General Microbiology Course
(sterilization and disinfection)
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Sterilization and Disinfections

Objectives

The difference between sterilization and disinfections

Chemical and physical methods of disinfection and sterilization

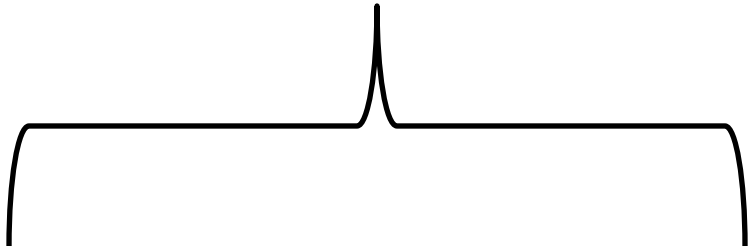
Principles and application of each method

Common disinfectants and antiseptics used in healthcare settings

Sterilization control

Definitions

Cleaning in general is divided into two major groups



Sterilization

Disinfection

Decontamination
Preservation
Sanitisation
Aseptic techniques

Definitions

Sterilization

- ✓ Destruction of **ALL** forms of life, including the bacterial spores, viruses, prions
- ✓ No degrees of sterilization: an all-or-nothing process
- ✓ Physical or chemical methods

Disinfection

- ✓ Is the process of **elimination** of **most pathogenic microorganisms** (excluding bacterial spores) **on inanimate (non-living) objects**.
- ✓ Disinfection can be achieved by physical or chemical methods.
- ✓ Chemicals used in disinfection are called **disinfectants**.
- ✓ **The purpose**: to prevent transmission of certain microorganisms with objects, hands or skin and prevent spreading the infection
- ✓ **Disinfection & Sterilization** are not synonymous.

Definitions

Disinfection

Sanitization:

✓ Removal of microbes that pose a threat to the public health, food industry, and water conditioning health.

✓ **Sanitizer**: is an agent, usually a detergent, that reduces the numbers of bacteria to a safe level

Preservation:

preventing methods of microbe-caused spoilage of susceptible products (pharmaceuticals, foods)

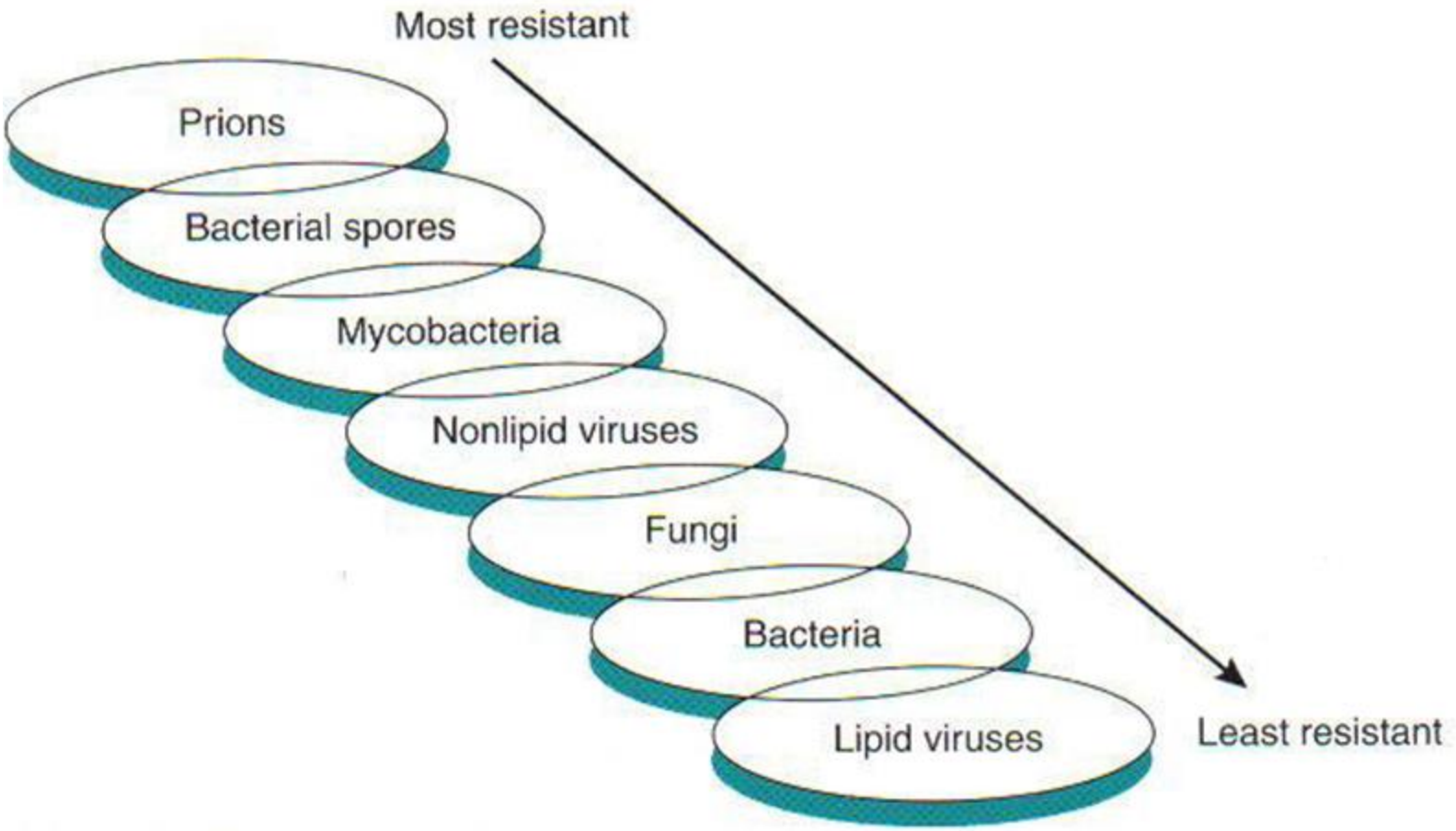
Aseptic techniques:

✓ prevent microbial contamination of materials or wounds by the employment of techniques such as usage of gloves, air filters, UV rays…etc.

✓ **Antisepsis:** disinfection of living tissues(e.g., in a wound),achieved through the use of antiseptics

✓ **Antiseptics:** are applied (do not kill spores) to reduce or eliminate the number of bacteria from the skin

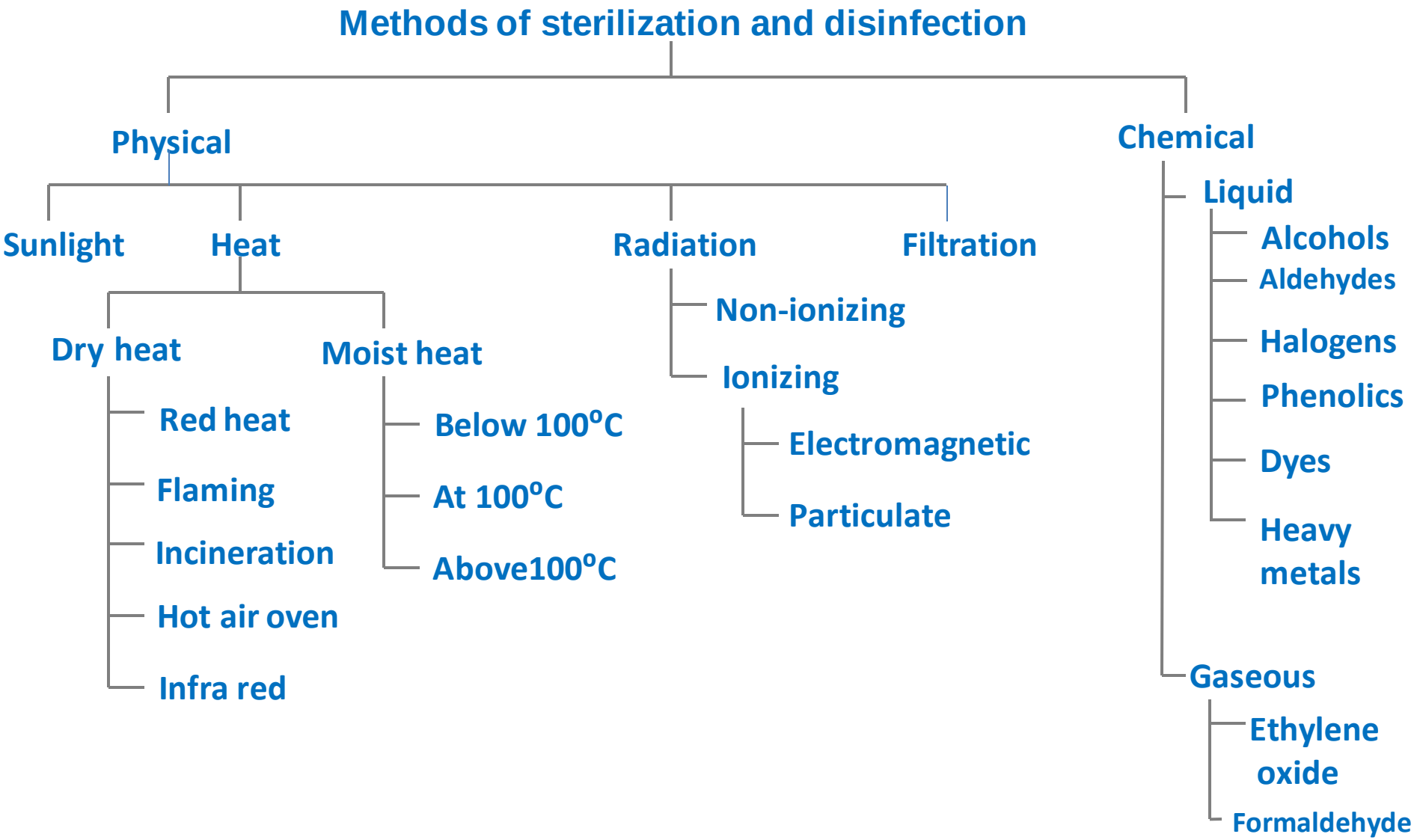
The resistance to killing



Prions:

The most known resistant to the action of heat, chemicals, and radiation. Prions can withstand temp. exceeding 121ºC for several hours while immersed in acid or basic solutions

Methods of sterilization and disinfection



Levels of disinfectants

High-level disinfectants

activity against bacterial spores

Intermediate-level disinfectants

tuberculocidal activity but not sporocidal

low-level disinfectants

A wide range of activity against microorganisms but no sporocidal or tuberculocidal activity

Methods of sterilization and disinfection

Medical materials are categorized into three types:

Critical materials

- invade sterile tissues or enter the vascular system
- most likely to produce infection if contaminated and therefore require High-level disinfectants.

Semi-critical materials

- have contact with mucus membranes
- require high-level disinfection agents

Noncritical materials

- have contact with intact skin
- require intermediate-level to low-level disinfection

Methods of sterilization and disinfection

Physical methods

Dry heat

1- Red heat

Articles such as bacteriological loops, tips of forceps, and spatulas are sterilized by holding them in Bunsen flame till they become red hot. This method is limited to those articles that can be heated to redness in flame.

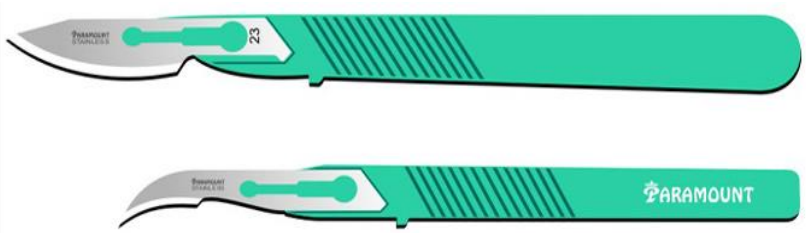
Methods of sterilization and disinfection

Physical methods

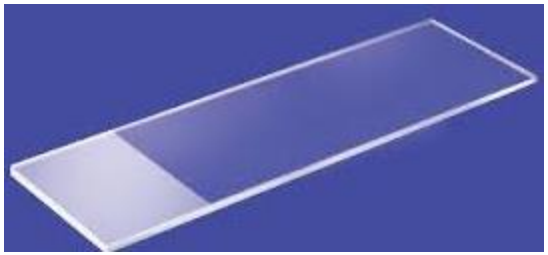
Dry heat

2- Flaming:

This is a method of passing the article over a Bunsen flame a few times, but not heating it to redness. Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure. This method too is limited to those articles that can be exposed to flame.



scalpels



glass slides

mouth of test tubes

Flasks

Methods of sterilization and disinfection

Physical methods

Dry heat

3- Incineration:

This is a method of destroying contaminated material by burning them in incinerator. Articles such as soiled dressings; animal carcasses, pathological material and bedding etc should be subjected to incineration.

4- Hot air oven:

requires much longer exposure times and higher temperatures than moist heat

- **2 hours at 160°C in dry air ovens or**
- **30 min. at 180°C**

Application: Metallic instruments (like forceps, scalpels, scissors), glasswares (such as petri-dishes, pipettes, flasks, all-glass syringes), swabs, oils, grease, petroleum jelly and some pharmaceutical products.

Methods of sterilization and disinfection

Physical methods

Moist heat

1- Below 100ºC

A. Pasteurization

- Do not kill spores
- Employed in food and dairy industry.

✓ LTH(low temperature holding) batch method: 63-65ºC for 30min.
✓ UHT(ultra-high temperature): 135ºC for 1-2 sec.

B. Vaccine bath: The contaminating bacteria in a vaccine preparation can be inactivated by heating **in a water bath at 60ºC for one hour.** Only vegetative bacteria are killed and spores survive.

Methods of sterilization and disinfection

Physical methods

Moist heat

2- At 100ºC

Boiling:

- kills most microorganisms in 10 min. at 100C except spores and certain bacterial toxin
- Tyndallization: an exposure of 100°C for 20 minutes on 3 successive days; sporicidal

3- At temperature above 100ºC:

By using autoclave

Destroy **ALL** microorganisms and their spores

-steam under 1 atm of pressure, at 121ºC, 15 minutes of exposure in autoclaves

Destroy ALL microorganisms **(including prions)** and their spores

-Using longer times: 135ºCfor at least 1hour under 2 atm

Application: the sterilization method of choice for heat-stable objects

Methods of sterilization and disinfection

Boiling and pasteurization achieve disinfection but not sterilization (do not eliminates spores)!!!

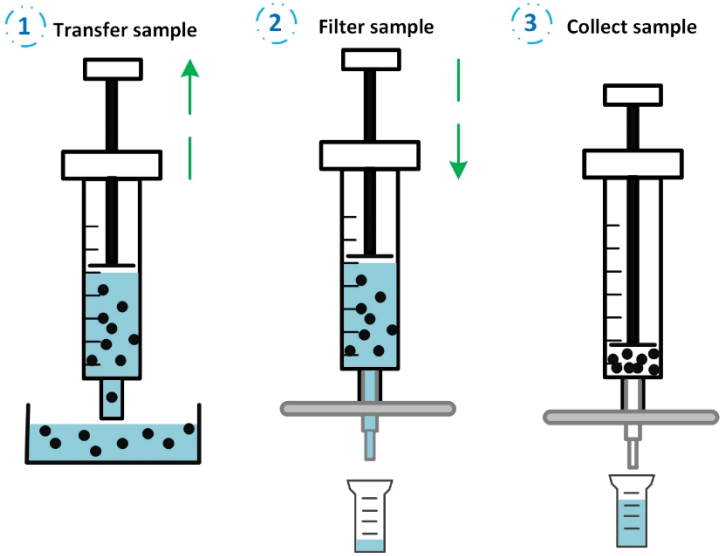
Autoclave

Methods of sterilization and disinfection

Filtration

➢ **of liquid**

- the membrane filters composed of plastic polymers or cellulose esters containing **pores of certain size**
- liquid is pulled (vacum) or pushed (pressure) through the filter matrix, organisms larger than the size of the pores are retained



pore size of:

- **0,45 and 0,80 µm-most bacteria, yeasts and molds**
- **0,22 µm-for criticial sterilizing, e.g. parenteral solutions**
- **0,01 µm-for retaining small viruses**

Application: parenteral solutions (serum), vitamins, vaccines and antibiotic solutions

Methods of sterilization and disinfection

Filtration

- **of air**
- filters remove microorganisms larger than **0,3µm**
- **Application: in laboratory hoods and in rooms of immunocompromised patients**

Air filters

Methods of sterilization and disinfection

Radiation

A. Ionizing radiation

- gamma rays or electron beams
- short wavelength and high energy

Application: for the medical industry: the sterilization of disposable supplies (syringes, bandages, catheters and gloves),and heat-sensitive pharmaceuticals,

B. Non-ionizing

- Rays of wavelength longer than the visible light are non-ionizing
- in the form of **ultraviolet rays (UV)(280-200 nm)**
- long wavelength and low energy
- poor penetrability
- the use is limited

Application: disinfect smooth surfaces with ultra violet lamps and to reduce airborne pathogens (hospital wards, operation theatres, virus laboratories)

Methods of sterilization and disinfection

Chemical methods

- Liquid
  - Alcohols
  - Aldehydes
  - Halogens
  - Phenolics
  - Dyes
  - Heavy metals
- Gaseous
  - Ethylene oxide
  - Formaldehyde

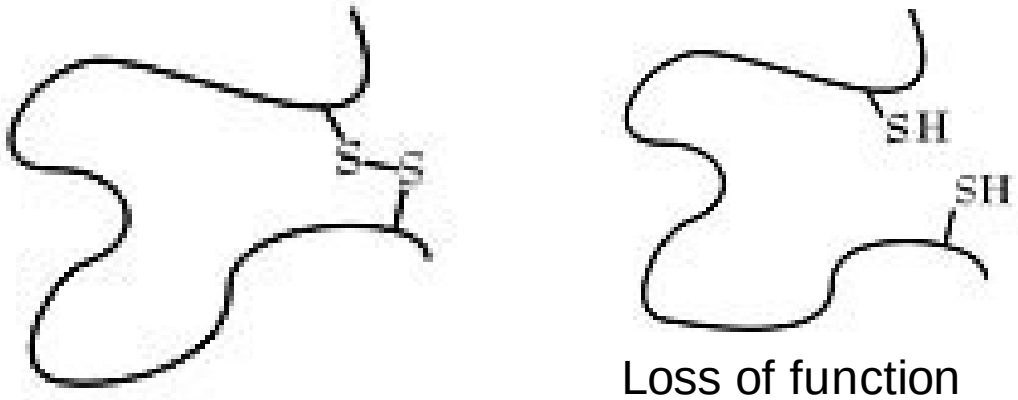
Methods of sterilization and disinfection

Chemical methods

Exert their killing effect by the following mechanisms:

- R**eaction with components of the cytoplasmic membrane** (surfactant compounds, alcohols)
- D**enaturation of cellular proteins** (alcohols, phenols, aldehydes, oxidants)
- R**eaction with the thiol (-SH) groups of enzymes** (heavy metals)
- D**amage of RNA and DNA** (aldehydes, oxidants, dyes)

  - The agent can exert one or a combination of actions on microorganisms



Loss of function

Methods of sterilization and disinfection

Chemical methods

1- Alcohols

- **Ethanol 70%,isopropanol 70%, propanol 60%**
- Inactivate microorganisms by denaturing proteins
- Wide spectrum against **bacteria and fungi but not sporocidal!**
- **Tuberculocidal and virucidal for most viruses (15 min.)**
- Alcohols may be contaminated with spores –should be filtered through a 0,22 µm filter
- The most effective concentrations are between 60%-90% (water is needed in chemical reactions)
- **Application: surgical and hygienic disinfection of the skin and hands**

Methods of sterilization and disinfection

Chemical methods

2- Aldehydes

- **Formaldehyde (HCHO)the most important**
- Is a water-soluble gas -**formalin (35% solution of this gas in water) or glutaraldehyde (disinfectant and sterilizer!!!)**
- Denaturate proteins and nucleic acids
- Broad-septrum: **against bacteria, fungi, and viruses**
- C**hemosterilizer in higher concentrations (sporicidial)**
- **Application: -disinfection of surfaces and objects (plastic and rubber items)**
- The **sterilizer of choice for heat-sensitive medical equipment**

Methods of sterilization and disinfection

Chemical methods

3- Halogens: (chlorine, iodine, and their derivatives)

Chlorine

- Used in the form of **hypochlorite(e.g. liquid sodium hypochlorite -household bleach)**
- **Broad-spectrum activity, sporocidal required the long exposure time**
- Corrosive
- **Applications: disinfection of water and swimming pool, cleaning and washing products**

Methods of sterilization and disinfection

Chemical methods

3- Halogens: (chlorine, iodine, and their derivatives)

Iodine (2 forms)

- **tincture (alcohol and iodine)**
- **iodophores (iodine and surfactants)**

- denatures proteins by oxidative effects
- **bactericidal, not sporocidal**
- less irritant than pure iodine

- **Application: as antiseptics, disinfection of skin and small wounds**

Methods of sterilization and disinfection

Chemical methods

3- Halogens: (chlorine, iodine, and their derivatives)

Phenols

- Denaturate proteins
- **Broad-spectrum, but not sporocidal, not virucidal**

- **Application: widely used, disinfection of hospital, institutional, and household environment (soaps)**

Sterilization control

- To ensure that potentially infectious agents are destroyed by adequate sterilization regimes

Three levels:

1- **Physical:** measuring device control (temp., time, pressure)

2- Chemical: substances that undergo a colour change or have melting points within the sterilizing range

- **Browne's tubes and Bowie Dick tape**

give an immediate indication of a successful or non- successful sterilization

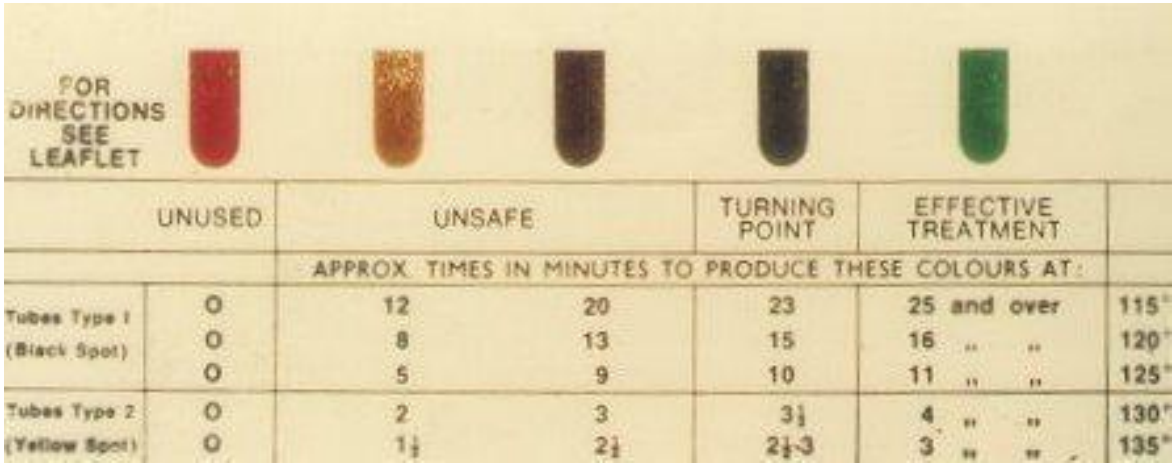
3- biological:

- *Bacillus stearothermophilus spores(10000 to million organisms)*
- Survives steam heat at 121^{o}C for 5 min. and is killed at 121^{o}C in 13 min.
- The sterilization process is validated by culturing to detect surviving spores

Sterilization control

Browne's tube are glass tubes that contain heat sensitive dyes. These change color after sufficient time at the desired temperature.

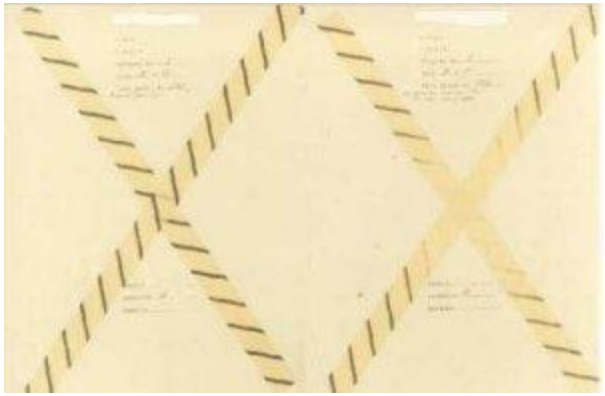
- Before heat exposure, the contents of the tube appear **red.**
- As heating progresses, the color changes to **green.**
- Only when the tube is **green sterilisation conditions can be considered adequate.**



	UNUSED	UNSAFE		TURNING POINT	EFFECTIVE TREATMENT	
		APPROX. TIMES IN MINUTES TO PRODUCE THESE COLOURS AT:				
Tubes Type 1	O	12	20	23	25 and over	115°
(Black Spot)	O	8	13	15	16 " "	120°
	O	5	9	10	11 " "	125°
Tubes Type 2	O	2	3	3½	4 " "	130°
(Yellow Spot)	O	1½	2½	2½-3	3 " "	135°

Sterilization control

Indicators Bowie Dick tape is applied to articles being autoclaved. Before heat exposure, the tape is uniformly buff in color. After adequate heating, the tape develops dark brown stripes. The pack on the left has been properly sterilized; that on the right has not.



The color-changing indicator of tape is usually lead carbonate based, which decomposes to lead(II) oxide


Pass
3M Comply™ SteriGage™
Steam
Chemical
Integrator
REJECT
ACCEPT
Fail
Fail
1243
Class 5