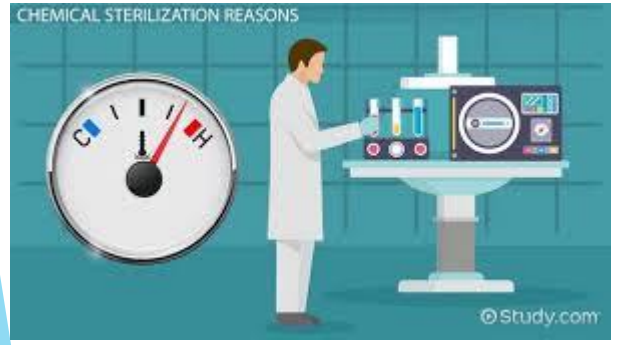
STERLIZATION & DISINFECTION

Sterilization: The inactivation of all self-propagating biological entities (e.g. bacteria, viruses, prions) associated
with the materials or areas under consideration 100% killing.

Disinfection: The reduction of pathogenic organisms to a level at which they no longer constitute a risk.

Antisepsis: it is used to describe disinfection applied to living tissue such as a wound.



Cleaning: it is a soil-removing process which removes many micro-organisms. The reduction in contamination by cleaning process is difficult to quantify other than visually. However, it has wide applications in the hospital environment and disinfection.

Decontamination: it is a general term for the treatment used to make equipment safe to handle, and includes microbiological, chemicals, radioactive and other contaminations

Procedures that kill micro-organisms have important applications in practical microbiology and in practice of medicine and surgery.

Laboratory work with pure cultures requires the use of apparatus and culture media that have been rendered sterile.

The need to avoid infections in the patients requires the use of equipment, instruments, dressing and parenteral drugs that are free from all living micro-organisms, or at least from those that may give rise to infections.

Sterilization and disinfection

- The choice of method of sterilization or disinfection depends on:

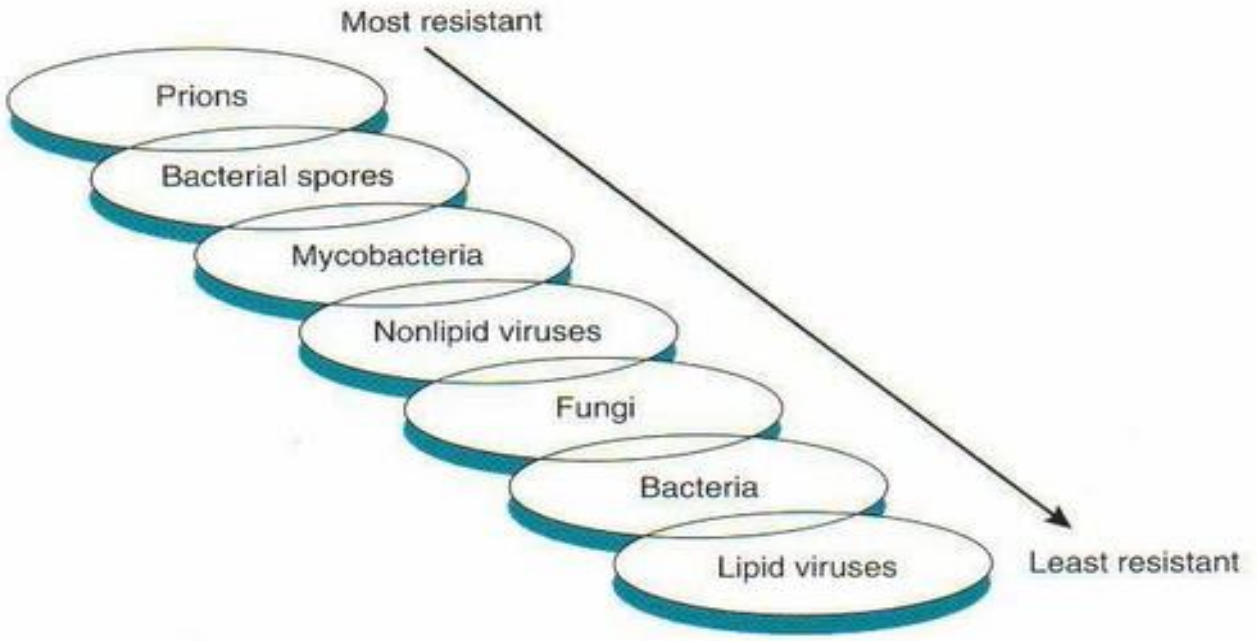
- The nature of the item to be treated
- The likely microbial contamination (degree of soilage)
- The risk of transmitting infection to patients or staff in contact with the item.
- ** Process must not damage the device.

Factors that influence the degree of killing

- Types of organisms
- Number of organisms
- Concentration of disinfecting agent
- Presence of organic material (e.g., serum, blood)
- Nature (composition) of surface to be disinfected
- Contact time
- Temperature
- pH
- Biofilms
- Compatibility of disinfectants and sterilants

Types of organisms

- Organisms vary in their ability to withstand chemical and physical treatment, e.g.,
- -**Spores**–have coats rich in proteins, lipids and carbohydrates
- -**Mycobacteria**–cell walls are rich in lipids
- -**Biofilms** -microorganisms living together in communities
- -**Prions**–the most resistant known organisms to the action of heat, chemicals, and radiation !!!
- Prions can withstand temp. exceeding 121C for several hours while immersed in acid or basic solutions



Different types of organisms and their resistance to killing agents

Number of organisms

- **Microbial load-the total number of organisms which determine the exposure time of killing agent**
- -is composed of organisms with varying degrees of susceptibility to killing agents
- -not all organisms die at the same time
- -higher numbers of organisms require longer exposure

Concentrating of disinfecting agent

- A proper concentration of disinfecting agents ensure the inactivation of target organisms, e.g.
- Povidone-iodine should be diluted with water before use because there is not enough free iodine to kill microorganisms in concentrated solution.
- The free iodine in the diluted solution, slowly liberated from the povidone-iodine complex and kill microorganisms.

Presence of organic material
(such as blood, mucus, pus)

- **affects killing activity** by inactivating the disinfecting agent, e.g,
- -by coating the surface to be treated, prevents full contact between object and agent (Glutaraldehyde)
- -easily inactivate bleach (Sodium hypochlorite)
- **For optimal killing activity,** instruments and surfaces should be cleansed of excess organic material before disinfection !!!

Nature (composition) of surface to be disinfected

- **some medical instruments** are manufactured of **biomaterials that exclude** the use of certain disinfection and sterlization methods because of possible damage, e.g.,
- -**endoscopic instruments** cannot be sterilized by the heat in an autoclave

Contact time

- the amount of time a disinfectant or sterilant is in contact with the object is **critical** e.g.,
- -**Betadine** (alcohol and iodine) must be in contact with object for at least **1 to 2 min.** to kill microorganism.
- -**the spores of bacteria and fungi** need a much **longer time**

Temperature

- **Disinfectants** are generally used **at room temp. (20-22°C)**
- ✓their activity is increased by an increased temp.
- and decreased by a drop in temp.

pH

- The pH of the material to be desinfected or sterilized can have an **effect on the activity** of disinfecting or sterilizing agent

Biofilms

- **communities of microorganisms**
- ✓can be on a surface of either inanimate or animate objects, e.g.,
- -**catheters (criticial place!), pipes** that carry water and **dionizing colums** used to make processed water
- ✓make disinfection more difficult
- ✓**the concentration of the disinfectant and the contact time** need to be **increased**

Compatibility of disinfectants

- **a common mistake is to believe that two disinfectants are better than one !**
- ✓some of them may inactivate other, e.g.,
- -the bleach and quaternary ammonium compound together negate the activity of both

STERILIZATION AND DISINFECTION

Sterilization methods

- **Physical methods**
- •Moist heat in autoclaves
- •Dry-heat in ovens
- •Gamma irradiaton
- •Filtration
- •Plasma sterilization
- ✓**Chemical agents**
- •Ethylene oxide
- •Glutaraldehye (high concentration)

Disinfection methods

- **Chemical agents**
- •Alcohols
- •Aldehydes
- •Halogens
- •Phenols
- •Surfactants
- •Heavy metals
- •Dyes
- •Oxidants
- ✓**Physical methods**
- **•Boiling and pasteurisation**
- **•Ultraviolet radiation**

Dry Heat

- **Most common method**
- Dry Heat kills microorganisms by destroying their oxidative processes.

1. Red heat:

The item to be sterilized is directly held in the flame and heated till it becomes red hot.
Application :- Bunsen burner used for sterilizing bacteriological loops, knives, blades

2. Flaming:

killing of organisms present on the surface of slides, mouth of culture tubes, …

Incineration:

Incinerator is a huge metal compartment used for burning all the objects that cannot be cleaned. Used for disposal of hospital waste

Hot air oven:

Hot air oven expose items to 160-170 °C for 1-2 hour. It has electric element in chamber as source of heat plus a fan to circulate air for even distribution of heat in chamber. Used to sterilize items that are lacking water such as metals and glassware.

Moist heat

- Steam is non-toxic and non-corrosive, but for effective sterilization it must be:

1 -Saturated: which means that it holds all the water it can in the form of a transparent vapor.

2 -Dry, which means that it does not contain water droplets.

- When dry saturated steam meets a cooler surface it condenses into a small volume of water and liberates the latent heat of vaporization.

- The energy available from this latent heat is considerable

- For example, 6 L of steam at a temperature of 134°C (and a corresponding pressure of 3 bar absolute) will condense into 10 mL of water and liberate 2162 J of heat energy.

Moist Heat

- Moist heat kills microorganisms by denaturation of proteins.

- Moist heat at temperature below 100°C:
  - Pasteurization
  - Inspissation
- Moist heat at temperature at 100°C:
  - Boiling
  - Tyndallisation
- Moist heat at temperature above100°C:
  - Autoclaving

➢ Moist heat at temperature below 100°C:

- **Pasteurization:**

  - ➢ Used heat at temperatures sufficient to inactivate harmful organism in milk.
  - ➢ Temperature may be 72°C for 20 secs. Followed by rapid cooling (Flash method) or 63°C for 30 mins. (Holder method).

- **Inspissation:**

  Exposure of the media to humid heat at 75°C for 2 hours on three successive days (sterilization of media contain proteins ,e.g. serum

➢Moist heat at temperature at 100°C:

- **Boiling:**

Simple boiling at 100°C for 5-10 min. is used to sterilize some glassware, forceps, scalpels .

- **Tyndallisation:**

Exposure to steam(100°C) for 20-30 min. for three consecutive days . It is used for materials which can not withstand prolonged boiling (media containing sugar and gelatin).

➢Moist heat at temperature above100°C:

- **Autoclaving:**

➢ Autoclaving is the standard (most efficient and reliable method) sterilization method in hospitals.

➢ it works under the same principle as the pressure cooker where water boils at increased atmosphere pressure i.e. because of increase pressure the boiling point of water is >100 °C.

- The autoclave is a tough double walled chamber in which air is replaced by pure saturated steam under pressure.

- Equipment subjected to high pressure saturated steam at **121 °C for around 15–20 minutes** depending on the size of the load and the contents.

- Autoclave is used to sterilize most of the instruments and culture media.

Monitoring of autoclaves:

Chemical- it consists of heat sensitive chemical that changes color at the right temperature and exposure time.

Autoclave tape

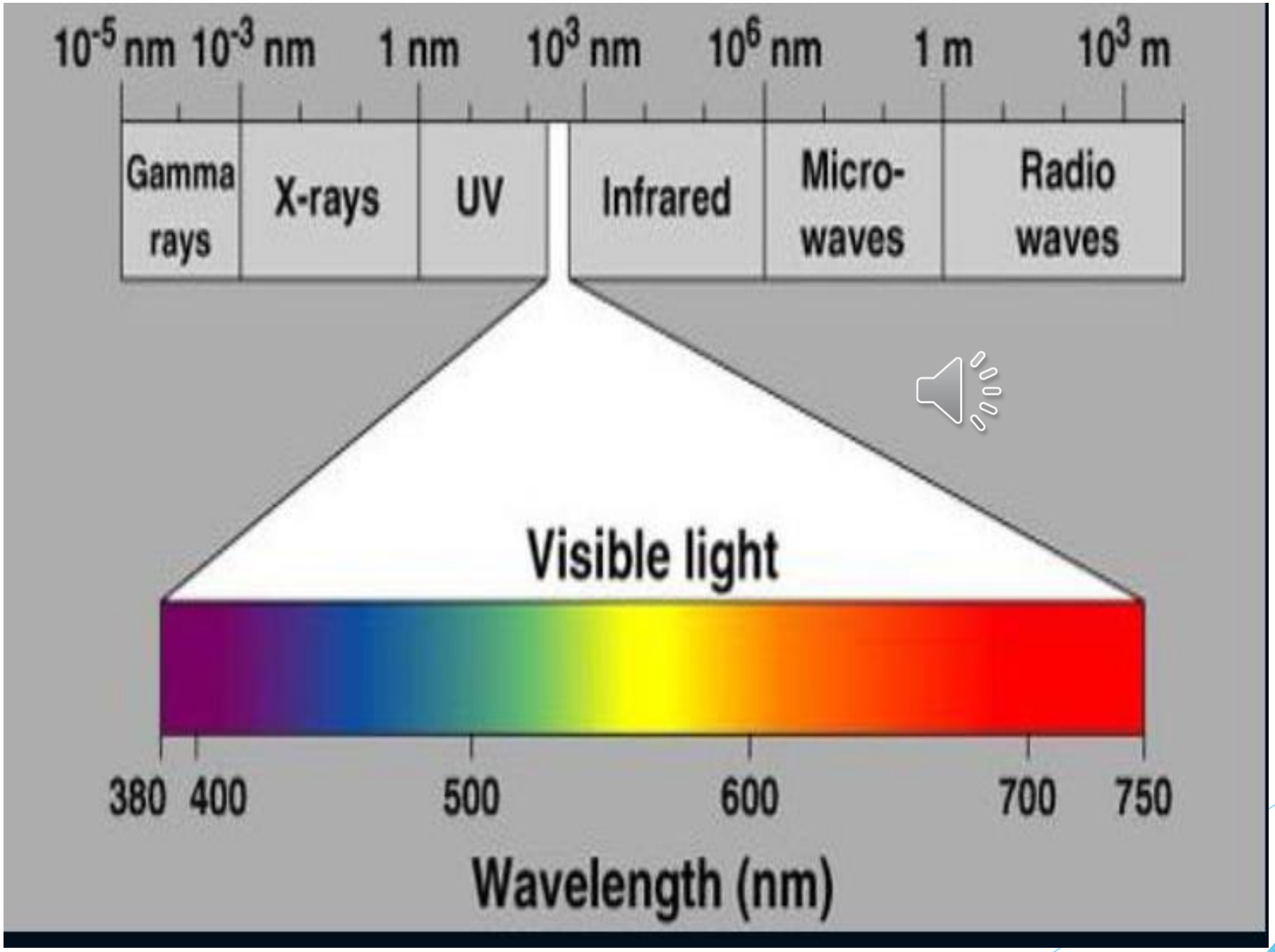
Browne's tube.

Biological – where a spore-bearing organism is added during the sterilization process and then cultured later to ensure that it has been killed. These biological indicators contain Bacillus stearothermophilus spores.

Radiation

U.V. rays

- Bactericidal
- Not efficient for complete sterilization
- Reduce number of bacteria in certain areas such as operating theaters


10^{-5} nm
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1 nm
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10^{6} nm
1 m
10^{3} m
Gamma rays
X-rays
UV
Infrared
Micro-waves
Radio waves
Visible light
380
400
500
600
700
750
Wavelength (nm)

Ionizing radiation:

- Rays of short wave length and high penetration power e.g. Gamma rays.
- In moderate doses lethal to microorganisms.
- Sterilize pre-packed disposable plastic items that cannot stand heat such as plastic syringes, catheters, gloves.

Infra-red rays:

- Act through heating, temp. reach 180°C.
- sterilize mainly glass ware and syringes

Filtration

➢ Mechanism

mechanically removes microorganisms by passage of a liquid or gas through a screen like material with small pores. May be done under either negative or positive pressure.

➢ Application

Filtration is the preferred method of sterilizing certain solutions, that likely to be damaged by heat e.g. IV fluids. Antibiotic solutions, vaccines, enzymes, and some culture media.

***Positive Pressure Environment**: Air pressure in the room under positive pressure is higher than outside, so contaminants (particles, viruses, bacteria) are kept out. The positive pressure environment is used to protect patients in operating theatres, so that infection does not enter open body cavities, or other conditions linked to a compromised immune system, being nursed in isolation rooms. Positive pressure rooms - also often known as a protective room.

***Negative Pressure Environment**: The air pressure in the room under negative pressure is lower than outside so that contamination from the room does not flow out into surrounding areas. The negative pressure environment is used for airborne infection control to protect people from patients with very contagious disease .

➢ Most common types:

- **Membrane Filters:** Uniform pore size. Used in industry and research. Different sizes:
- 0.22 and 0.45um Pores: Used to filter most bacteria. Don't retain spirochetes, mycoplasmas and viruses.
- 0.01 um Pores: Retain all viruses and some large proteins.

Chemicals

Ethylene oxide

- Denature proteins and DNA by cross-linking functional groups.
- Ethylene dioxide used in sterilizing heat sensitive materials such as surgical instruments and plastics. It is used for sterilizing endoscopes and anesthetic apparatus
- Highly inflammable ,potentially explosive gas.

Aldehydes:

- Denature proteins and inactivate nucleic acids.
- Formaldehyde as gas used to sterilize operation theatres and other spaces.
- Glutaraldehyde a chemical relative of formaldehyde less irritating and more effective than formaldehyde.
- Glutaraldehyde used to disinfect hospital instruments, including endoscopes and respiratory therapy equipment

Halogens

- The halogens, particularly iodine and chlorine, are effective antimicrobial agents.
- They damage enzymes via oxidation or by denaturing them
- iodophores (Betadine®), chlorine treatment of drinking water, bleach.

Oxidizing agents

- Peroxides and ozone, kill by oxidation of microbial enzymes
- Hydrogen peroxide can disinfect and sterilize surfaces of objects
- Ozone treatment of drinking water

Alcohol

- Powerful disinfectant and antiseptic
- Effectively kills bacteria and fungi **but does not inactivate spores**
- Mode of action: denatures proteins, dissolves lipids and can lead to cell membrane disintegration
- Swabbing of skin with 70% ethanol prior to injection
- Most commonly used alcohols are ethanol and Isopropanol.

Spaulding Classification for disinfectants

- According to their efficiency disinfectant can be classified into 3 categories:

- A) High level disinfectant
- B) Intermediate level disinfectant
- C) Low level disinfectant

A) High Level Disinfectant

An agent that kills all microbial pathogens except a small numbers of spores. It includes-

- Heat sterilization, including steam or hot air.
- Ethylene oxide gas
- Hydrogen peroxide gas plasma
- Glutaraldehyde-based formulations
- Hydrogen peroxide 7.5%
- Per-acetic acid, concentration variable but 0.2% or greater is sporicidal .

B) Intermediate Level Disinfectant:

- A chemical that kills all microbial pathogens including mycobacteria and non enveloped viruses except spores.
- Ethyl or isopropyl alcohol (70-90%)
- Sodium hypochlorite (5.25%-6.15%) provides **>100 ppm available chlorine).**
- Phenolic germicidal detergent solution
- Iodophor germicidal detergent solution

C) low level Disinfectant:

Kills only vegetative bacteria, fungi and lipid envelope virus

- Quaternary ammonium germicidal detergent solution