Sterilization

Is the complete destruction or elimination of microbial life based on a probability function.
Sterilization methods
(Methods of inactivating microorganisms)

Physical
- Dry heat
- Moist heat
- Radiation

Chemical
- Gas sterilization

Mechanical
- Filtration
Kinetics of Sterilization

Survival Curve

If a population of organisms is exposed to a lethal agent, the proportion of surviving can be plotted at any time against the exposure.

Survival Curves are diagramed for each species for different sterilization conditions.
**Survival Curve** is often exponential (typical of first order kinetics).

\[ N_t = N_0 \times 10^{-t/D} \]

- \( N_0 \) is the initial number of m.o.
- \( t \) is the sterilization time
- \( N \) is the number of m.o. after exposure time \( t \)
- \( D \) is the **decimal decay time** (the time interval required to reduce the microbial population by 90%, to 1/10 or by one logarithmic value).
**Inactivation Factor**: is the degree to which the viable population of organisms is reduced by the applied treatment.

\[
\text{Inactivation Factor} = \frac{\text{Initial viable count}}{\text{Final viable count}}
\]

\[
\text{Inactivation Factor} = 10^{t/D}
\]

\( t \): the treatment time in minutes
Dry heat treatment
(Sterilization & depyrogenation)

The aim of sterilization is to destroy the ability of m.o. to survive & multiply.
The aim of depyrogenation is to destroy the chemical activity of the by-products.

The most common operating conditions are:

**Sterilization**
- 160°C for 120-180 min
- 170°C for 90-120 min
- 180°C for 45-60 min

**Deyrogenation**
- 230 °C for 60-90 min
- 250 °C for 30-60 min
Dry heat treatment

Red heat incineration

IR radiation

Flaming

Hot air oven
Hot air sterilization

Method: The preparation to be sterilized is distributed in its final container, which are either finally or temporarily closed to exclude m.o. in Hot air oven

Articles in oven can receive heat by direct transfer from the hot air circulating, by radiation from the oven walls and by conduction.
Hot air sterilization

**Mechanism:** Oxidation of essential cell constituents such as enzymes & other proteins leading to irreparable degradation.
Hot air sterilization

**Applications:** Heat stable, non-aqueous products

- Oily vehicles (soft & hard paraffin, ethyl oleate, waxes)
- Oily injections containing heat stable drugs.
- Oily suspensions if the suspended solid doesn’t dissolve at high temperatures or recrystallize into larger Aggregates on cooling.
- Glycerol
- Maize starch, powders, dusting powders containing talc.
- Paraffin gauze dressings.
- Implants of steroids with m.p. ≥ 150 °C
- Glassware: test tubes, pipettes & flasks
- Laboratory equipments: Forceps & throat swaps.
- Silicone rubber
Hot air sterilization

Not for sterilization of:

1. Aqueous fluids, potential lethal explosive power will generate.
2. Rubber goods and lab coats, due to burning.
3. Culture media, the liquid would boil to dryness.
Moist heat treatment

- Steam sterilization (autoclaving)
- Tyndallization

Heating with a bactericide
Steam sterilization

The sterilizing medium is pressurized saturated steam most commonly at 121°C for 15 min (corresponding to an inactivation factor of $10^7$-$10^{12}$).

Saturated steam at 121°C has a pressure of 2.05 abs atm.
Steam sterilization

**Mechanism:** When heat is applied in presence of sufficient water, disulphide bonds & hydrogen bonds between the protein strands can be broken and the strands have sufficient mobility to form new linkages resulting in denaturation of the protein.
Steam sterilization

**Mechanism:** When heat is applied in presence of sufficient water, disulphide bonds & hydrogen bonds between the protein strands can be broken and the strands have sufficient mobility to form new linkages resulting in denaturation of the protein.

Spores show higher resistance to moist heat because they contain dipicolonic acid which protect the proteins against thermal displacement.
Steam sterilization

**Mechanism:** When heat is applied in presence of sufficient water, disulphide bonds & hydrogen bonds between the protein strands can be broken and the strands have sufficient mobility to form new linkages resulting in denaturation of the protein.

Moist heat must make direct contact with the m.o. (directly or indirectly).
Steam sterilization

Applications: Heat stable, aqueous products

• Aqueous injections.
• Distilled water
• Saline solution
• Bulky cotton dressings.
• Lab coats & aprons.
• Glassware & medical devices (they are required to be dry & are generally sterilized in hot air ovens).
• Some plastic & rubber materials (thermoplastic).
• Solid & liquid media.
Steam sterilization

Not for sterilization of:

1. The inside of an empty closed container.
2. Anhydrous oil based solutions in closed containers.
Heating with a bactericide

It consists of heating the solution with a bactericide in their final containers at 98-100°C for 30 min.
Heating with a bactericide

**Applications:** aqueous preparations unstable at elevated temperatures of moist heat sterilization.

- Aqueous injections, eg. morphine.
- Solutions of extreme pH values (even without bactericide), eg. Procaine injection & Phenobarbitone injection, B.P.
- Solutions of drugs with intrinsic antimicrobial activity.
- Eye drops.
- Surgical instruments in solutions of Na carbonate or borax.
Heating with a bactericide

Not for sterilization of:

1. Any injection reaching the cerebrospinal fluid, eg. Intrathecal and intraatrium.
2. Intracardiac injections.
3. IV injections greater than 15 ml in volume.
4. Intraocular injections.
Sterilization by Ionizing Radiations

Particle radiation

Electromagnetic radiation

UV radiation

Gamma radiation
Gamma Radiation

It is done by exposure of the product in its final container to $\gamma$-radiation emitted from radioactive materials such as Cobalt-60 or Cesium-137.

Sterilization doses starts from 2-25 KGy for suitable period of time.
Mechanism: Ionizing radiations kill or inactivate m.o. by ionization of & altering the molecular structure or spatial configuration of the biologically active macromolecules involved in cell replication (loss of reproductive capability).

As with heat, spores are more resistant in the dry state than in the fully hydrated conditions.
Applications: Heat sensitive products, densely packed material of any geometry and Pre-packed products.

• Several drugs in the dry state, eg. Some anticancer drugs, vitamins, antibiotics and hormones (cold process).
• Ready-packed disposable surgical equipment (Deep penetration power).
• Catgut & adhesive dressings.
• Ointments
• Latex Gloves
• Contact lens solutions.
Gamma Radiation

Not for sterilization of:

1. Aqueous solution (drug degradation could be mediated by free radicals in water).
2. Teflon & polypropylene (severe damaged).
Gas sterilization

The process involves exposure of materials to a variety of gases or vapors of germicidal properties

- Ethylene oxide (EtO)
- Ozone
  - Formaldehyde
  - Propylene oxide
Gas sterilization

Ethylene oxide occurs as a vapor at room temperature & pressure (B.P.=11°C). It is colorless & heavier than air.

Mechanism: Alkylation of –SH, -OH, -COOH, NH₂ groups in enzymes, proteins & nucleic acids of m.o.

The reaction must be activated by the presence of water vapor. It increases by temperature & EtO conc.

Direct contact with the m.o. is a must.
Gas sterilization

Ethylene oxide occurs as a vapor at room temperature & pressure (B.P.=11°C). It is colorless & heavier than air.

\[
\begin{array}{c}
\text{CH}_3 \quad \text{CH}_3 \\
\quad \text{O} \\
\text{Oxygen bridge}
\end{array}
\]

Normal EtO conc. is 400-1200 mg/ml at 30 - 60 °C & relative humidity > 30% for ≥ 3 hours.
Gas sterilization

**Applications**: objects sensitive to temperatures greater than 60 °C and / or radiation such as plastics.

- Surgical equipment made wholly or partially of plastic.
- Medical products packed in plastic or paper.
- Absorbable dusting powders.
- Rubber articles (gloves).
- Woolen blankets.
Gas sterilization

Limitations for gas sterilization:
1. EtO is highly flammable. So it is used as a mixture with inert gases e.g. CO₂ or N₂.
2. Different process variables are involved.
3. Slow process.
4. Toxic (sterilized items should be left long to eliminate absorbed EtO residues).
5. Doesn’t penetrate some plastic & nylon wraps (must be left open during exposure).
6. Not for liquids (solutions or emulsions).
Sterilization by filtration

It is the removal of m.o & particulate matter from a fluid stream followed by aseptic transfer to the final container.

Membrane filters have become the filters of choice. They are thin, strong and homogenous polymeric structures which retain m.o. by the process of physical sieving.

Membrane filters of 0.1 and 0.22 µm pore size are used.
Membrane filters are manufactured from a variety of polymers, such as cellulosic esters (MCE), polyvinylidene fluoride (PVF) and polytetraflouroethylene (PTFE).

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>PVF, MCE</td>
</tr>
<tr>
<td>Aqueous, extreme pH</td>
<td>PVF, MCE</td>
</tr>
<tr>
<td>Oil</td>
<td>PVF, PTFE</td>
</tr>
<tr>
<td>Organic solvent</td>
<td>PVF</td>
</tr>
<tr>
<td>Gases</td>
<td>PVF, PTFE</td>
</tr>
</tbody>
</table>
Sterilization by filtration

Membrane filters are manufactured from a variety of polymers, such as cellulosic esters (MCE), polyvinylidene fluoride (PVF) and polytetrafluoroethylene (PTFE).

Membrane filters are sterilized by autoclaving, in-situ steaming or by EtO.
Sterilization by filtration

Applications: Thermolabile materials where none of the foregoing methods is applicable.

- Large volume solutions.
- Eye drops if the dropper bottle doesn’t withstand heating.
- Drugs unstable in aqueous solution, e.g. hormones & enzymes, filtration in non-aqueous solvents then removal.
- Paraldehyde Injection.
- Serum
- Solutions of antibiotic.