Sterilization and Disinfections

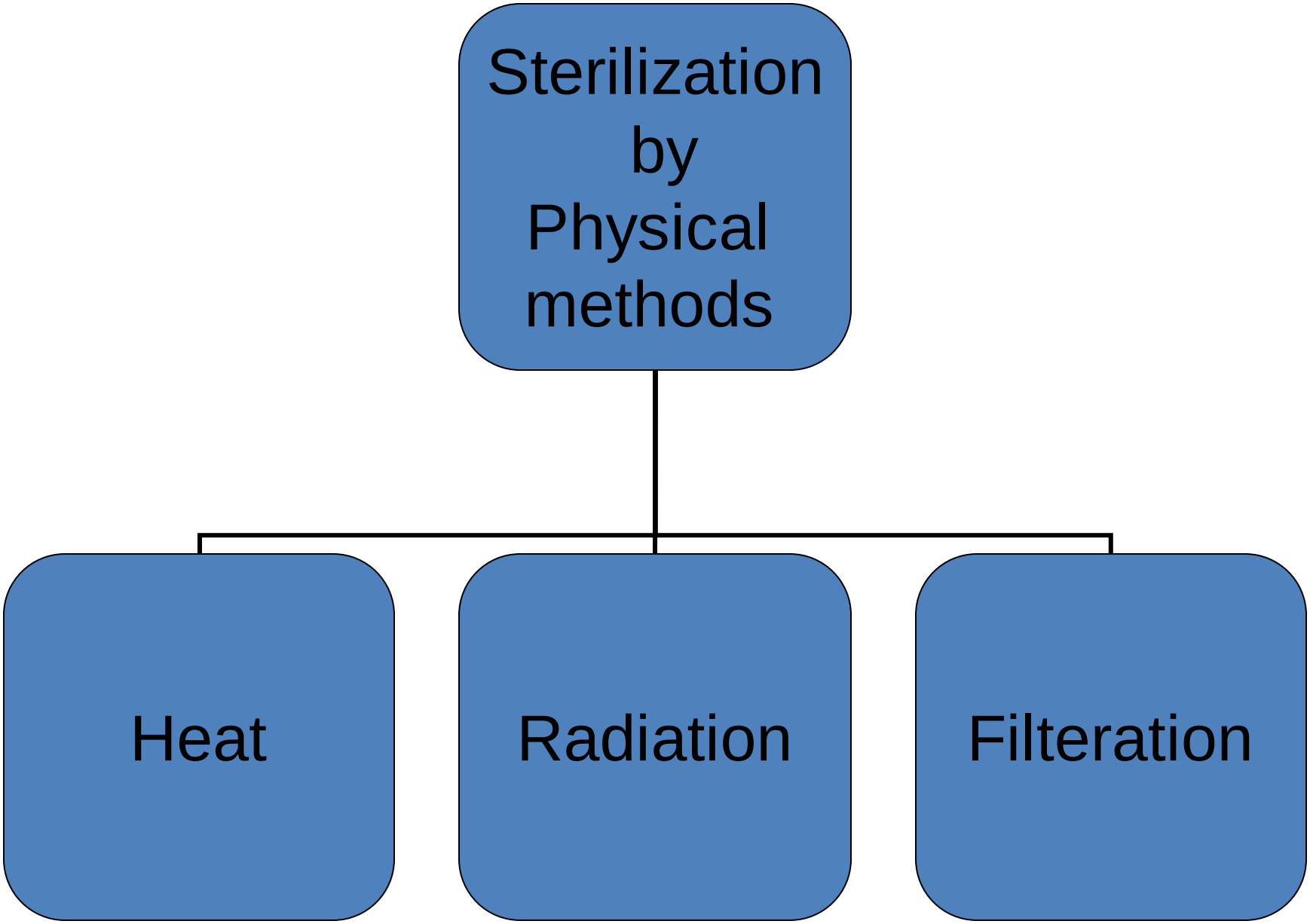
Sterilization

Freeing of an environment from all living microorganisms includes bacteria and their spores, fungi, parasites and viruses.

Sterilization means germ free objects.

Sterilization occurs by:

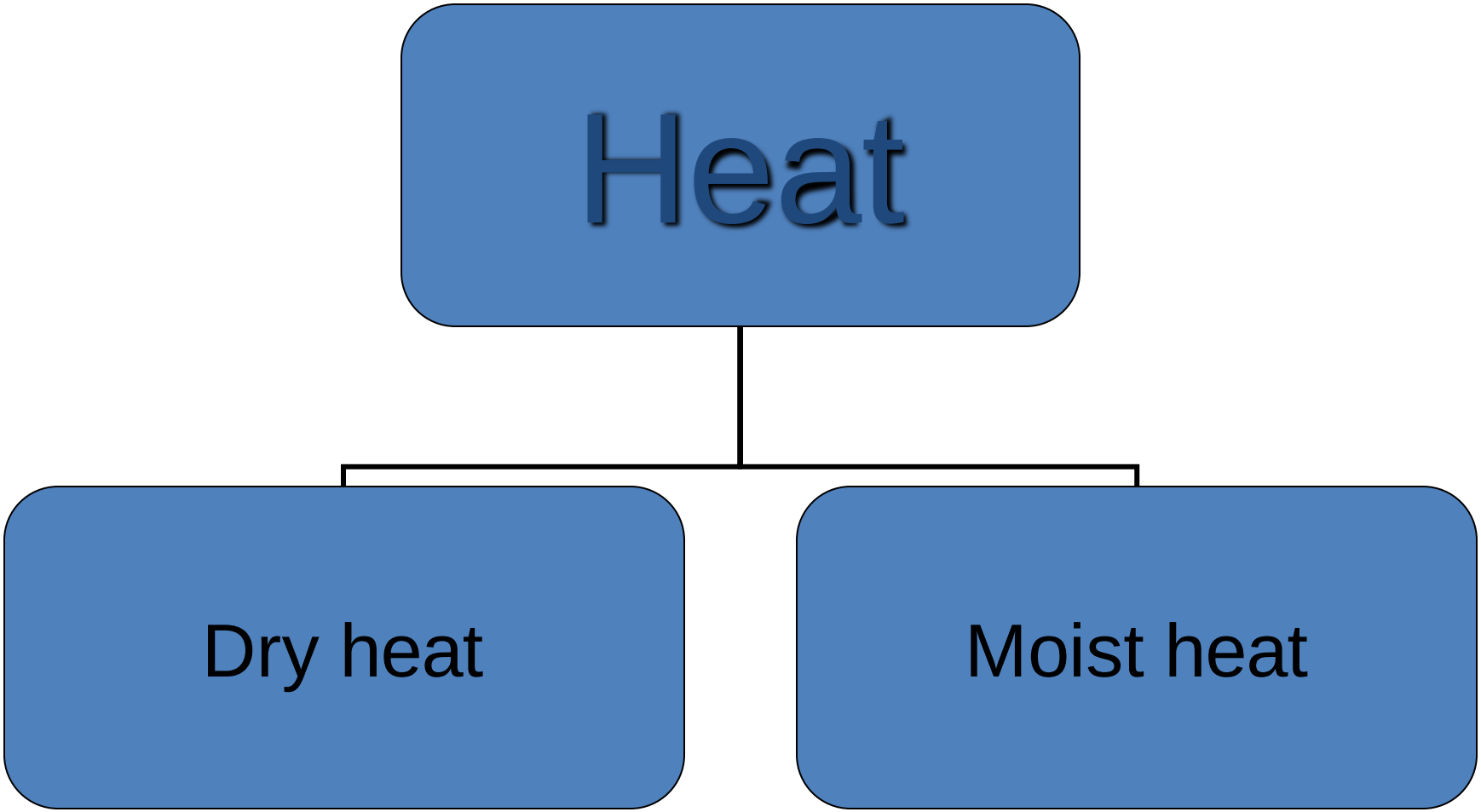
- Physical methods.
- Chemical methods.


Sterilization by Physical methods
Heat
Radiation
Filteration

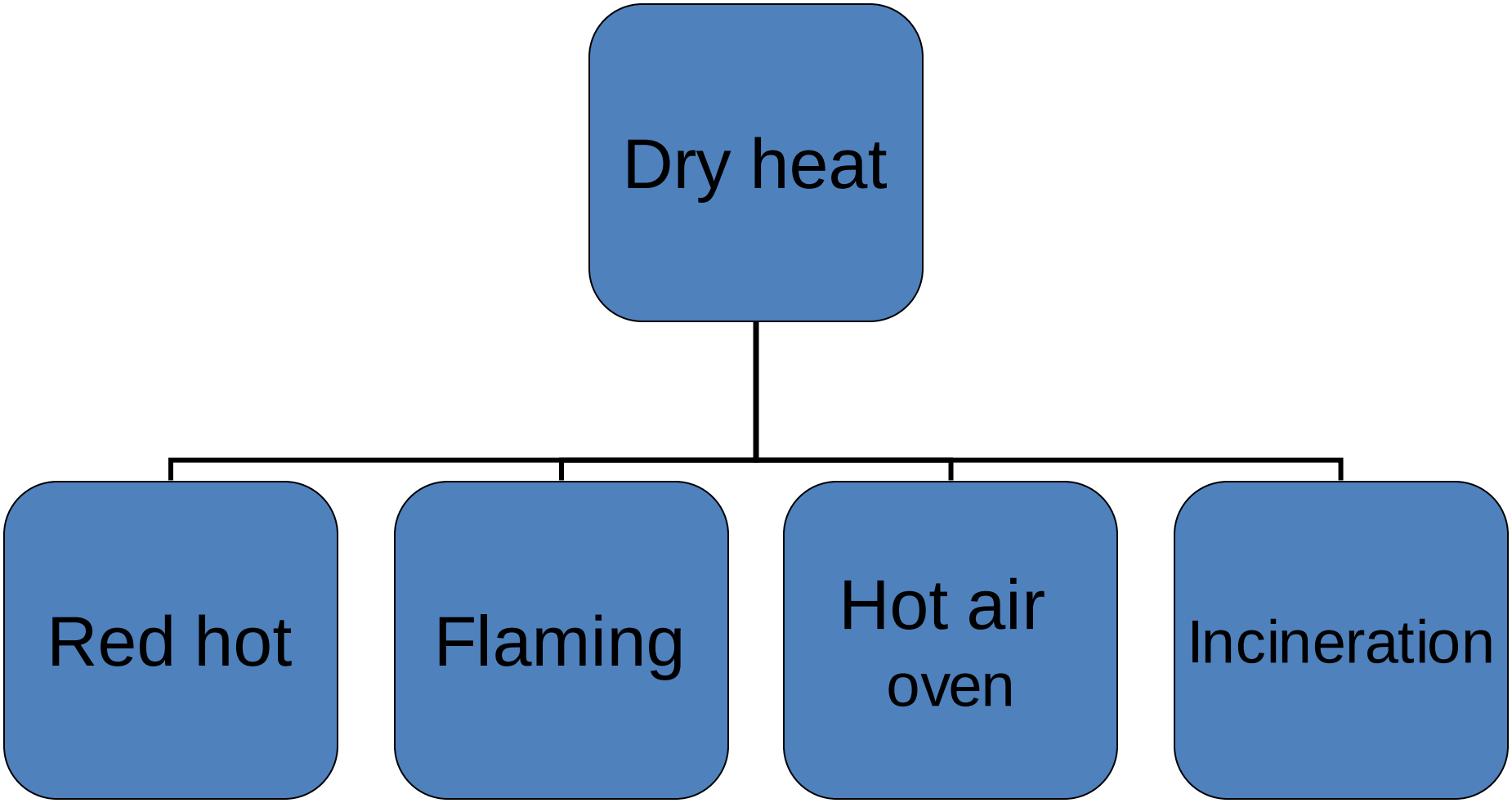
Physical methods

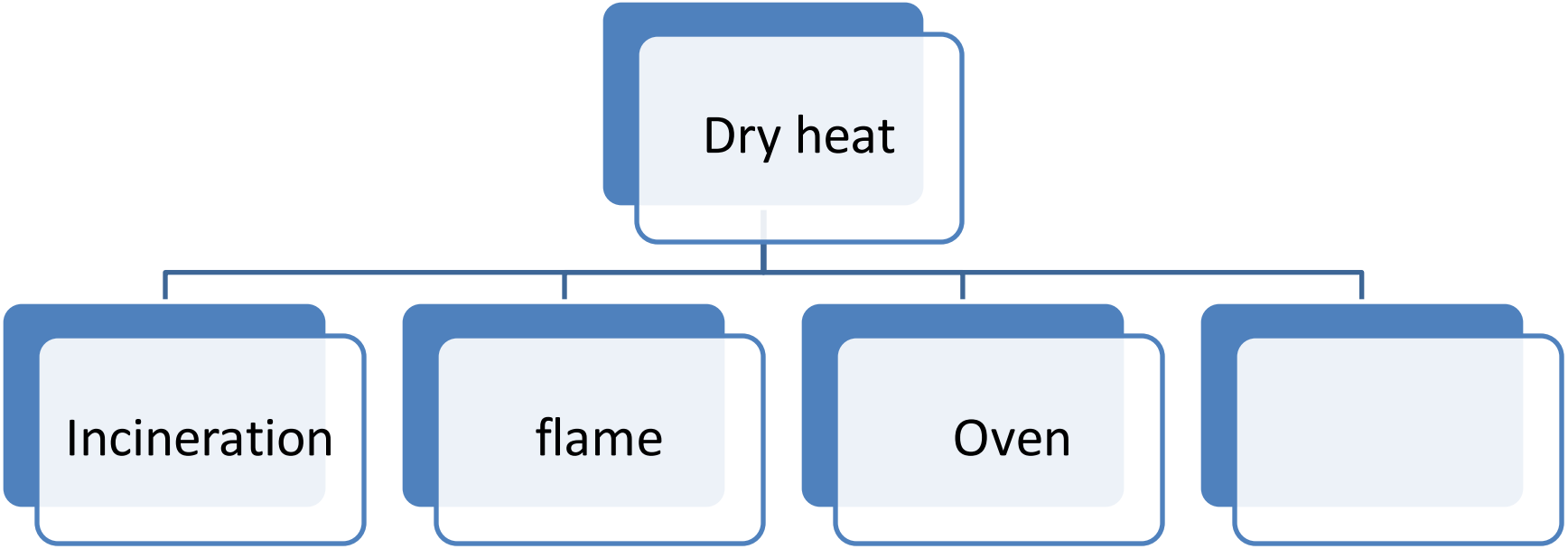
- Heat:
  - Exposure of the objects to heat will kills microbes by coagulation of protein, denaturation of enzymes and oxidation.
- Filtration:
  - Sterilization through removing of microbes from fluids by exposing to small size filter. Used for heat sensitive fluids like serum, antibiotic, suger, and urea.
- Radiation:
  - Exposure to irradiation causes denaturation of proteins and enzymes.

sterilization by heat



Dry heat




Dry heat
Incineration
flame
Oven

Red hot

- Exposure of wires and forceps to the Bunsen flame until it becomes red hot, then cool down and use.
- Used for loop, forceps, and metal rods.

Flaming

- Slowly passing of an objects to the Bunsen flame will reduce the number of microorganisms.
- Used for sterilization of the mouth of bottle, flasks, containers and test tubes, smear slides etc,,,.

Flaming

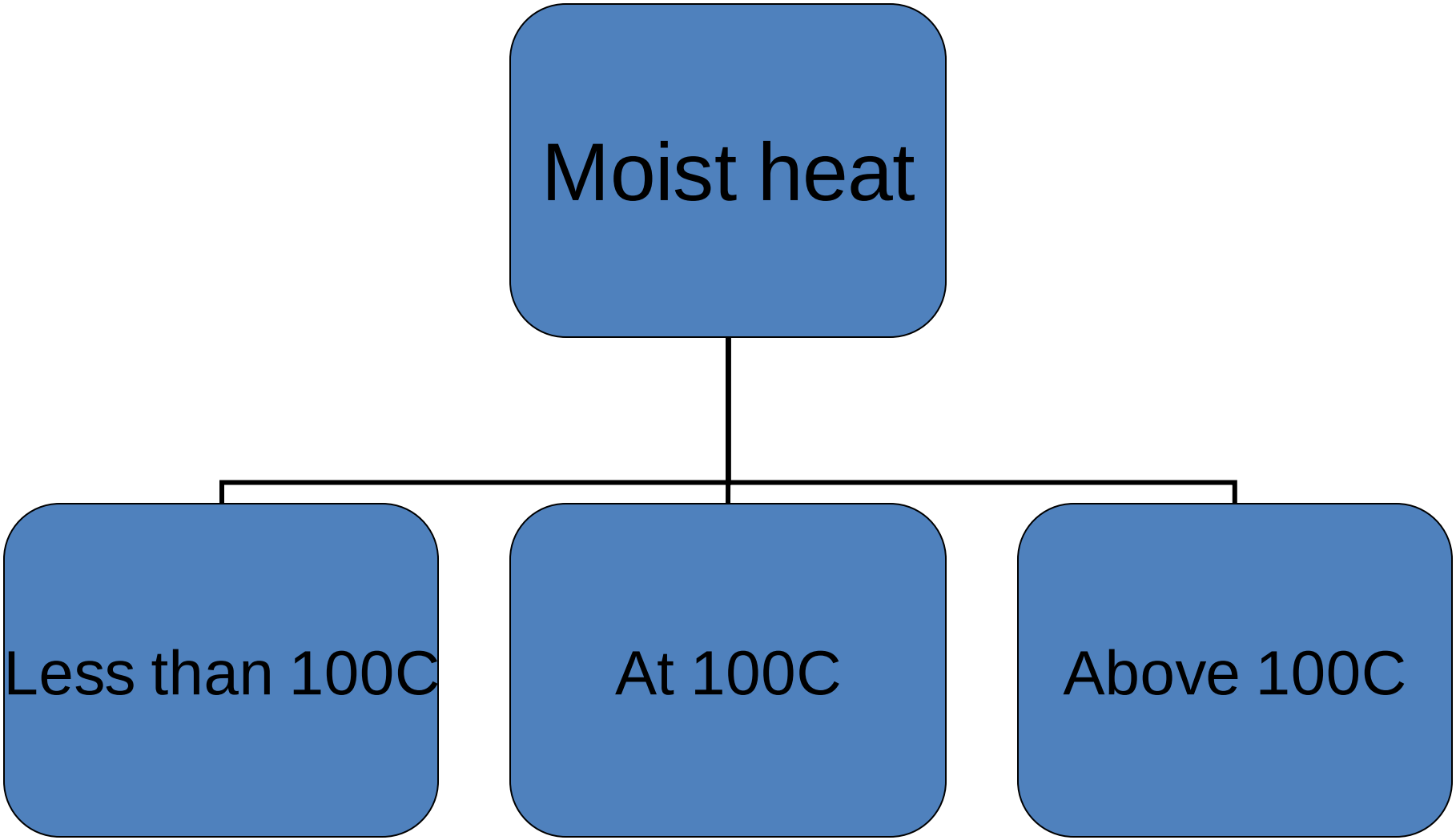
Hot air oven

1. Instruments consist of heater, oven.
2. Used for sand, powder, metal, glass.
3. Thermal death point and Thermal death time:
    1. 160C for 60 min.
    2. 180C for 30 min.

Incineration

- Is treating of an objects to heating over 250 until become black.
- Done for used equipment.

Moist heat



Less than 100C

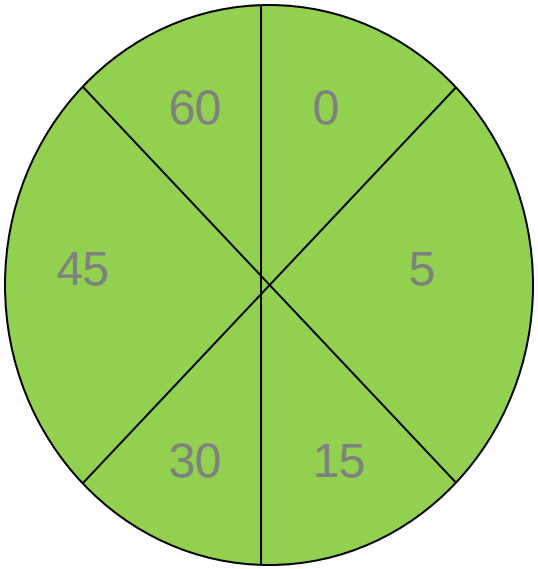
- Pasteurization of milk:
  - Holding method (65C for 30 min)
  - Flash method (72C for 20 sec)
- Preparation of vaccine:
  - By heating at 56C for 30-60 min.

At 100 C

- Steaming:
  - Single exposure of the microbe to steam at 100C for 90 min.
- Boiling:
  - Boiling water is the most common form of application of moist heat but is not capable of killing endospores or killing all viruses
  - At 100º C for 30 min.

Boiling plate test

- A pure culture of a single microorganism is grown in a broth.
- Divide the plate into 6 parts 0,5,15,30,45,60 min.
- Culture the org. on 0 part.
- Incubate the culture tube in water bath 100º C.
- After 5 min culture org. in 5 part, and so on, until you complete 1 hr.
- Incubate 24 hr.



Above 100C (Autoclaving)

- Depends on steam and pressure.
  - Steam is a hot air able to penetrate through things.
  - Pressure will rise the temperature from 100C to 121C.
- Moist heat is more effective than dry heat at a given temperature or length of exposure. also more penetrating than dry heat
- Make complete killing of bacteria, their spores, fungi and their spores, parasites and viruses including Envelop and non Envelop virus.
- Thermal death point and thermal death time:
  - 121C for 15 - 20 min.
  - Flash autoclaving at 134C for 4-5min.

Autoclave indicator

- Chemical indicator:

1. Autoclave tape.
2. Brown’s tube (diack tube).

- Biological indicator: Spore strip (thermophilic organism).

Radiation

– Sterilization by radiation kills microbes by causing mutation to the cellular protein and disrupting cellular elements.

Types of radiation:

1) UV (not a good sterilizing method).

2) Ionizing , most medical disposables (syringes, needles).

Filtration

- Sterilization by mechanical removal of pathogenic microorganism by passing through membrane filter. Unable to filter viruses according to their small size.
- The pore size is less than 0.45 µm(bacteria size 100-1 µm).
- Used for sterilization of heat sensitive fluids like serum, glucose, urea, and Amino acids.

Disinfection

Chemical methods of sterilization

Disinfection:

- Is removing of pathogenic microorganism or reducing their number on the exposed area.
- Unable to destroy spores and some could not kill non envelop viruses.

Antiseptic:

is a chemical agent that is applied to living tissue to kill microbes.

Factors affects disinfection action:

- Type of disinfection.
- Concentration of disinfectant.
- Type of microorganism.
- Number of microorganism.
- Time of exposure.
- Temperature.

Phenolic group of disinfectant:

- E.g.: Phenol crystal, Dittol, Lysol, Cresol.
- Remain active, stable and persist for long period of time.
- Active against G+ve, G-ve, Mycobacterium & viruses.

Alcohols:

- E.g.: Anti-bacterial, sanitizer.
- They able to act and Evaporate, short period of time..

Dyes:

- Crystal violet and Eosine are very effective antiseptic.

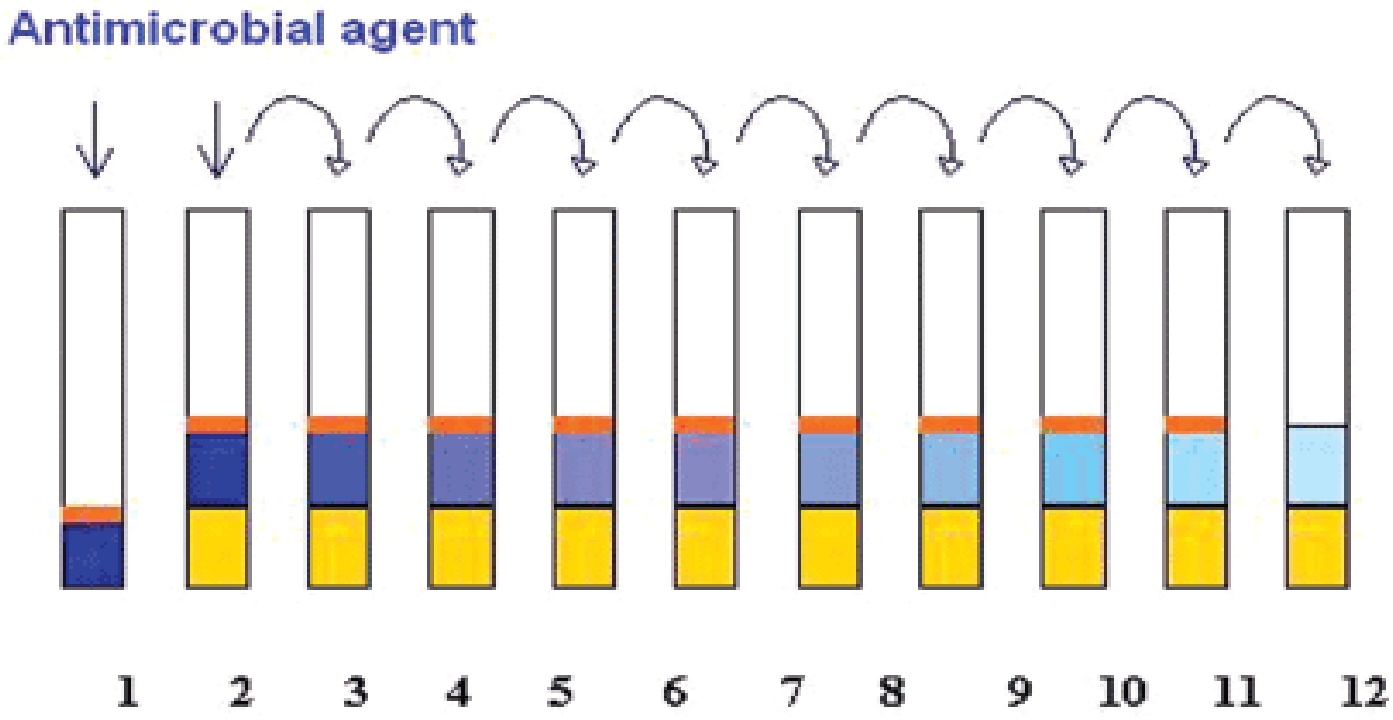
Surface active agents:

- Soap and other detergent make mechanical remove of microbe by scrubbing of dead tissue so reduce their number.

MIC test

- A pure culture of a single microorganism is grown in a broth.
- The antimicrobial agent is diluted in a number of times, 1:1, through a sterile diluent (usually Mueller-Hinton broth).
- Add 2 drops of the organism onto the antimicrobial dilution.

- Incubate 24 hr in the incubator.
- After incubation, the dilution observe growth, The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.


Antimicrobial agent
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