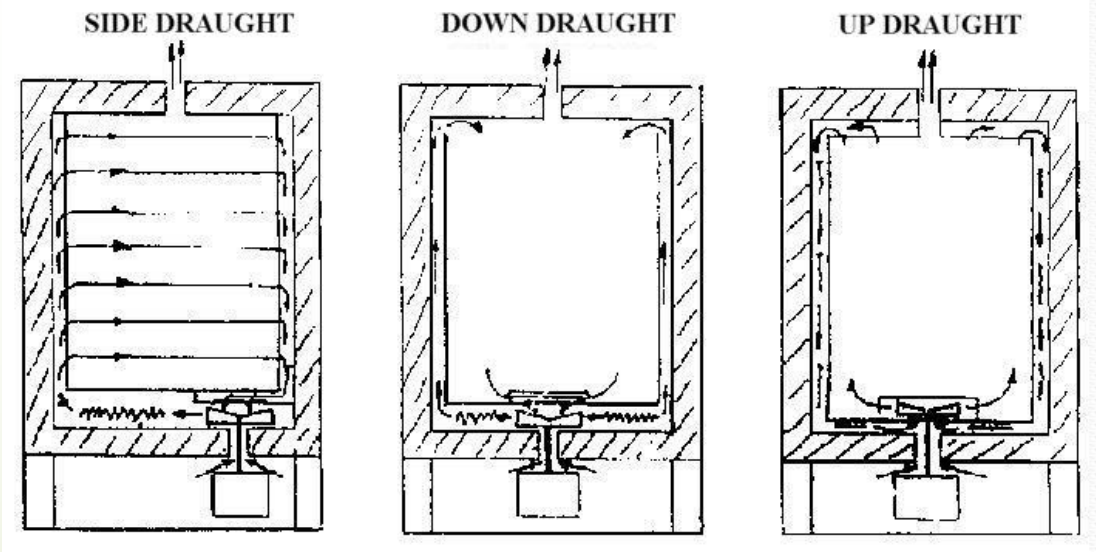
Dry Heat Sterilization: Operation

1. Hot Air Oven : bring to Operating Temperature
2. Loading- in which the objects or items are packaged and loaded in the sterilizer.
3. Heat-up Time(depend upon volume)
4. Sterilization Period

Force Draught Oven

- Object is to overcome viscous drag of air adherent on the surface of articles
- Air flows 700 ft^3/min at 200^0C
- Assist sweeping away the static air film & improve the heat transfer





Infra red conveyor oven

Difference Between Moist Heat & Dry Heat Sterilization

Moist Heat sterilization	Dry Heat sterilization
The moist heat sterilization have water and steam.	The dry heat sterilization have no use of water and steam.
Sterilization with coagulation of protein.	Sterilization with oxidation.
This process is under pressure.	This process is on direct flame.
This process takes less time.	This process takes more time.
Moist heat are boiling and autoclave.	Dry heat are flame and incineration.

FILTRATION

- Help to remove bacteria from heat labile liquids.
- TYPES:
  - Candle filter
  - Asbestos filter
  - Sintered glass filter
  - Membrane filter

Sterilization by Filtration:

- Filtration through a bacteria-proof filter is a suitable method for the sterilization of injections containing thermolabile medicaments.
- However, the solid or medicament must be stable in solution or compatible with water.
- The process involves four stages :

1. Filtration of the solution through a bacteria-proof filter.
2. Aseptic distribution of the filtered solution into previously sterilized containers.
3. Aseptic closure of the containers.
4. Testing of samples for sterility.

Filtration Sterilization

- Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles.
- The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.
- Ex:HEPA FILTERS

Membrane Filters

- The **membrane filter** is the most common type of filtration system used in modern microbiology laboratories. These are made from high tensile strength polymers of cellulose acetate, cellulose nitrate, polycarbonate, polyester, polypropylene or polysulfone.

Laminar Flow Bench

- As viruses pass through ordinary filters, it can be used to obtain bacteria free filtrates of virus isolation.

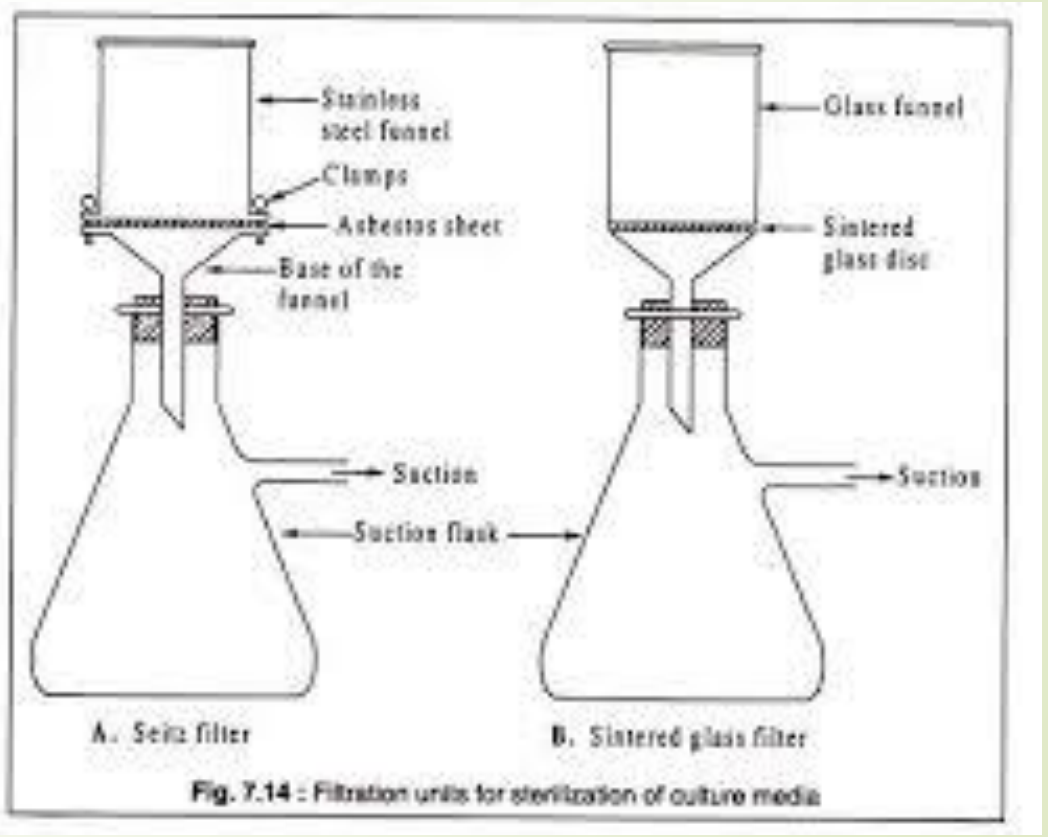


Fig. 7.14 : Filtration units for sterilization of culture media

Irradiation

Radiation used for sterilization is of two types

1. Ionizing radiation, e.g., X-rays, gamma rays, and high speed electrons .
2. Non-ionizing radiation, e.g. ultraviolet light, and infrared light.

These forms of radiation can be used to kill or inactivate microorganisms.

Ionizing radiation

- X-rays, gamma rays and cosmic rays are highly lethal to DNA and other vital constituents.
- They have high penetration power.
- There is no appreciable increase in temperature, thus referred to as cold sterilization.
- Commercial plants use gamma radiation for sterilizing plastics, syringes, swabs, catheters etc.

Non-ionizing Radiations

Two types of non-ionizing radiations are used for sterilization

- **Ultraviolet -**

  Short range UV(UVC) is considered **"germicidal UV".**

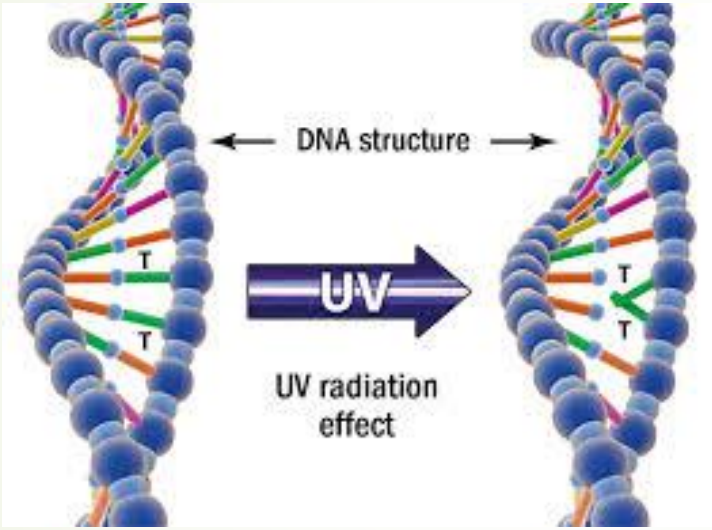
  At a wavelength of **2537 Angstroms** UV will destroy micro-organismal DNA.

  Used mainly for air purification and water purification in hospitals.

- **Infrared –**

  It is most commonly used to purify air, such as in the operating room. Infrared is effective, however, it has no penetrating ability.

Ultraviolet Radiations



Photochemical reaction provokes dimerization of DNA and RNA bonds, which inhibits the ability of microorganisms to replicate. This process is known as inactivation of microorganisms.

Chemical Sterilization

- is the process of removal of microorganisms by the use of chemical bactericidal agents.
- Under such conditions, chemical either in liquid or gaseous state can be used for sterilization.
- it is crucial to ensure that the materials undergoing sterilization are compatible with the chemical being used.

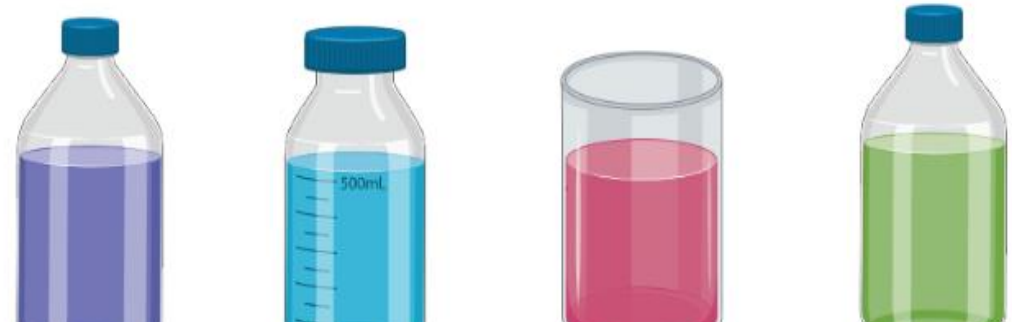
Chemical Sterilization

Gaseous Sterilization

- Ethylene oxide
- Formaldehyde
- Nitrogen dioxide (NO_2)
- Ozone

Liquid Sterilization

- Hydrogen peroxide
- Glutaraldehyde
- Hypochlorite



Gaseous sterilization

- involves the process of exposing equipment or devices to different gases in a closed heated or pressurized chamber.
- Gaseous sterilization is a more effective technique as gases can pass through a tiny orifice and provide more effective results.
- Gases are commonly used along with heat treatment which also facilitates the functioning of the gases.
- The mechanism of action is different for different types of gases.

Ethylene Oxide Sterilization (ETO)

- Used almost exclusively to sterilize medical products that cannot be steam sterilized or sensitive to radiation.
- *Mechanism of action:* It destroys micro-organisms by alkylation and cause denaturation of nucleic acids of micro-organisms.
- At 30 °C - 60°C with relative humidity above 30 % and gas conc. between 200 and 800 mg/l for at least 3 hours.

Ethylene Oxide Sterilization (ETO)

- applied to sterilize, pasteurize, or disinfect different types of equipment and surfaces because of its wide range of compatibility with different materials.
- widespread method used for almost 70% of all sterilizations and around 50% for disposable medical devices.
- Ethylene oxide kills all known microorganisms, such as bacteria (including spores), viruses, and fungi (including yeasts and molds), and is compatible with almost all materials even when repeatedly applied.

- Ethylene oxide is a colorless liquid with a boiling point of 10.7 °C.
- Highly penetrating gas with sweet ethereal smell.
- Highly inflammable & in conc. greater than 3%, highly explosive.
- By mixing with inert gases such as CFC or CO2, explosive tendency is eliminated.
- Plastics, rubber & photographic equipments can be sterilized by this method.
- Also used for mass sterilization of disposable items, plastic syringes,needles,catheters,blades etc.

Formaldehyde

- Formaldehyde is another important highly reactive gas which is used for sterilization.
- This gas is obtained by heating formalin (37%w/v) to a temperature of 70-80°C.
- It possesses broad-spectrum biocidal activity and has found application in the sterilization of reusable surgical instruments, specific medical, diagnostic and electrical equipment, and the surface sterilization of powders.
- Formaldehyde doesn't have the same penetrating power of ethylene oxide but works on the same principle of modification of protein and nucleic acid.
- As a result of the low penetrating power, its use is often limited to paper and cotton fabrics.
- Formaldehyde can generally be detected by smell at concentrations lower than those permitted in the atmosphere and thus can be detected during leakage or other such accidents.

Nitrogen dioxide

- Nitrogen dioxide is a rapid and effective sterilant that can be used for the removal of common bacteria, fungi, and even spores.
- NO_2 has a low boiling point (20°C) which allows a high vapor pressure at standard temperature.
- This property of NO_2 enables the use of the gas at standard temperature and pressure.
- The biocidal action of this gas involves the degradation of DNA by the nitration of phosphate backbone, which results in lethal effects on the exposed organism as it absorbs NO_2.
- An advantage of this gas is that no condensation of the gas occurs on the surface of the devices because of the low level of gas used and the high vapor pressure. This avoids the need for direct aeration after the process of sterilization.

Performance Test/ Sterility indicators

Instrumental Test-Thermocouple,thermistor

Biological indicator- Spores of bacillus stearothermophilus are used as the test organisms as it is toughest organism for an autoclave to destroy.

Its spores require an exposure of 15 mins at 121^0c to be destroyed.

Chemical indicator- Strips or tapes that change color once the correct conditions have been met.

Chemical indicators

- are devices used to monitor the presence or attainment of one or more of the parameters required for a satisfactory sterilization process or
- used in a specific test of sterilization equipment.
- For example, when placed inside packs, chemical indicators are used to confirm that sterilant achieved good penetration in the items being sterilized.
- used as part of comprehensive quality control program, the use of biological indicators and physical monitors, to assure that the conditions for sterilization were met.
- they can capture failures, such as malfunctioning equipment and technician errors, that could result in a non-sterile device.
- The use of chemical indicators in sterilization provides confidence in the effective reprocessing of medical devices.

Chemical indicators

- Chemical indicators use one or more chemicals that undergo either a physical or chemical change, that is visible to the human eye, after exposure to predetermined critical parameters such as time, temperature and sterilant.
- chemical indicator uses a chemical pellet that changes from a solid phase to a liquid phase when it is exposed to steam. Once in a liquid state, the material wicks along a paper strip and is visible through the window in the chemical indicator.
- The second type of chemical indicator utilizes one or more chemical reactions to bring about a chemical change.
- This physical or chemical change is observed and interpreted as a pass or fail result and can help Sterile Processing Staff and Surgical Technologists decide whether to release a set of instruments for use.

TYPES OF CHEMICAL INDICATORS	WHAT THEY INDICATE	APPLICATION EXAMPLE
TYPE 1 CHEMICAL INDICATORS: PROCESS INDICATORS	•Exposure to the sterilization process •Help differentiate between processed and unprocessed loads	Indicator tape or indicator labels that are placed on the outside of a pack
TYPE 2 CHEMICAL INDICATORS: SPECIFIC USE	•For use in specific tests as defined by standards	Bowie Dick test used to check the efficiency of the air removal and steam penetration within the chamber
TYPE 3 CHEMICAL INDICATORS: SINGLE VARIABLE	•React to one critical parameter	A chemical pellet which melts at a specific temperature

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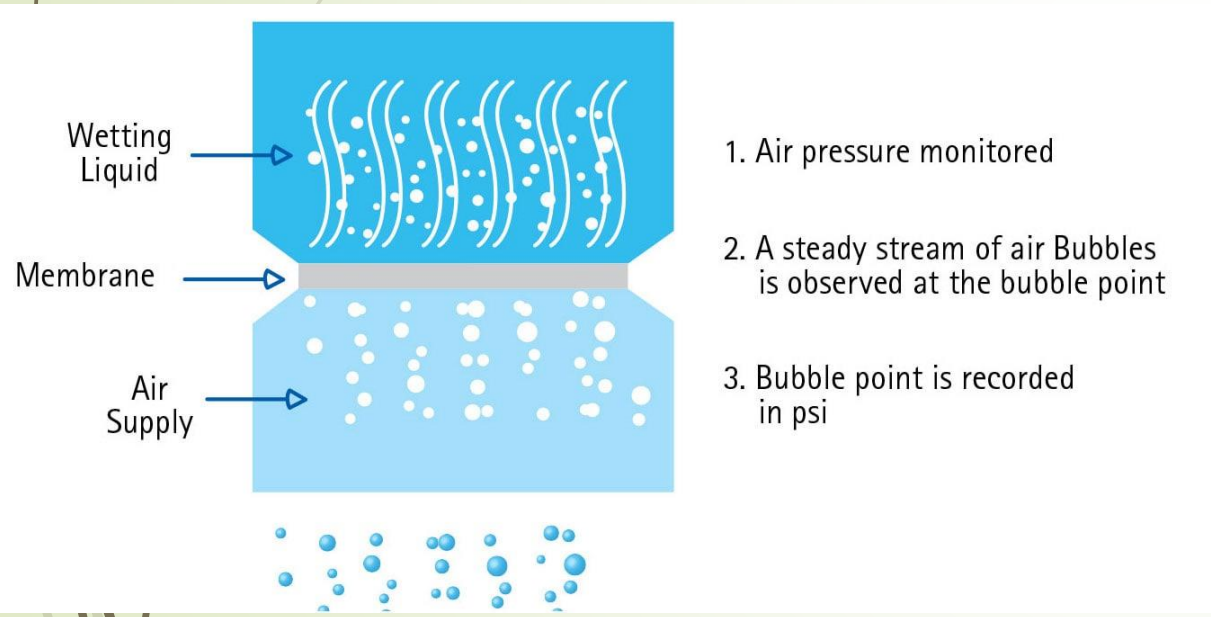
Bubble Point Procedure for Filters



1. Wet the filter with the appropriate fluid, typically water for hydrophilic membranes or an alcohol for hydrophobic membranes.
2. Place the filter into the holder and cover the membrane with the wetting fluid.
3. Pressurize the system to about 80% of the expected bubble point pressure which is stated in the manufacturer's literature.
4. Slowly increase the pressure until a single, continuous stream of bubbles comes through the membrane.
5. Read the bubble point pressure from the pressure gauge.

Bubble Point Procedure for Filters

- **Where:**
- P = bubble point pressure
  d = pore diameter
  k = shape correction factor
  cos θ = liquid-solid contact angle
  ó = surface tension

$$P = \frac{4k \cos \theta}{d} \quad \sigma$$

Evaluation of the efficiency of sterilization methods

Survival of Bacterial Spores during heat processing (Decimal Reduction)

- Thermal destruction of bacteria or spores takes place in definite pattern.
- Suspension of bacterial spores exposed to constant lethal temp.
- Logarithms of the number of the surviving spores are plotted against time on linear scale (or the number of survivors in a log scale against time in linear scale) a straight line graph will be obtained.
- *Destruction of microbial population subjected to sterilization process follow logarithmic progression*
- the D-value will change as the temperature changes.

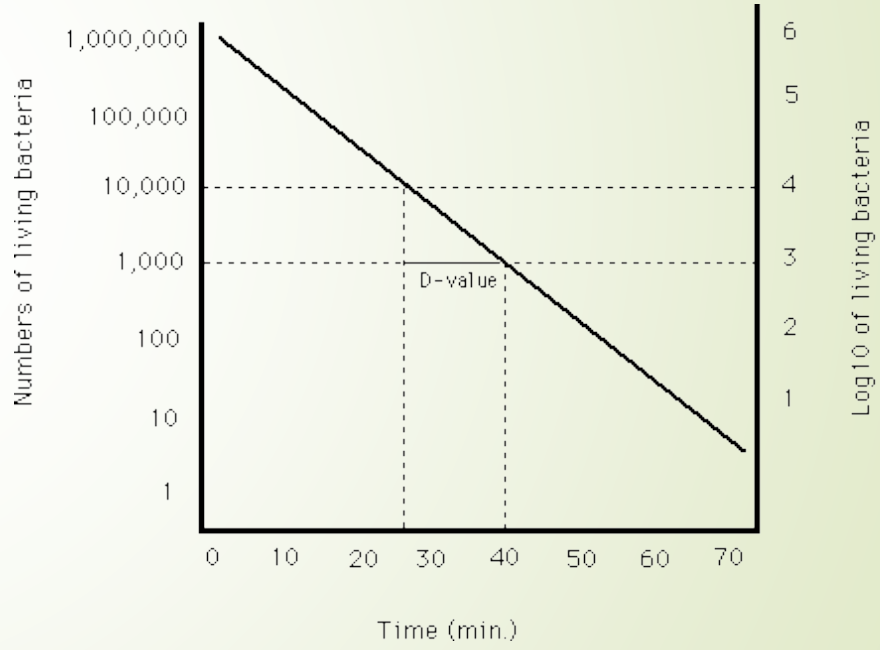
Thermal Death Rate Curve or Decimal Reduction Time Curve or Survival Curve

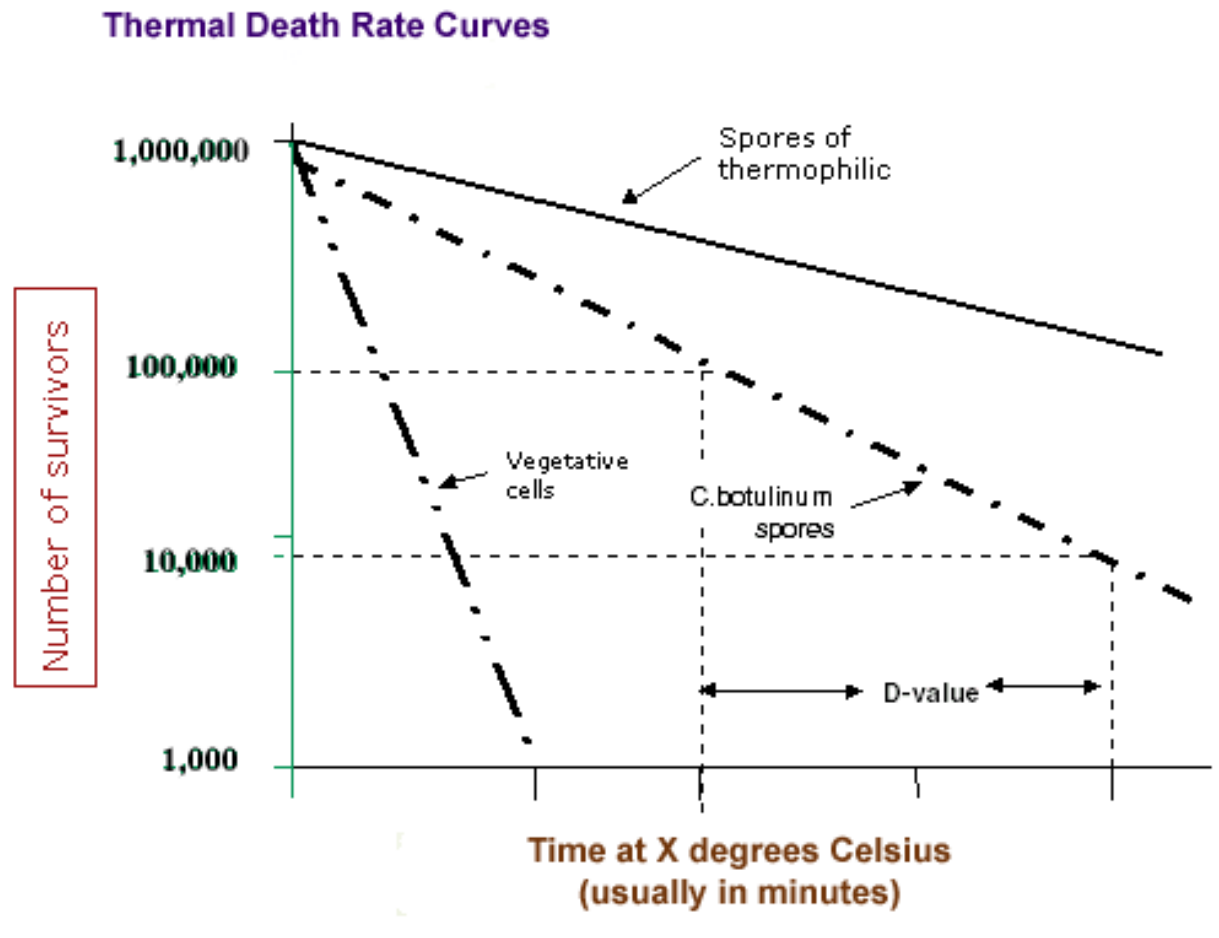
- The slope of the curve _

Decimal Reduction Time (D) or

Death Rate.

- D is equal to time in minutes required to reduce the number of survivors to one tenth of the original at a specified temperature.
- Time required for the curve to traverse one log cycle.
- D_{121}˚C
- Higher the load longer the time.



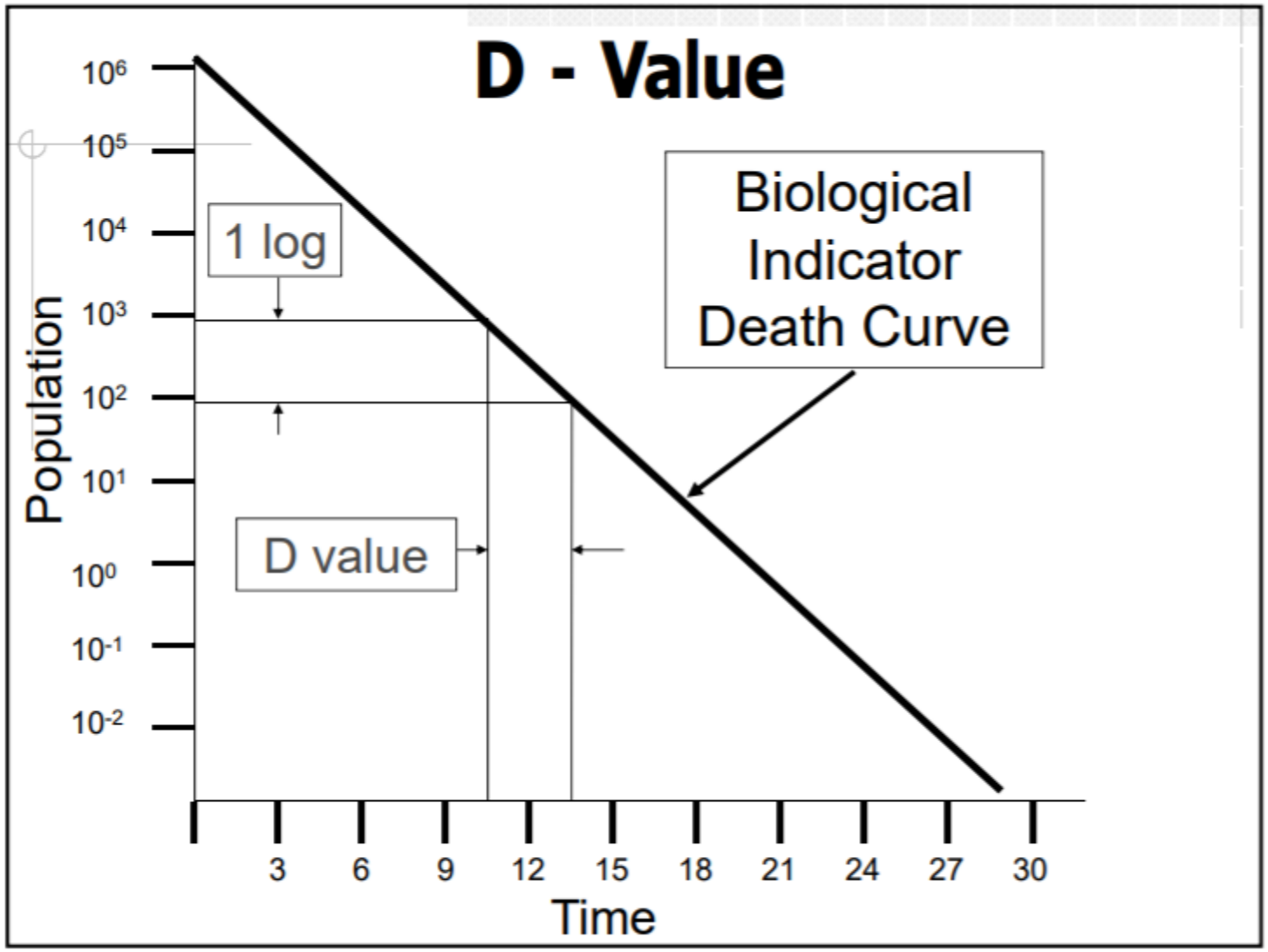

Thermal Death Rate Curves
1,000,000
100,000
10,000
1,000
Number of survivors
Spores of thermophilic
Vegetative cells
C.botulinum spores
D-value
Time at X degrees Celsius
(usually in minutes)

D Value

Organism	D value min @ 121.1˚C
Bacillus Stearothermophilus	4-5
C. thermosaccharolyticum	3-4
Desulfotomaculum nigrificancs	2-3
Clostridium botulinum type A & B	0.1-0.25
C. sporogenes (P.A. 3679)	0.1 - 1.5
B. coagulans	0.01 - 0.07

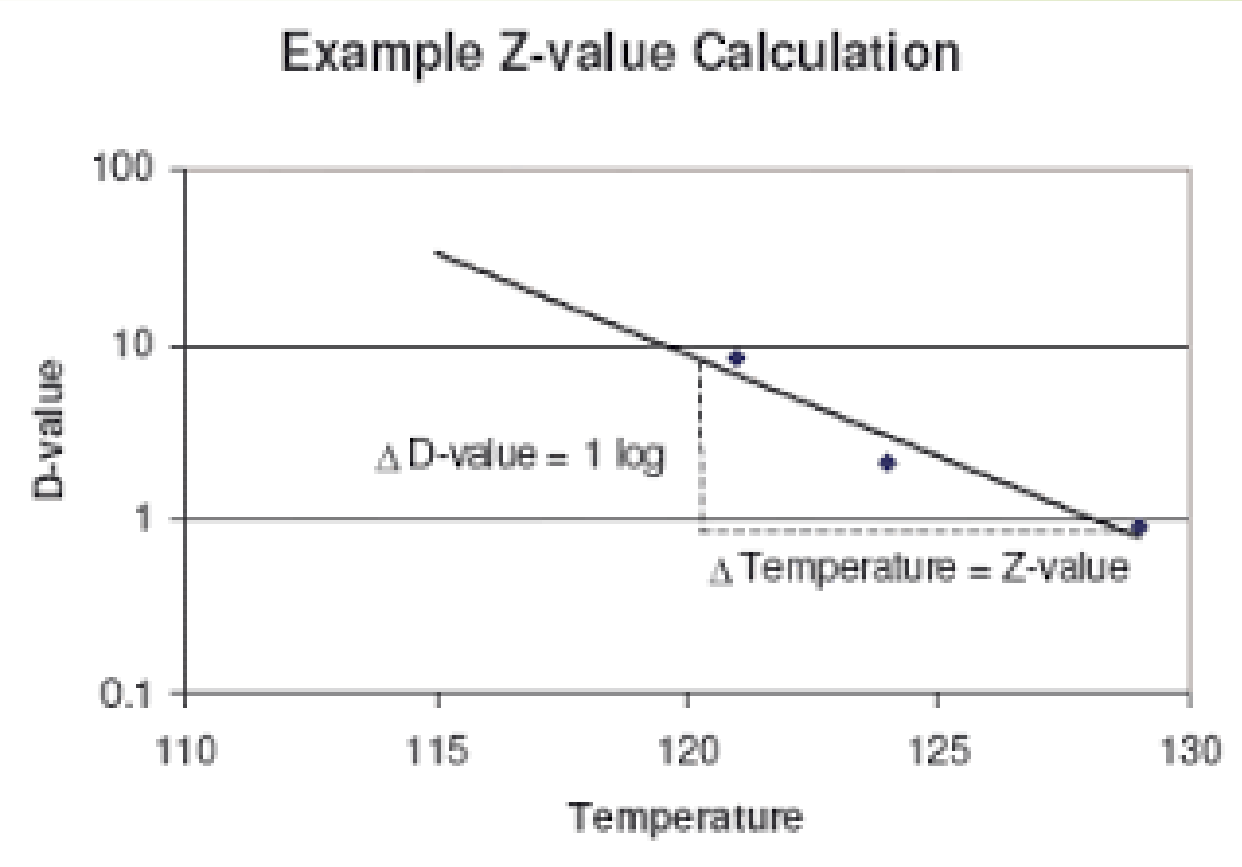
The D-Value

- The D-value is the time required to reduce a population of microorganisms by one log or a 90% reduction in count.
- A D-value is only meaningful if referenced to specified lethal conditions.
- For example D-values should always be referenced to a temperature, without that reference they have no meaning, i.e., moist heat $D_{121.1°C}$ or dry heat $D_{170°C}$.
- For D-values in gases / liquids the agent concentration, RH and temperature must be indicated, i.e., $D_{900\ PPM,\ 75\%\ RH,30°C}$


D - Value
Biological Indicator Death Curve
1 log
D value
Population
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
3
6
9
12
15
18
21
24
27
30
Time

Z Value

- The z-value is defined as the temperature coefficient of microbial destruction, i.e. as the number of degrees of temperature which causes a 10-fold variation of D (or, more generally, of the sterilization rate).



Z-Value

- *Z* value: The change in temperature, in °C, necessary to cause a tenfold change in the *D* value of an organism under specified conditions
- Z-values are calculated from the slope of the curve of D-value vs temperature
- **Z- value is the measurement of the sensitivity of an organism to changes in temperature**

- Z-value:

is a term used in microbial thermal death time calculations. It is the number of degrees the temperature has to be increased to achieve a ten fold (i.e. 1 log10) reduction in the D-value.

- F-value:

The F value is a measurement of sterilization effectiveness.

It is defined as number of minutes to kill number of microorganism with specified Z-value at a specific temperature