SNS COLLEGE OF ALLIED HEALTH SCIENCES

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DEPARTMENT OF CARDIO PULMONARY PERFUSION CARE TECHNOLOGY

COURSE NAME : MICROBIOLOGY

TOPIC : STERILIZATION AND DISINFECTION

STERILIZATION

- It is defined as the killing, destroying or removal of all microorganisms, including bacterial spores, which are highly resistant.
- Sterilization describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.
- Sterilization can be achieved by physical, chemical and physiochemical means.
- Chemicals used as sterilizing agents are called chemisterilants.

DISINFECTION

- Disinfection is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects.
- Disinfection can be achieved by physical or chemical methods.
- Chemicals used in disinfection are called disinfectants.
- Different disinfectants have different target ranges, not all disinfectants can kill all
- microorganisms.

- Disinfectants vary in their tissue-damaging from the corrosive phenol-containing compounds, which should be used only on inanimate objects, to less toxic materials such as **ethanol** and **iodine** which can be used on skin surface.
- Chemicals used to kill microorganisms on the surface of skin and mucous membrane are called **antiseptics**.
- Surgical instruments that can be damaged by moist heat are usually sterilized by exposure to **ethyl oxide gas** and most intravenous solution are sterilized by **filtration**.

Cleaning:

Cleaning is removal of visible soil (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.

Decontamination:

Decontamination removes pathogenic microorganisms fromobjects so they are safe to handle, use, or discard.

- **Decontamination is the process of removal of contaminating pathogenic microorganisms**.
- It is the use of physical or chemical means to remove, inactivate, or destroy living organisms on a surface so that the organisms are no longer infectious.
- **Sanitization is the process of chemical or mechanical cleansing, applicable in public health systems.**
- **Usually used** by the food industry. It reduces microbes on eating utensils to safe, acceptable levels for public health.

- Methods of sterilization/ disinfection
  - Physical
    - Sunlight
    - Heat
      - Dry heat
        - Red heat
        - Flaming
        - Incineration
        - Hot air oven
        - Infra red
      - Moist heat
        - Below 100°C
        - At 100°C
        - Above 100°C
    - Vibration
    - Radiation
      - Non-ionizing
      - Ionizing
        - Electomagnetic
        - Particulate
    - Filtration
      - Earthenware
      - Asbestos
      - Sintered glass
      - Membrane
  - Chemical
    - Liquid
      - Alcohols
      - Aldehydes
      - Phenolics
      - Halogens
      - Heavy metals
      - Surface active agents
      - Dyes
    - Gaseous
      - Formaldehyde
      - Ethylene oxide
      - Plasma
  - Physio-chemical

PHYSICAL METHODS OF STERILIZATION:

Sunlight:

- The microbicidal activity of sunlight is due to the presence of ultra violet rays in it.
- It is responsible for spontaneous sterilization in natural conditions.
- It is more effective in killing germs due to combination of ultraviolet rays and heat.
- By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes.

Heat:

- Heat is considered to be most reliable method of sterilization of articles that can withstand heat.
- Heat acts by oxidative effects as well as denaturation and coagulation of proteins.
- Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

DRY HEAT:

- **Red heat:**
- Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot. This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame.

- **Flaming:**
- This is a method of passing the article over a Bunsen flame, but not heating it to redness.

  Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times.
- 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. Cracking of the glassware may occur.

- **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.
- This technique results in the loss of the article, hence is suitable only for those articles that have to be disposed.
- Burning of polystyrene materials emits dense smoke, and hence they should not be incinerated.

- **Hot air oven:** This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160o C) for duration of one hour in an electrically heated oven.
- Since air is poor conductor of heat, even distribution of heat throughout the chamber is achieved by a fan.
- Articles sterilized: 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.

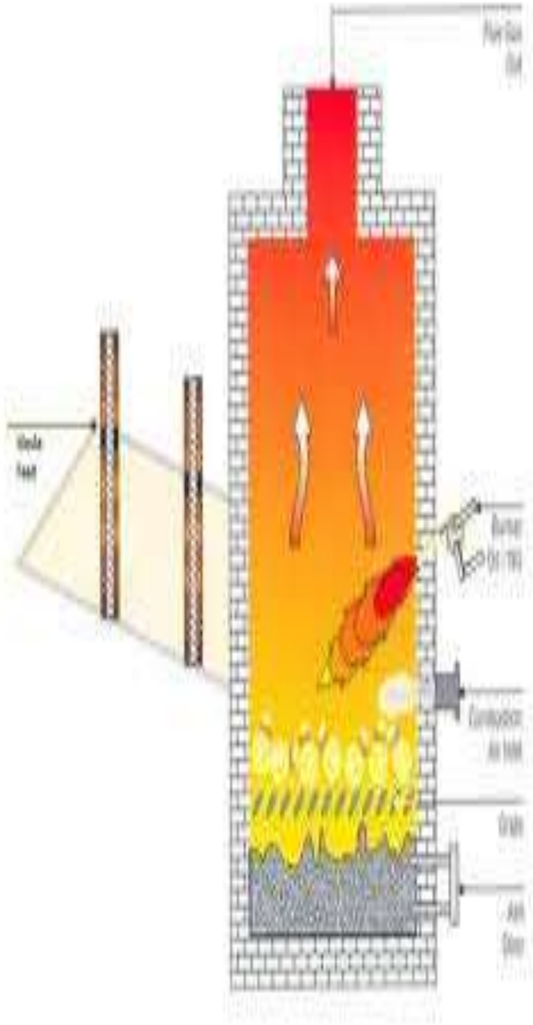
- Sterilization cycle: This takes into consideration the time taken for the articles to reach the sterilizing temperature, maintenance of the sterilizing temperature for a defined period (holding time) and the time taken for the articles to cool down.
- Different temperature-time relations for holding time are 60 minutes at 160oC, 40 minutes at 170oC and 20 minutes at 180oC. Increasing temperature by 10 degrees shortens the sterilizing time by 50 percent.
- The hot air oven must not be opened until the temperature inside has fallen below 60oC to prevent breakage of glasswares.

RED HEAT

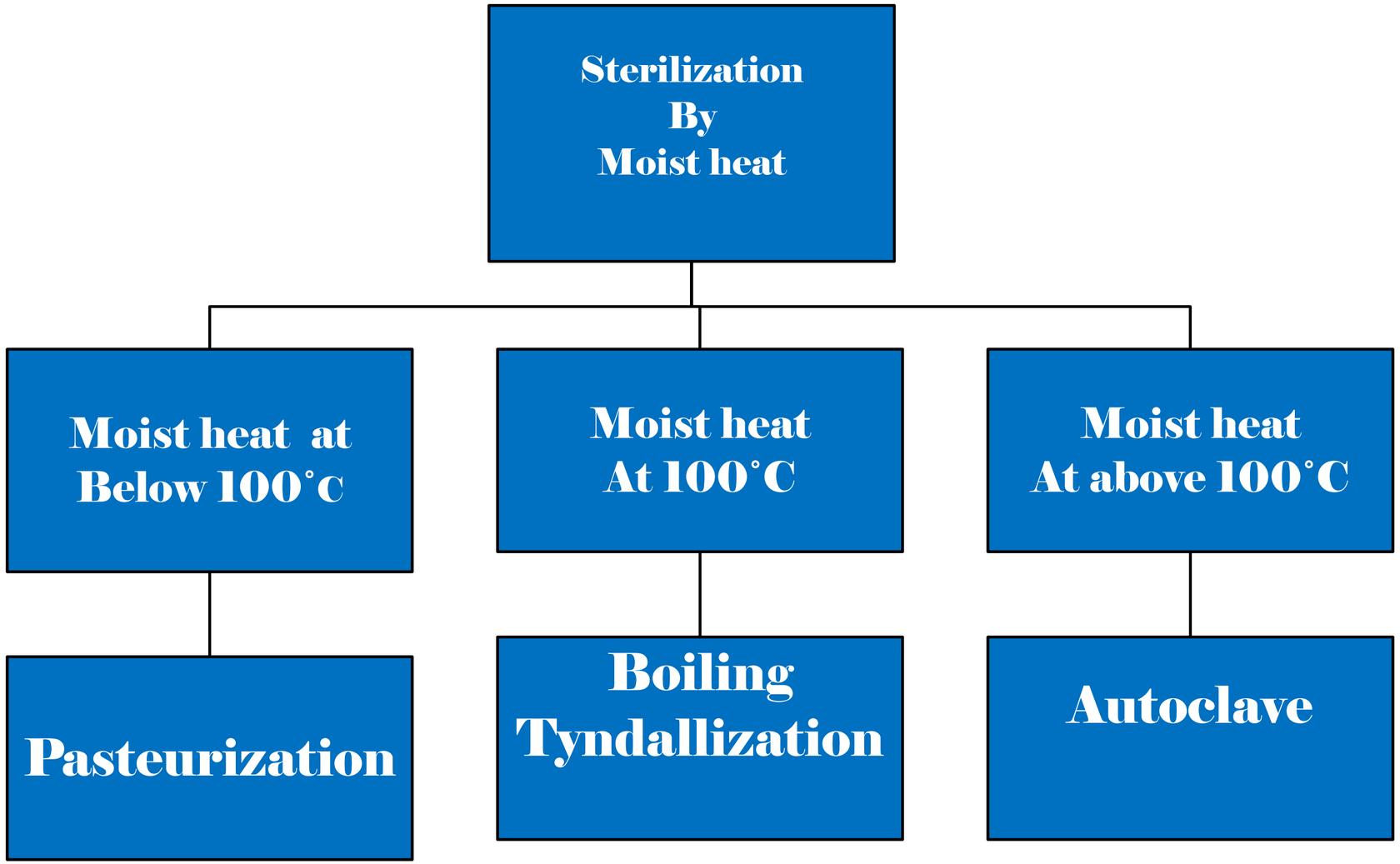
- A simple & effective method.
- Loops or wires, needle , glass slides, cover slips
- Instruments held on a Bunsen flame till red-hot.

Flaming

Incinerator



MOIST HEAT


Sterilization
By
Moist heat
Moist heat at
Below 100˚C
Pasteurization
Moist heat
At 100˚C
Boiling
Tyndallization
Moist heat
At above 100˚C
Autoclave

MOIST HEAT:

- Moist heat acts by coagulation and denaturation of proteins.

At temperature below 100oC:

Pasteurization:

- Originally employed by Louis Pasteur, followed in food and dairy industry.
- There are two methods of pasteurization, the **holder** method (heated at 63oC for 30 minutes) and **flash** method (heated at 72oC for 15 seconds) followed by quickly cooling to 13oC.
- Other methods - Ultra-High Temperature (UHT), 140oC for 15 sec and 149oC for 0.5 sec.
- It is suitable to destroy most milk borne pathogens like Salmonella, Mycobacteria, Streptococci, Staphylococci and Brucella.

Vaccine bath:

- The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60oC for one hour.

Serum bath:

- The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56oC for one hour on several successive days.
- Proteins in the serum will coagulate at higher temperature.
- In both, vaccine and serum bath, only vegetative bacteria are killed and spores survive.

Inspissation:

- This is a technique to solidify as well as disinfect egg and serum containing media.
- The medium containing serum or egg are placed in the slopes of an inspissator and heated at 80-85oC for 30 minutes on three successive days.

At temperature 100oC:

Boiling:

- Boiling water (100oC) kills most vegetative bacteria and viruses immediately.
- Certain bacterial toxins such as Staphylococcal enterotoxin are also heat resistant.
- Some bacterial spores are resistant to boiling and survive; hence this is not a substitute for sterilization.
- The killing activity can be enhanced by addition of 2% sodium bicarbonate.
- When absolute sterility is not required, certain metal articles and glasswares can be disinfected by placing them in boiling water for 10-20 minutes.

Boiling

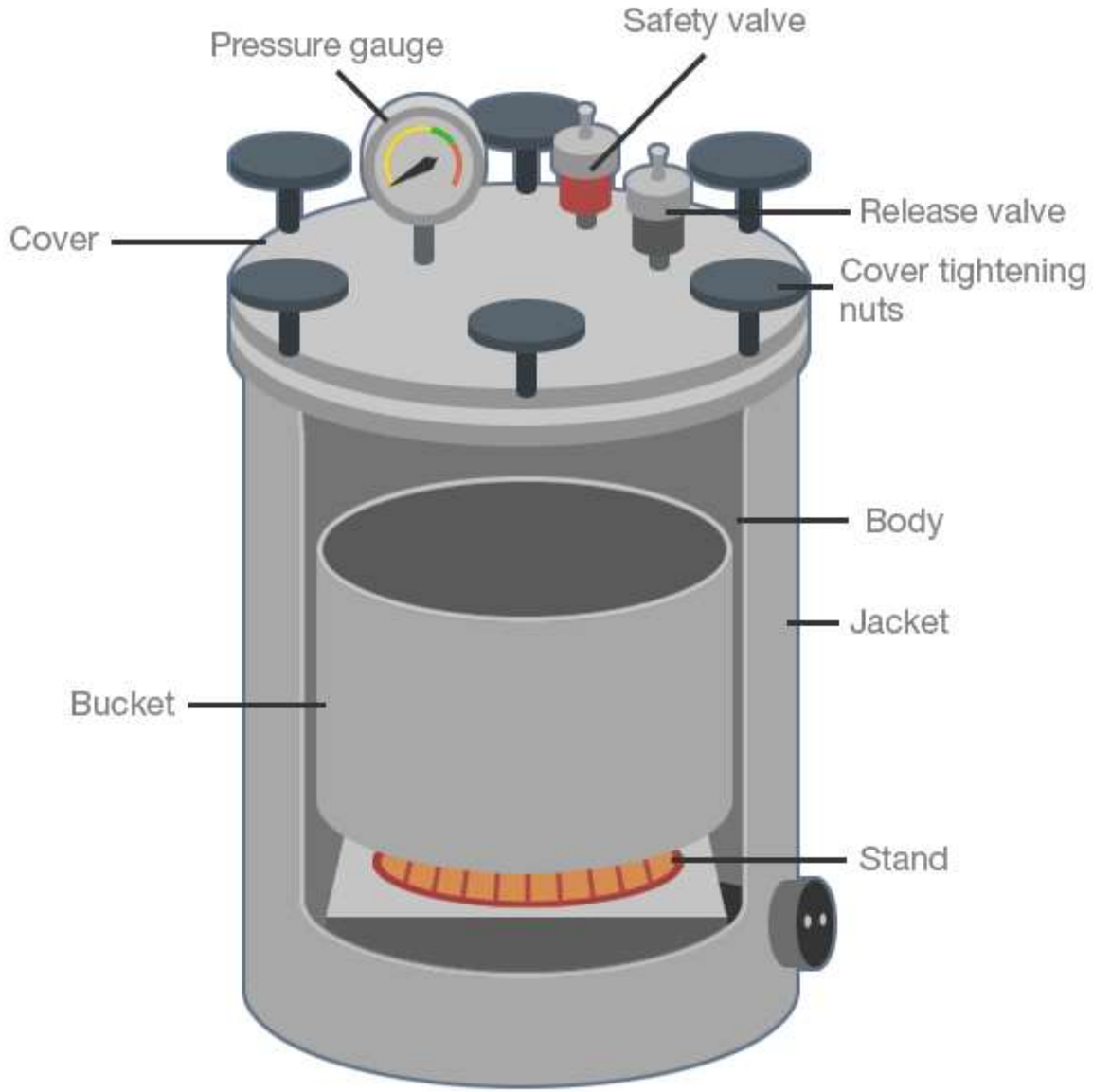
Steam at 100oC:

- Instead of keeping the articles in boiling water, they are subjected to free steam at 100oC.
- Traditionally Arnold's and Koch's steamers were used.
- An autoclave (with discharge tap open) can also serve the same purpose.
- A steamer is a metal cabinet with perforated trays to hold the articles and a conical lid.
- The bottom of steamer is filled with water and heated.
- The steam that is generated sterilizes the articles when exposed for a period of 90 minutes.

At temperature above 100oC:

Autoclave:

- Sterilization can be effectively achieved at a temperature above 100oC using an autoclave.
- Water boils at 100oC at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases.
- In an autoclave the water is boiled in a closed chamber.
- As the pressure rises, the boiling point of water also raises.
- At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121oC.
- Exposure of articles to this temperature for 15 minutes sterilizes them.
- Under autoclave conditions, pressurize steam KILLS bacterial spore, vegetative cells and other microbial forms.

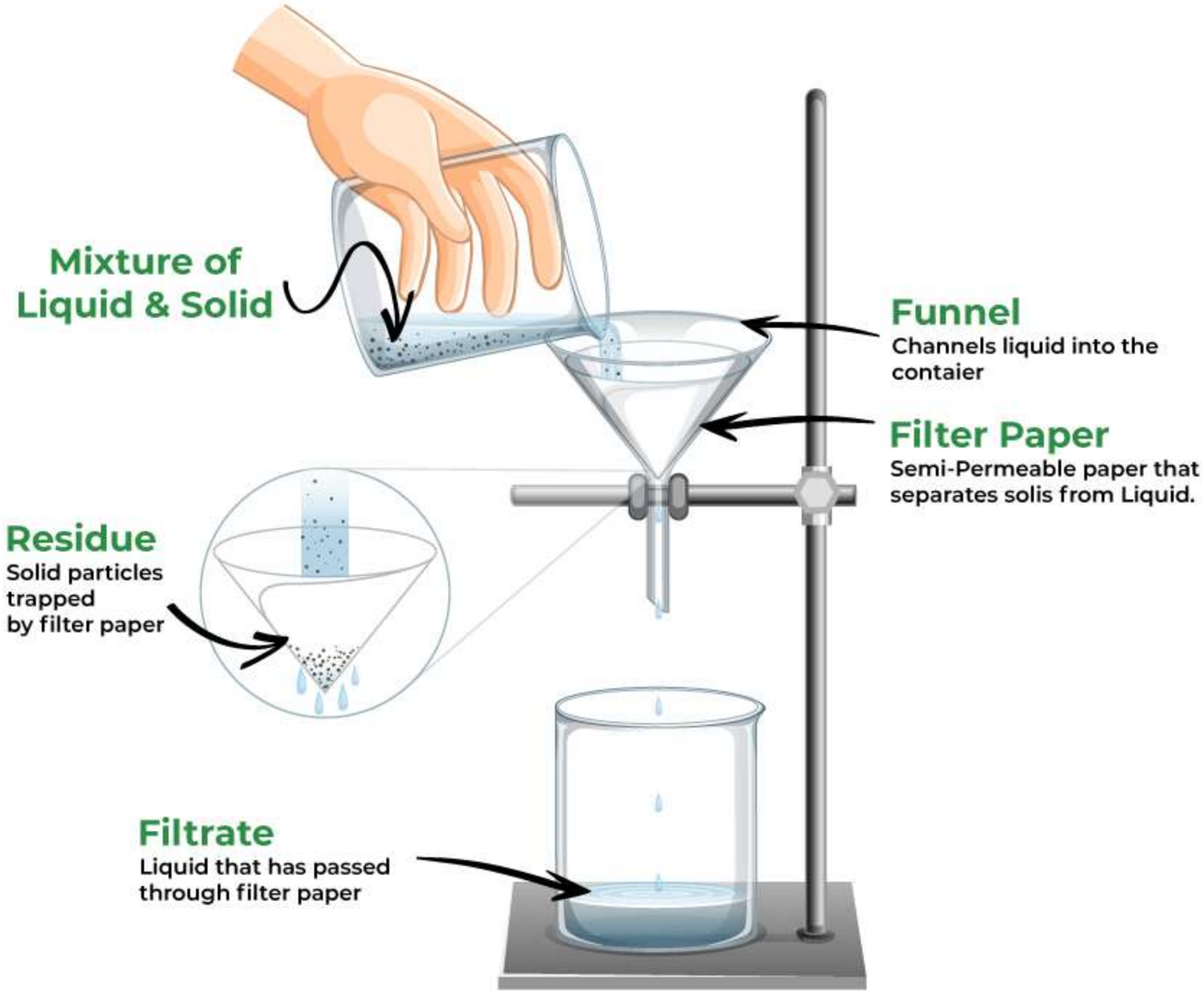

Pressure gauge
Safety valve
Cover
Release valve
Cover tightening nuts
Body
Jacket
Bucket
Stand

Different types of autoclave

- Simple “pressure-cooker type” laboratory autoclave
- Steam jacketed downward displacement laboratory autoclave
- High pressure pre-vacuum autoclave

FILTRATION

- Filtration does not kill microbes, it separates them out.
- Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved.
- It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution.
- Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydatid fluid.


Mixture of
Liquid & Solid
Funnel
Channels liquid into the contaier
Filter Paper
Semi-Permeable paper that separates solis from Liquid.
Residue
Solid particles trapped by filter paper
Filtrate
Liquid that has passed through filter paper

Different types of filters

- **Earthenware filters**
- a. Pasteur-Chamberland filter:
- b. Berkefeld filter:
- c. Mandler filter:
- **Asbestos filters:**
- **Sintered glass filters**
- **Membrane filters:**
- **Air Filters:** Air can be filtered using **HEPA (High Efficiency Particle Air)** filters.
- They are usually used in biological safety cabinets.
- HEPA filters are at least 99.97% efficient for removing particles >0.3 μm in diameter.

Assessment

1. List of methods involved in the dry heat?

2. List of methods involved in the moist heat?

3. What is Autoclave and Hot air oven?

4. Types of filters used for sterilization?

5. Size of the filters ranges from?

STERILIZATION/PHYSICAL/MICROBIOLOGY/MRS.MITHRA/SNSCAHS

Thank You