2nd year Medical Students - JU

Sterilization and disinfection

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Sterilization and 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**
- Term used to describe disinfection applied to living tissue such as a wound.

Methods of Sterilization

1 - Heat

- -Dry
- -Moist

2 - Radiation

- -U.V. rays
- -Ionizing radiation
- -I.R. rays

3 - Filtration

4 - Chemical agents (Sterilization and Disinfection)

HEAT

- The only method that is both reliable and widely applicable
- temperatures above 100°C to ensure that bacterial spores are killed.
- This temperature is insufficient to destroy prions.
- Shorter applications of lower temperatures, such as in pasteurization can effectively remove specific infection hazards (Milk,Juice).

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, …

- If you take an egg, crack it open and drop it into a pot of boiling water, you will cook the egg by poaching it. The white part of the egg starts to stick together and becomes hard, a process called **coagulation**, which occurs at 52℃. When the egg coagulates, it becomes denaturalized, thus killing all proteins inside the cells of the egg.

- Another way to cook an egg is to fry it in a frying pan. At first the egg will coagulate, but if you leave the egg in the pan and let it continue frying, it will all eventually turn black. This burning is called **oxidation**, and occurs at much higher temperatures than coagulation. Oxidation is a chemical process in which electrons are removed from the atom, and the end result is death of that organism.

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 vapour.

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.

Moist heat

- 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

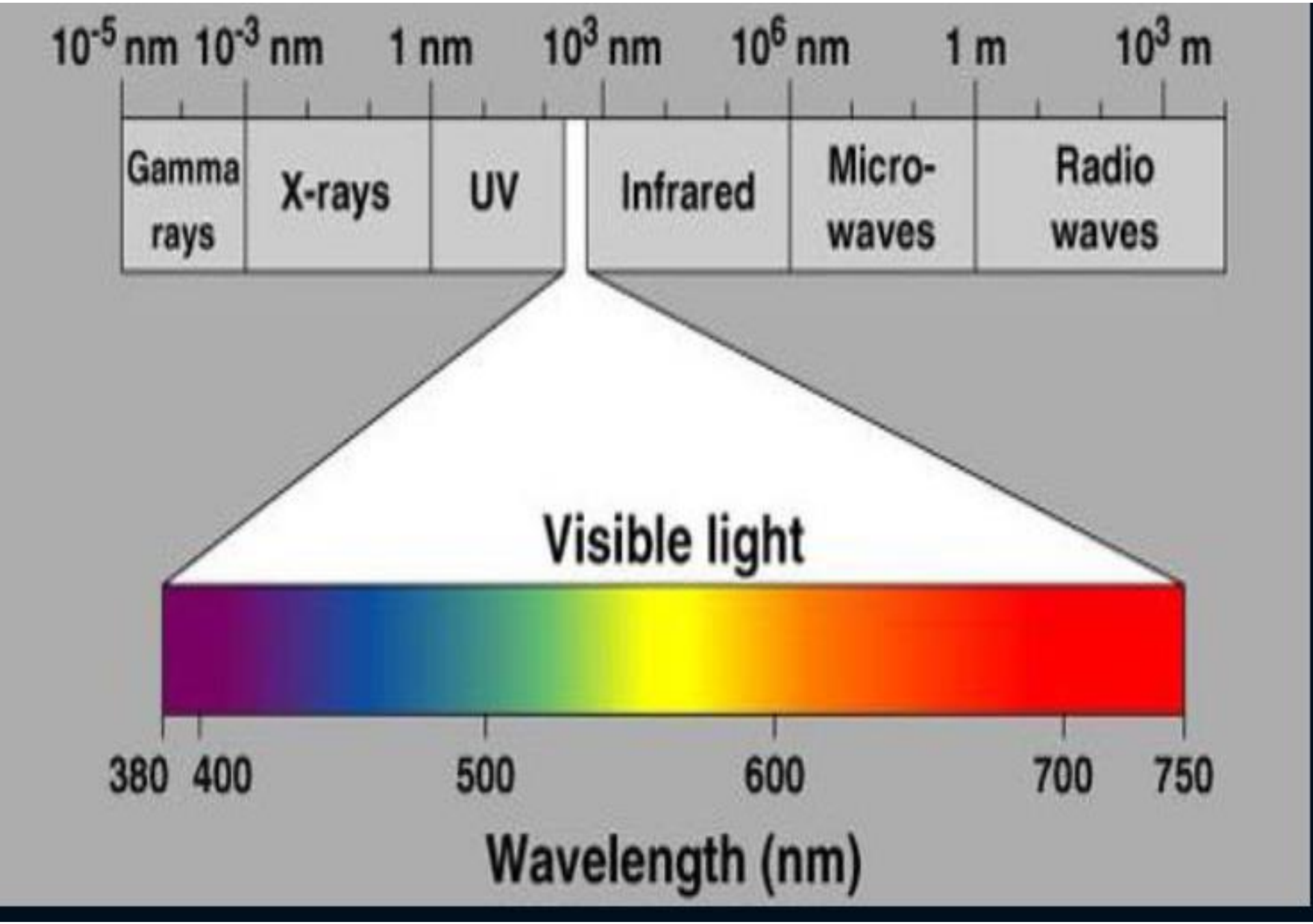
Browne's tube.

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

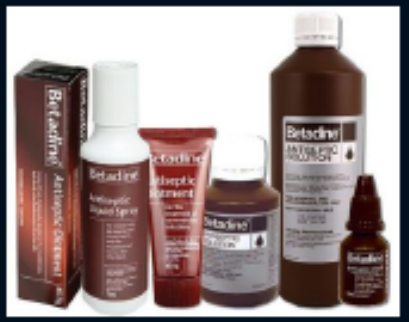
- Denature proteins and DNA by cross-linking functional groups.
- Ethylene dioxide used in sterilizing heat sensitive materials such as surgical instruments and plastics. It is used for sterilizing endoscopes and anesthetic apparatus
- Highly inflammable ,potentially explosive gas.

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.

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
- The risk of transmitting infection to patients or staff in contact with the item.

Measurement of microbial death

survivor curve, D value:dose required to inactivate 90% of the initial population. See figure

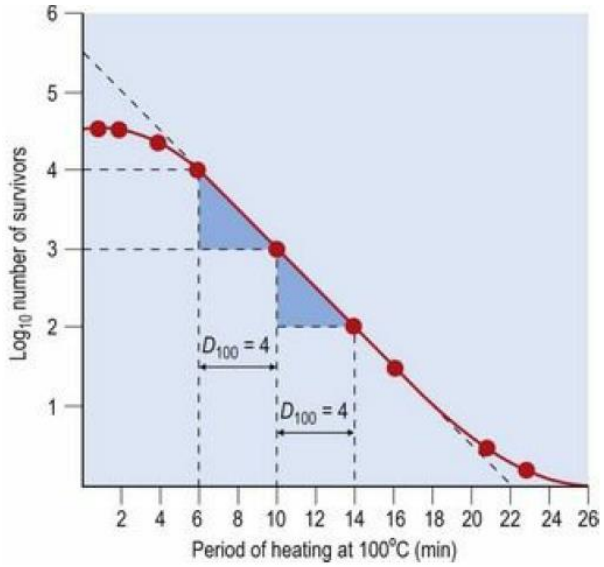


Fig. 4.3 Rate of inactivation of an inoculum of bacterial spores showing the decimal reduction time (*D* value) at 100°C and the nonlinear 'shoulder and tail' effects.

Resistance to Sterilization and disinfection

- Vegetative bacteria and viruses are more susceptible
- Bacterial spores are the most resistant, to sterilizing and disinfecting agents.
- Within different species and strains of species there may be wide variation in intrinsic resistance (structure difference, growth conditions)
- Prions: Highly resistance, use disposable instruments

Sterilization and disinfection

- Gluteraldehyde is least effective and consdiderd as a disinfectant
- Fibrooptic endoscopes

The End