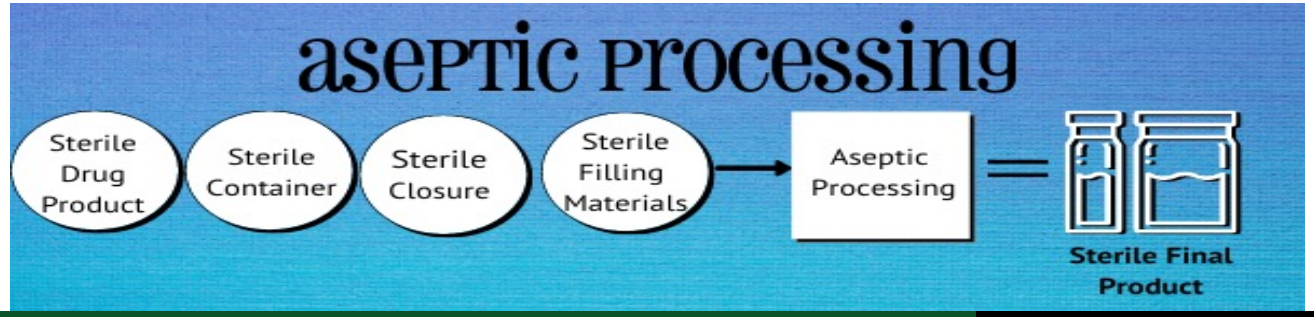
# Sterilization

# Sterile Technique

- Process designed to produce a **sterile state**.
- **Sterile State**: Absolute condition of total destruction or elimination of **all living** microorganisms.
- It is essential concept in production of sterile pharmaceutical products like **ophthalmic and parenteral**.
- However, absolute term is only theoretical term which cannot be achieved but only be approached.
- Ex: sterilization of a parenteral product, particularly steam under pressure where the probability of finding a non sterile vial is **one in each million vial**.
- So **sterile** indicates a probable condition of **complete freedom from viable microorganisms**.

# Aseptic Technique

- 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.
- Reduce the number of life forms, in a general example, by wash hands with soap.
- In any environment where human operators are present, **microbial contamination at some level is inevitable.→**
  - Even the foremost cautious clean-room environment design and operation will not eliminate the shedding of microorganisms if human operators are present.




# Sterilization Process

- Persons who work with sterilization should be aware of:

1. The **effectiveness and limitation** of the sterilization technique to be used.
2. The **effect of these techniques on the material** to be sterilized since some material may deteriorate by specific technique such as protein denaturation by heat.

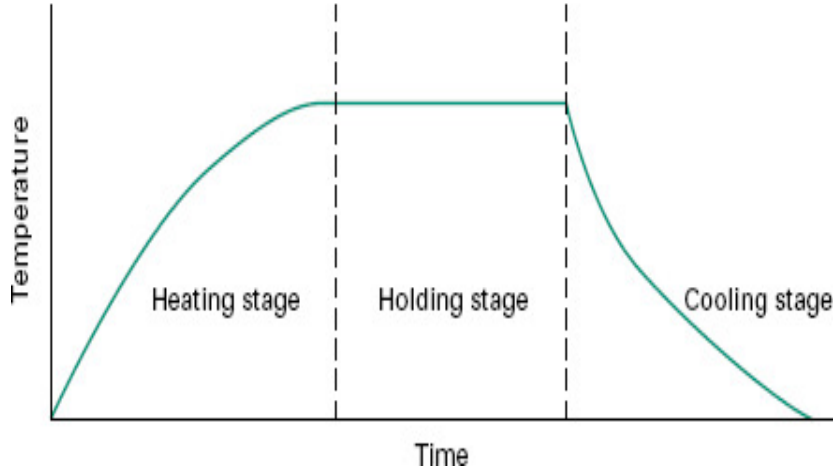
- So balance is needed between the most effective technique that will not affect our product.
- For example: it may be necessary to **add antibacterial agent** to a thermally sensitive product to enhance the effectiveness of a low-temperature sterilization process; thereby decomposition (by heat) is prevented while the combined effect of the antibacterial and the heat provide reasonable assurance that the product will be sterilized.

# Sterilization Process

- Microorganisms exhibit varying resistance to sterilization procedures.
- For example: **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 t**o be lethal to the most resistant spores of microorganisms, **with** additional treatment designed to provide a margin of safety against a sterilization failure.
- So sterilization procedures **must be validated** and this can be facilitated by using quantitative, theoretically principles such as: **Microbial Death Kinetic Expressions.**

# Kinetic of Microbial Death

- It is necessary to understand the kinetics of cell inactivation, and the calculation of parameters by which microbial destruction and growth inhibition are measured.
- The death of a population of cells exposed to heat or radiation is often found to follow or approximate to first-order kinetics.
- This mean that the percent decrease in microbial population (cell death) will depend on the amount of microorganisms remaining.

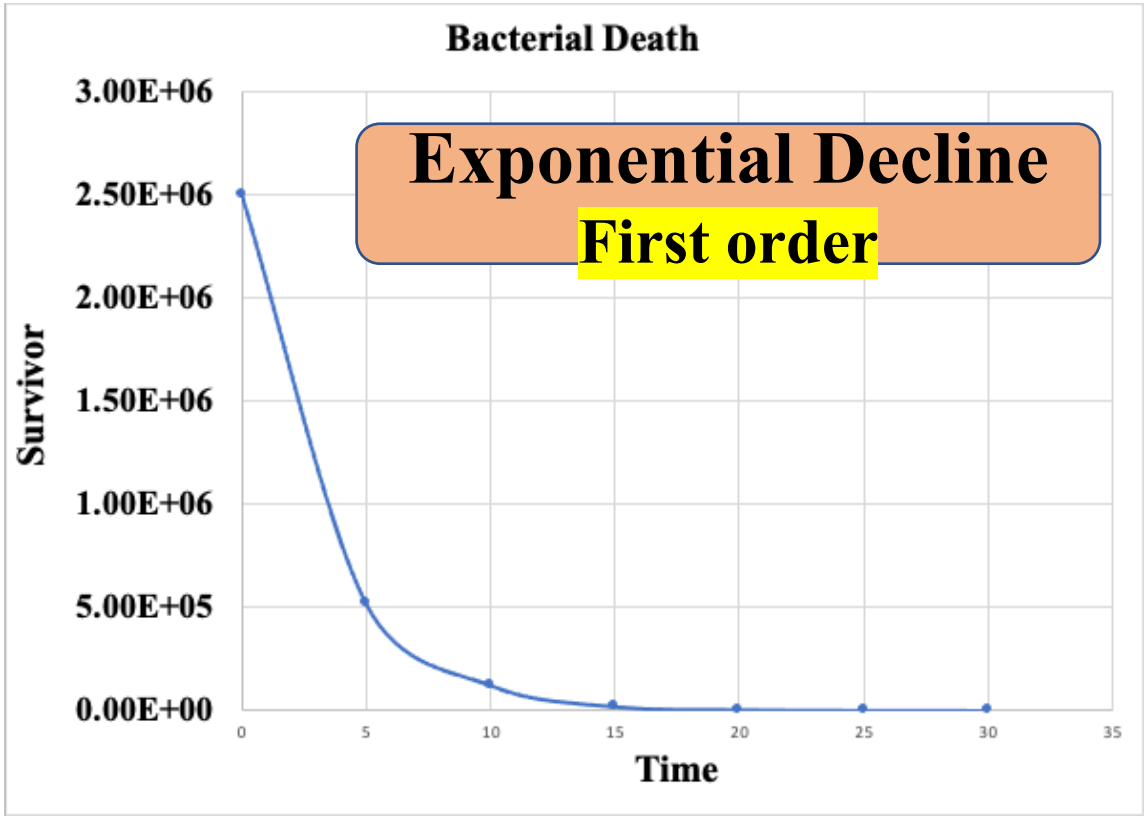


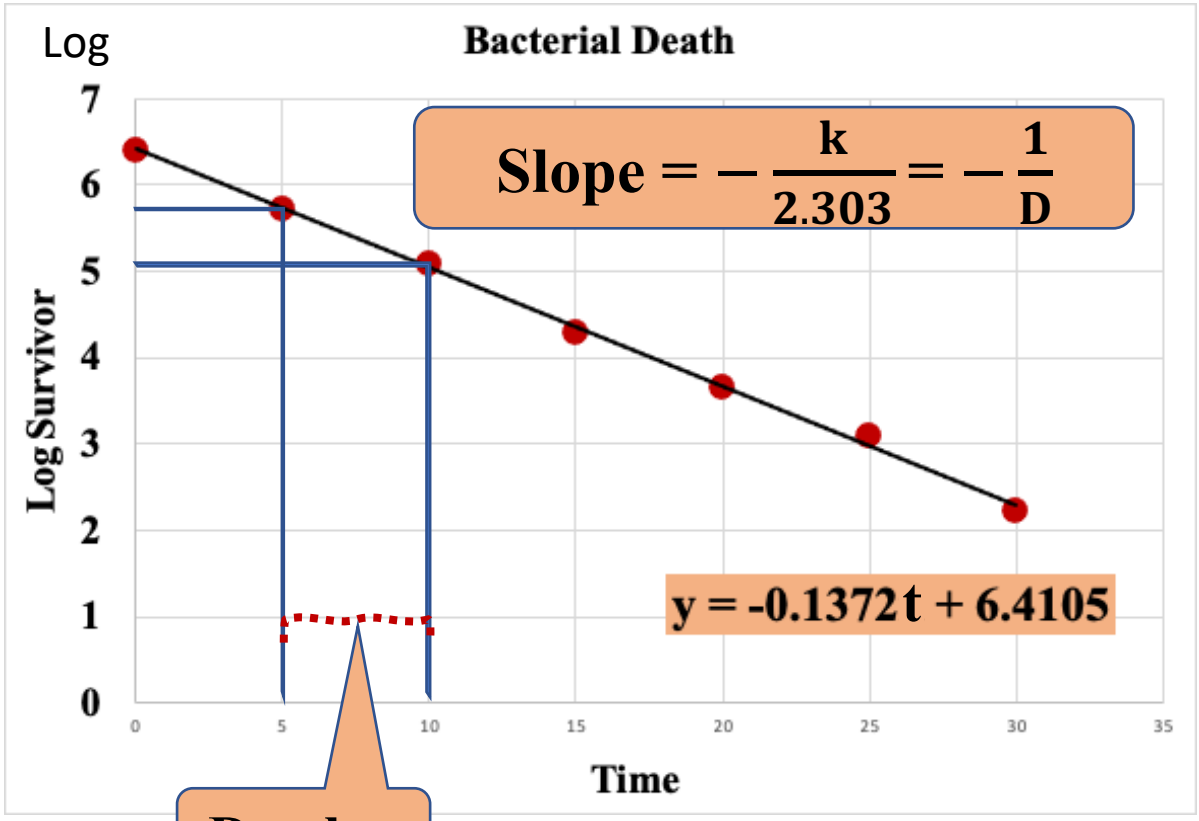
# D-Value

- The first term to know is the D value:
- D value (**decimal reduction time**): is the **time** (for heat, chemical, radiation, …) required for microbial population to decline by one decimal (one logarithmic unit, or by **90%** of the previous number)
- If we know D value **we can estimate the time** required for the sterilization.
- D value **measures the effectiveness of heat (or other methods)** at any given temperature, So its usually has a subscript showing the temperature like $D_{121}$.
- D value is **not very useful for sterilization using gases** (such as ethylene oxide) because of the complex interaction of heat, concentration of gas, and relative humidity.

# D value

- Death of Bacteria spores in pH 7 buffer at 95˚C
- There is a tabulated data of D value for Different microorganisms



# D value

- $N_o$= initial Microbial Population. $N_t$= Population at time t
- k= inactivation rate constant

$$N_t = N_o e^{-kt} \rightarrow \ln N_t = \ln N_o (-kt) \rightarrow \log_{10} N_t = \log_{10} N_o \left(\frac{-k}{2.303} t\right)$$

$$\text{Slope} = -\frac{k}{2.303} = -\frac{1}{D} \rightarrow D = -\frac{1}{\text{Slope}}$$

- D value have been calculated for various organisms.
- From these equations, killing of all microbial organisms will mean $N_u$=0 ➔
- ∵ Log 0 = ∞ ➔ *Guaranteed sterility* would therefore require an infinite exposure time. → theoretically impossible.

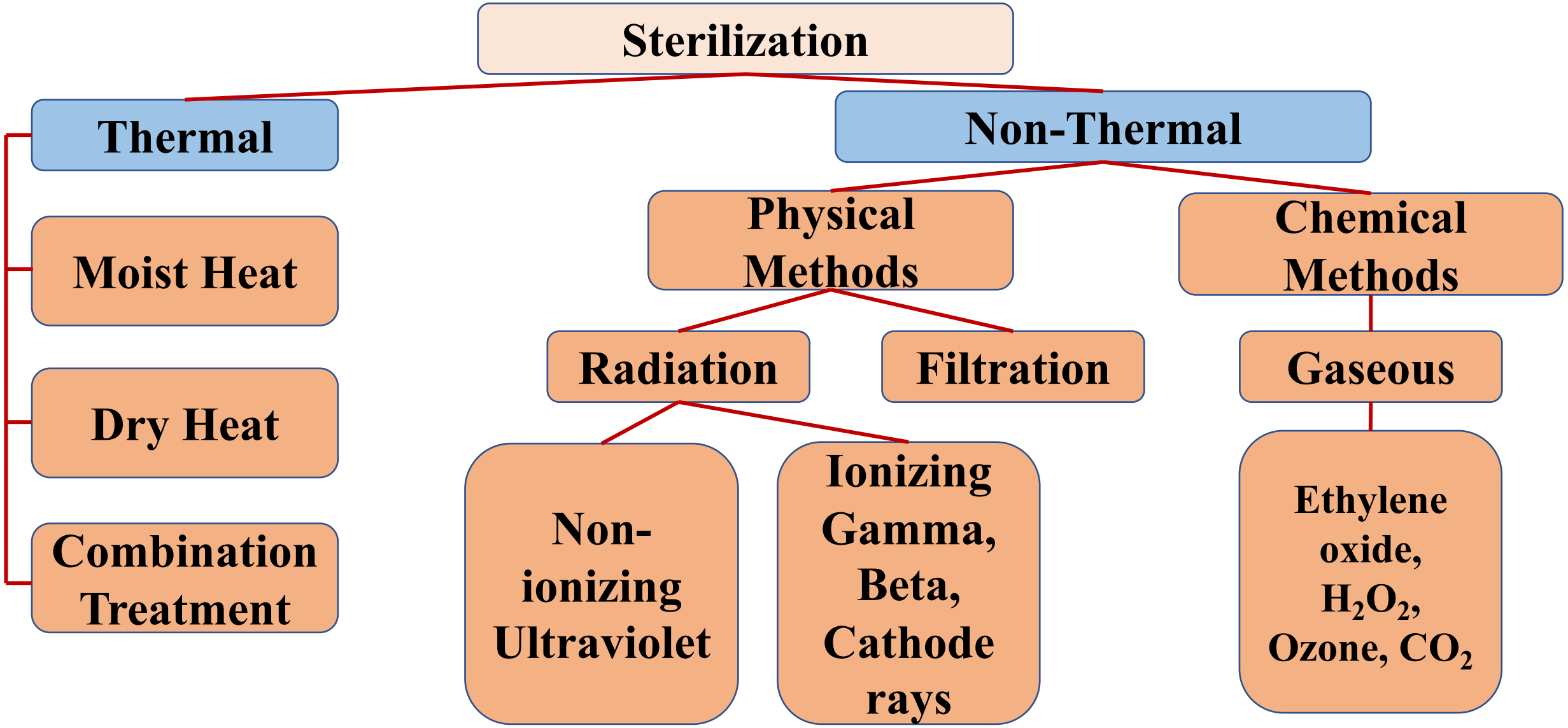
# Z-Values

- This **measure the effect of change in temperature** on death rate → it is the number of **degrees** (˚C, ˚F) required for a 1 log **(10-fold) reduction in the D-value** →
- It measures the extent to which a particular increase in temperature will reduce the D value.
- e.g. if the D value for *Bacillus stearothermophilus1* spores at 110 ˚C is 20 minutes and they have a Z value of 9 ˚C, this means that at 119˚C the D value would be 2.0 minutes and at 128 ˚C the D value would be 0.20 minutes.

$$Z = \frac{T_2 - T_1}{\text{Log } D_2 - \text{Log } D_1}$$

**T = Temperature**

# Sterilization Methods



# Physical Process of sterilization
# Thermal Methods

- Lethal effectiveness of heat on microorganisms **depends upon**:

1. Degree of heat.
2. Exposure period.
3. Moisture present.

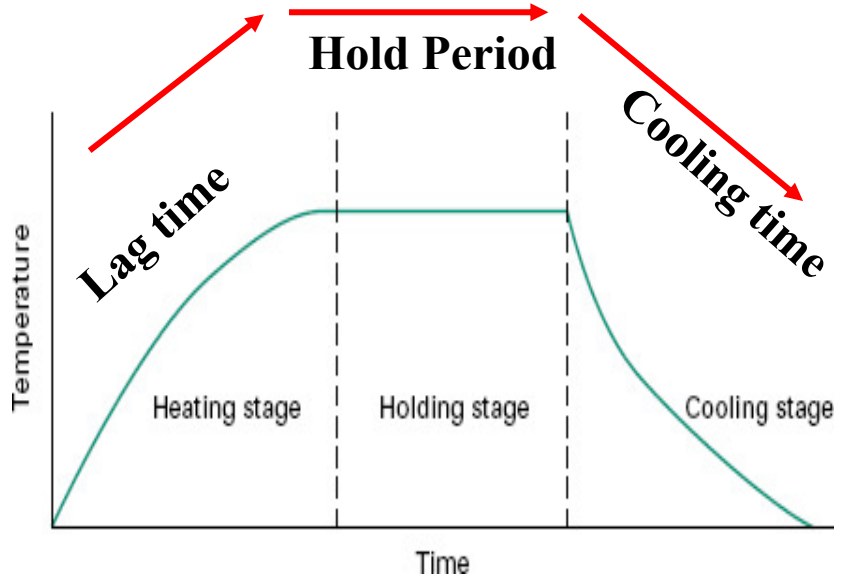

- Within the range of sterilization temperature, the time required to produce a lethal effect is **inversely** proportional to the temperature employed.
- Sterilization may be accomplished in **1 hour** with dry heat at a temperature of 170˚C, but may require as much as **3 hours** at a temperature of 140˚C.
- Thermal methods of sterilization may conveniently be divided into: Dry heat and moist heat.

# Dry Heat

- Substances that resist degradation at temp. above 140˚C may be rendered sterile by means of dry heat.
- 2 hr exposure to a temperature of 180˚C or 45 min at 260˚C kill **spores** as well as **vegetative** forms of **all** microorganisms.
- The state of hydration of a cell is an important factor determining its resistance to heat.
- **Mechanism**:
- Dry heat is believed to exert its lethal action upon microorganisms by **oxidizing proteins**, affecting particularly the reproductive process.

# Factors in Determining Cycle Time in Dry Heat Sterilization

- The cycle time is composed of three parts:
- Thermal increment time **(lag time)** of both the chamber and the load of material to **reach to the sterilization temperature**, assuming both start at room temperature.
- **Hold period** at the maximum temperature to achieve sterilization (actual sterilization process).
- **Cooling time** for the material to return to room temperature.



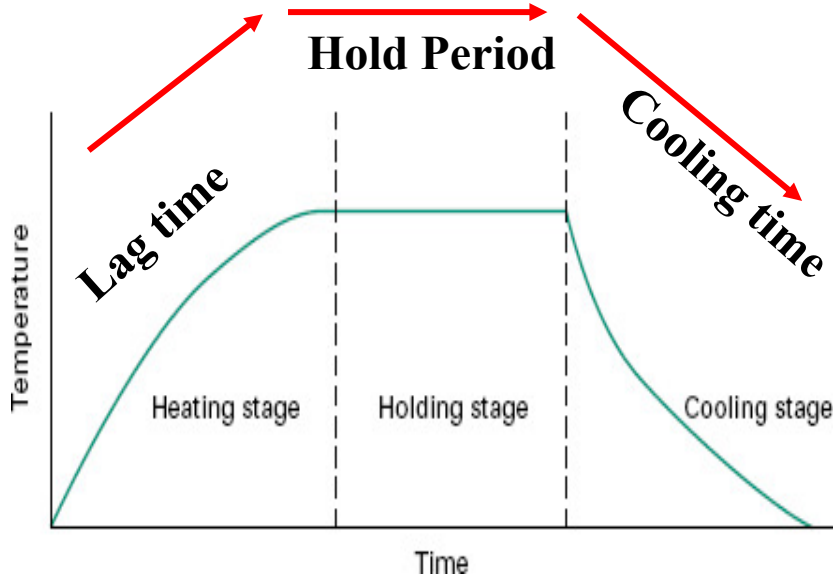
# Dry Heat Sterilization

- The lag time is the time required for all of the material to "catch up" with the temperature of the chamber.
- This time is **longer** with:

a) Larger quantities of material.

b) Poorer thermal conductance properties of the material.

- The effect of these factors on sterilization time must be studied carefully during validation studies to get an effective time cycle.



# Dry Heat Sterilizers
# Hot Air Ovens

1. **Hot Air Oven: Two types**

a) **Natural convection oven**: the circulation of air depends on the fact that the hot air will move up and cool air will move down.


- This will create an air current that distribute the heat around the oven.
- **Advantage**: simple design, reasonable cost.
- **Disadvantage**: air circulation is easily blocked with the container → resulting in:
  - Poor heat distribution efficiency.
  - Longer lag time.

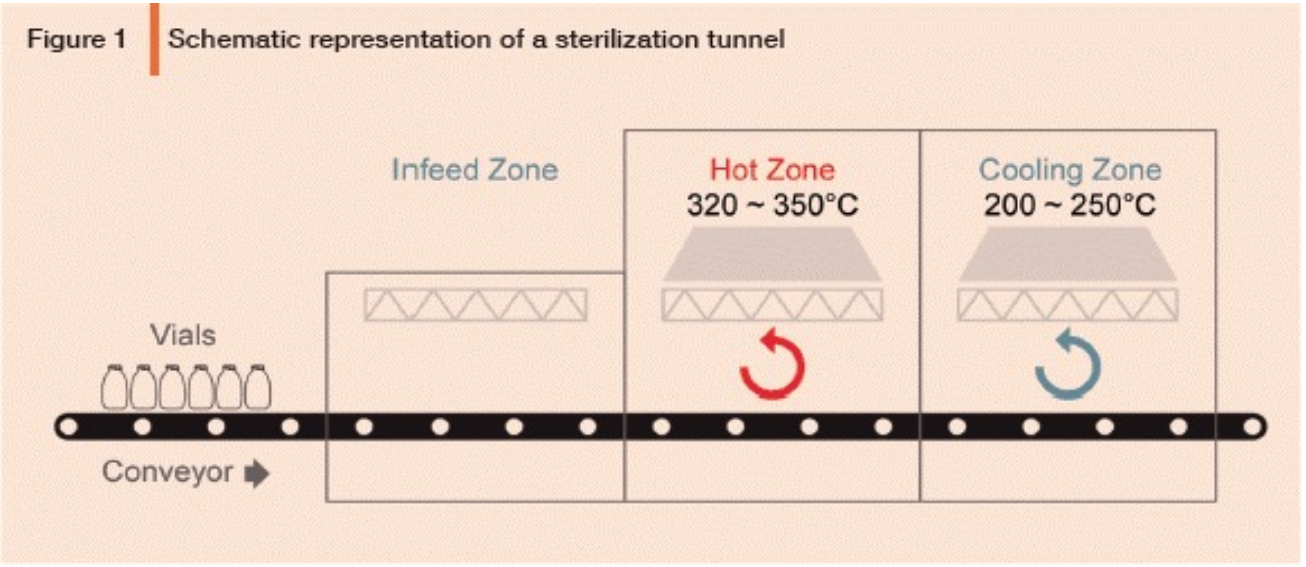
# Dry Heat Sterilizers
# Hot Air Ovens

**b) Forced Convection Oven**:

- The difference from the previous one is that it contains a **blower (fan)** to circulate the hot air around the oven which will provide better heat distribution.
- **Advantage**:
- Reasonable cost.
- Due to efficient heat distribution around the oven and the objects → shorter lag time → shorter time required for the whole sterilization process.

# Dry Heat Sterilizers
# Dry Heat Tunnel

- Designed to sterilize **glass bottles** and similar items.
- Contains a **moving belt** that moves the item under different temperature zones.
- The items is cooled by cold air before they exit the tunnel.




# Effect of Materials on Dry Heat Sterilization

- The elevated temperatures required for effective hot air sterilization in a reasonable length of time have an **adverse effect on many substances**:

I. **Cellulose materials** (paper and cloth) begin to char (burn) at a temperature of about 160◦C.

II. At these temperatures, many **chemicals are decomposed**, rubber is rapidly oxidized, and thermoplastic materials melt (material that becomes pliable or moldable at a certain elevated temperature and solidifies upon cooling).

III. Expansion of materials that heated from room to sterilizing temperatures. For example **Glassware must not be wedged tightly** in the oven chamber, containers for oils must be large enough to permit expansion of the oil.

- Due to these effects, **dry heat method is reserved for glass ware, metal-ware, anhydrous oils**, and materials that can withstands elevated temperature range without degradation.

# Advantages of Dry Heat

- Provide a completely **dry glass-ware** and **metal-ware** at the end of sterilization process. These dry containers are essential in the manufacturing of dosage forms fer example the anhydrous products.
- Dry heat **effectively destroys pyrogens**, usually requiring about **twice the hold time** for sterilization.

- Note: **to maintain sterility after sterilization**:

I. The openings of **equipment must be covered** with a barrier material such as aluminum foil.

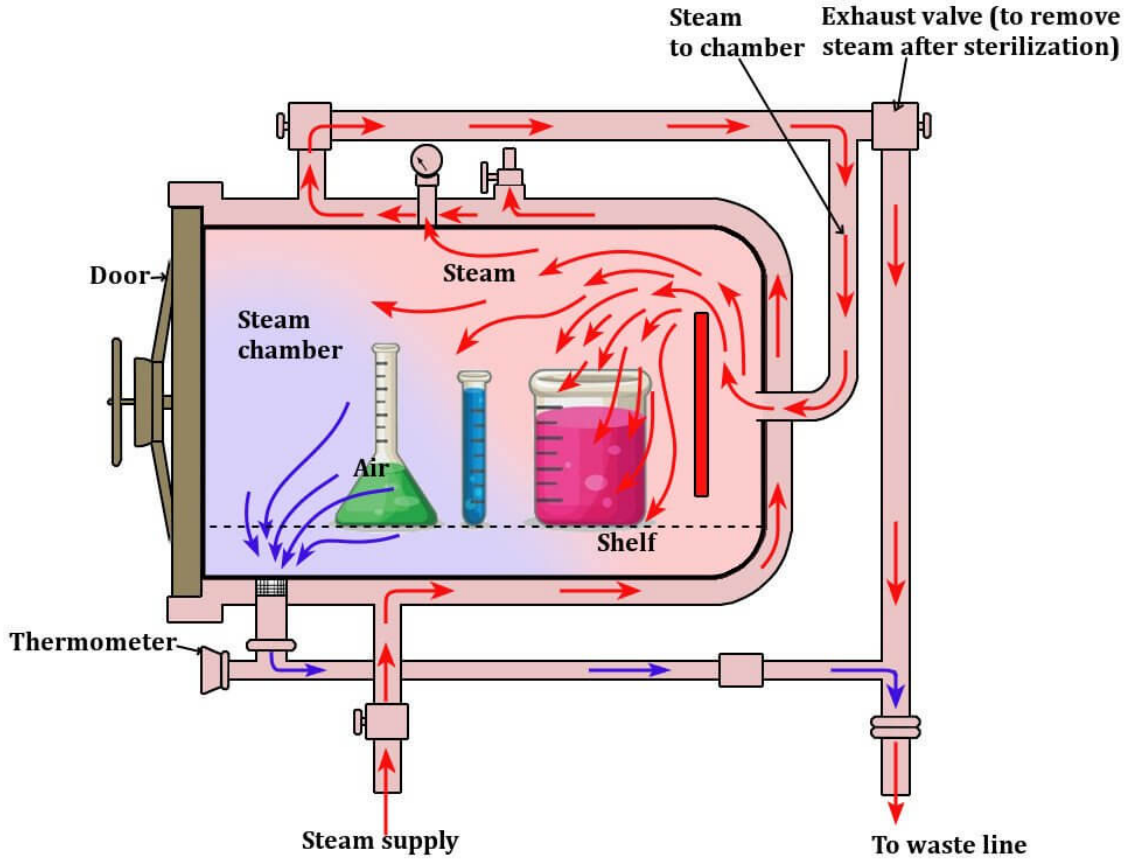
II. As an alternative, items to be sterilized may be placed in a **covered stainless steel box** or similar protective container

# Thermal Sterilization
# Moist Heat (Autoclave)

- **More effective** than dry heat for thermal sterilization. However, the normal moist heat cycles **do not destroy pyrogens**.
- **Mechanism**: Moist heat causes the **coagulation of protein** of living cells.
- Due to the **heat capacity of steam** is much greater than dry hot air, this mean when a saturated hot steam hit a cold object it **will liberate heat energy** much higher than dry air (about 500 times higher) → the object is heated much more rapidly by steam.
- This because that steam do have the same temperature of water that producing it **but has much higher latent heat**.
- This latent heat is available for transfer (without a decrease in temperature) when it condenses on to a cooler surface.

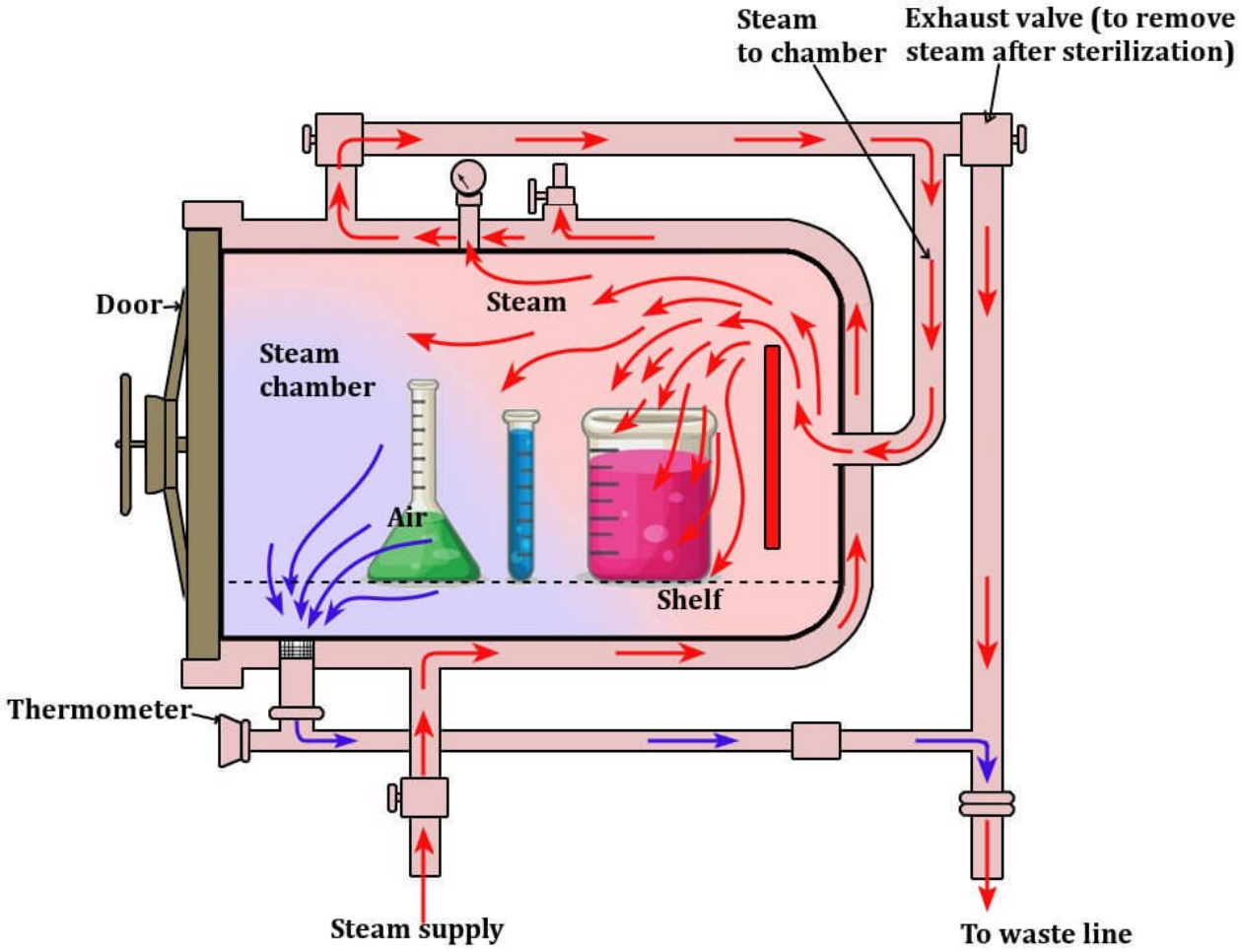
# Autoclave

- The density of steam is lower than that of air.
- Steam enters an autoclave chamber and **rises to the top → displacing air downward**.
- Objects must be placed in the chamber with adequate circulation space around each object → air can be displaced downward and out of the exhaust line from the chamber.
- Any trapped air in containers with continuous sides and bottoms or in tightly wrapped packs, **prevents penetration** of the steam to these areas and **thus prevents sterilization.**



# Autoclave

- The air trapped in this manner is heated to the temperature of the steam. But→
- Hot air in oven at a temp. of 120˚C requires a cycle time of 60 hrs to ensure a lethal effect on spores → A 20-min exposure at this temp. with hot dry air would be entirely inadequate.
- → containers must **not be tightly closed** inside the autoclave.



# Air-Steam Mixture

- Some times **pure steam** used in autoclave can cause damage to the item being sterilized due to inability to control pressure of pure steam. →
- So mixture of air and steam is used.
- **Advantage**: This mixture will have **lower thermal capacity** than pure steam **but** it will be easier to control pressure so can sterilize items such as flexible wall containers (such as plastic bags).
  - This is because we can control the pressure **outside the container** to be **equal** to the pressure inside the container which will protect these containers form collapsing or swelling and bursting.
- **Disadvantage**: these type of sterilizers required continuous mix of the air and steam because they have the tendency to stratify (separate).

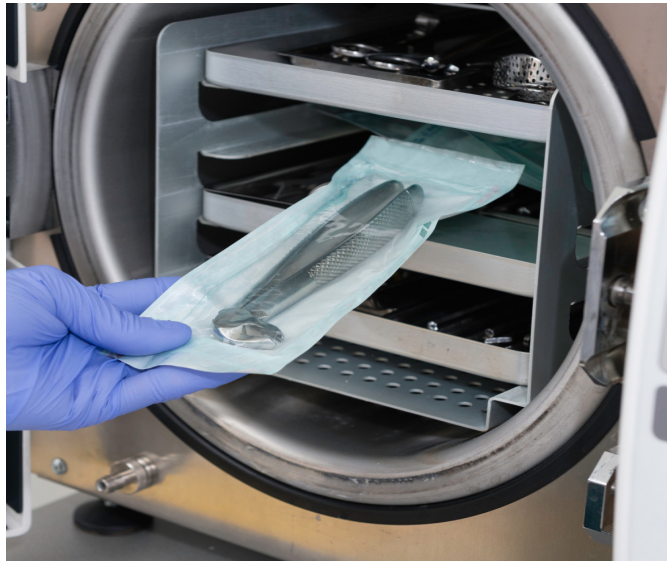
# Factors Determining Cycle Time in Moist Heat Sterilizers

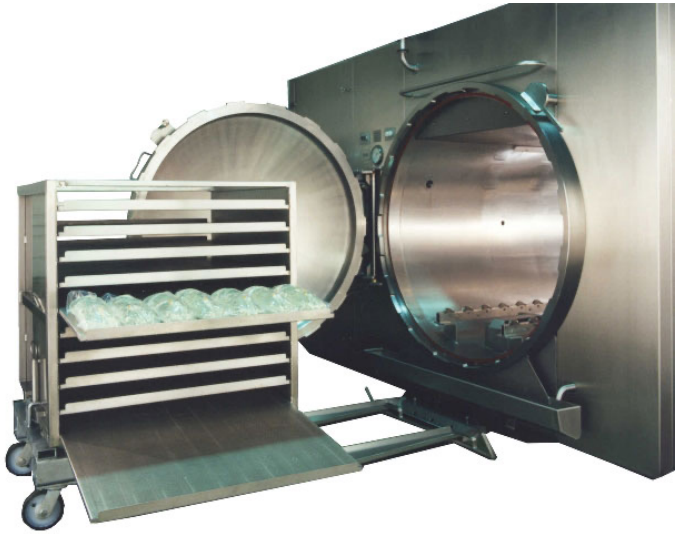
- **Spores and vegetative** forms of bacteria were effectively destroyed in an autoclave employing steam under pressure during an exposure time of:

I. 20 min at 15 pounds pressure (121˚C).

II. 3 min at 27 pounds pressure (132 ˚C).

- These times bases on the assumption that:
- Steam has reached the innermost point of the material to be sterilized.
- Temperature of the material is held for at least one half of that time interval.





# Factors Determining Cycle Time in Moist Heat Sterilizers

- **Note**: Bottles of solution (closed bottles), the heat must be conducted through the wall of the container, raise the temperature of the solution to that of its environment, and generate steam within the container from the water therein.
- → This will cause a significant **lag time** involved before the solution reaches the sterilizing temperature.
- **Ex**: its been found that autoclave at 121 ˚C for 20 min is effectively sterilize 1200 amp. Containing 5 ml each.
- And a bottle of 6 L requires 60 min at 121˚C.
- These two examples shows **the effect of amount of material** to be sterilized on the required time for sterilization.

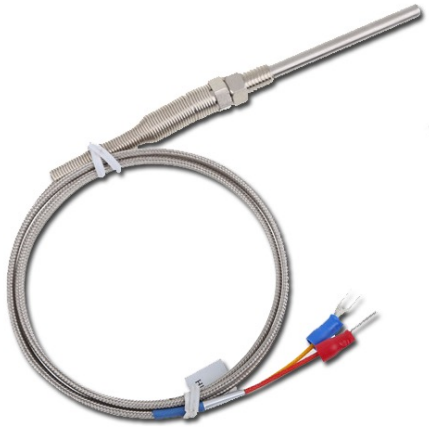
# Application of Moist Heat Sterilization

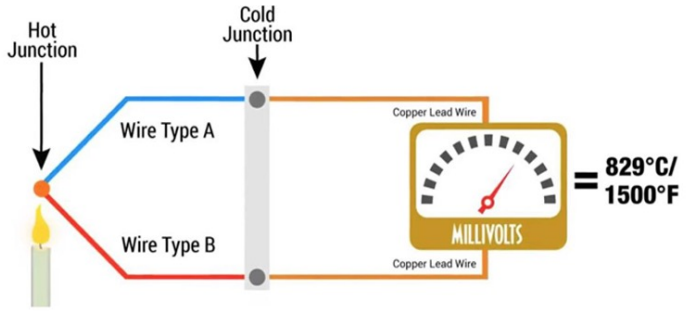
- Usually the moist heat under pressure is the **first choice** for thermal sterilization **whenever is possible**.
- As long as the container is autoclavable, moist heat can be applied for many materials:
- Sealed liquid product which **will stay sterile until** a break of the seal is happened.
  - Note: non-aqueous **sealed products** cannot be sealed in this way because there is no water inside to generate the required steam. → require other sterilization method such as **radiation**.
- Moist heat is applicable to equipment and supplies such as rubber closures, glassware.

# Evaluation of Thermal sterilization

- The effectiveness of sterilization technique **must be evaluated and validated** prior to its application in large scale processing.
- It also should be evaluated during the process.
- Indicator is used to evaluate the sterilization process such as:

**a) Thermocouple**: can be inserted in the sample (or close to it) to record the temperature throughout the process. This provides a continuous recording.

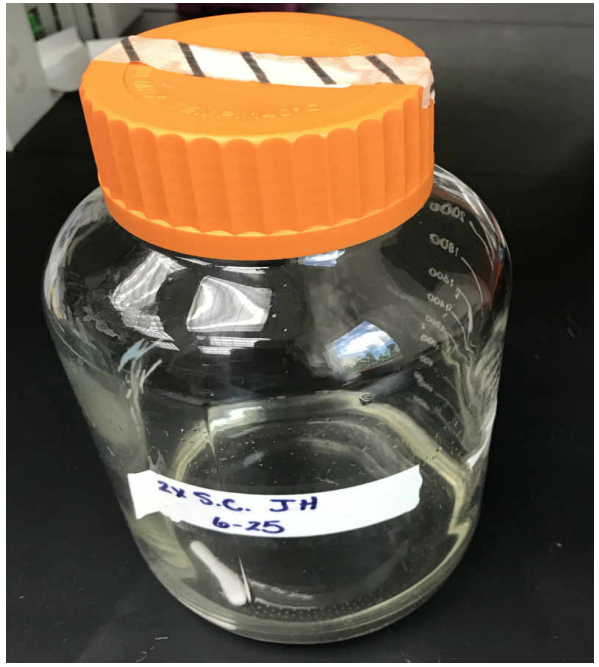





# Evaluation of Thermal sterilization

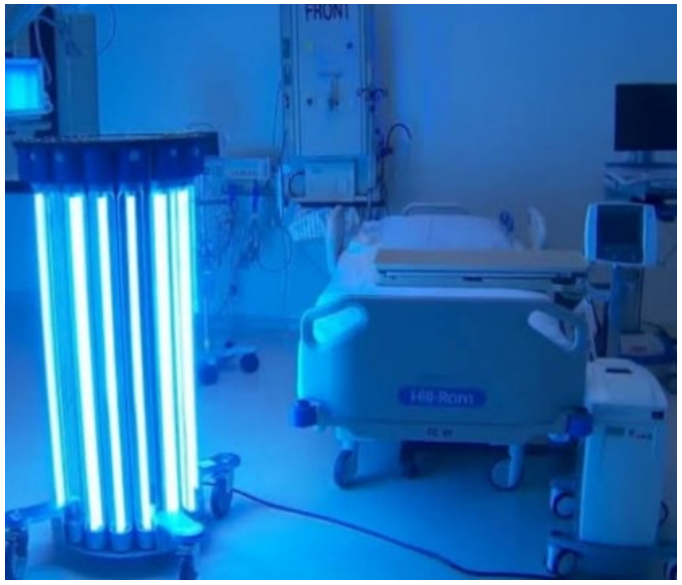
**b) Autoclave sterilization:** indicator used include:

1. Wax or chemical pellets that melt at 121˚C.
2. **Paper strips** impregnated with chemicals that change color under the influence of moisture and heat.
3. **Resistant bacterial spores** in sealed ampoules or impregnated in dry paper strips are used as biologic indicators:
   - Their destruction is evidence of a sterilization process.
   - **Their use** is mostly to **validate the equipment** and not commonly used for the day to day processing like number 1 and 2 because of the **variability** in resistance between different bacterial generations and **the safety** issues of placing a pathogen next to a material intended for human use.



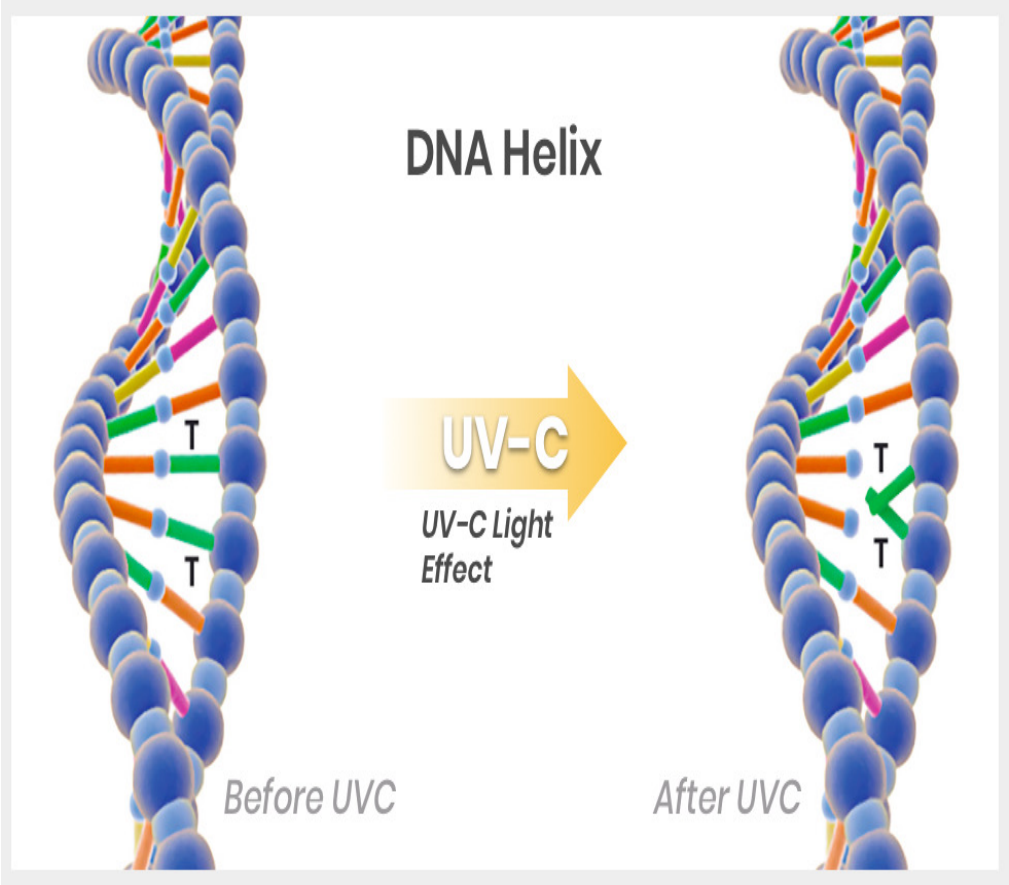

# Non-Thermal Methods
# Cold Sterilization

- **Radiation Sterilization**:
- **Ultraviolet radiation (UV):** a non particulate radiation**.**
- Commonly employed **to aid** in the reduction of contamination in the **air** and **on surfaces** within the processing environment.
- The **germicidal light** produced by mercury vapor lamps is emitted almost exclusively at a wave length of 253.7 nm.
- Ultraviolet light **penetrates clean air and pure water well**.
- **But** an increase in the salt content and/or the suspended matter in water or air causes a rapid decrease in the degree of penetration.
- For other application → due to the penetration is limited → any germicidal action is confined **to the exposed surface**.



# Ultraviolet Radiation (UV)

- **Mechanism**:
- When UV light passes through matter → Energy is liberated to the orbital electrons within constituent atoms → This absorbed energy causes a highly energized state of the atoms and alters their reactivity.
- When such excitation and alteration of activity of essential atoms occurs within the molecules of microorganisms (such as DNA) → organism dies or is unable to reproduce.



# Ultraviolet Radiation (UV)

- **Lethal Dose**:
- The **germicidal effectiveness** of ultraviolet light is a function of:

I. Intensity of radiation.

II. Time of exposure.

III. Susceptibility of the organism.

- Due to these factors, organism may recover after exposure to UV light if no enough radiation is delivered to the surface.

# Ultraviolet Radiation (UV)

- To maintain maximum effectiveness:

I. UV lamps must be kept **free from dust**, grease, and scratches because of the large reduction in emission intensity will occur.

II. Light **must Replaced** when emission levels decrease (about 30 to 50%), owing to energy-induced changes in the glass that inhibits the emission.

- **Uses**: UV lamps are used primarily:

a) Germicidal effect **on surfaces**.

b) Penetrating effect through **clean** air and water.

- Therefore they are frequently installed in rooms, airducts, water supplies.