

Practical Work No. 1: Milk Pasteurization

1) Sterilization

Sterilization is an operation applied to inert substances to ensure the **complete and permanent elimination of all microorganisms**, including:

- Vegetative forms, Spore-forming forms, Viruses

Sterilized materials must be protected until their next use to avoid recontamination.

The choice of technique depends on:

- The nature of the product or material to be sterilized, Cost, Local technical possibilities

A) Sterilization by Dry Heat

Oxidation of microbial proteins

a-1) Flaming : Uses the flame of a **Bunsen burner**

- Applied for:-Sterilizing the air in the working area, -Immediate sterilization of small equipment.

Examples:

- Platinum wire loop, Necks of tubes and flasks, Outside of Pasteur pipettes

a-2) Hot Air Oven (Pasteur Oven) : Destroys organic matter at high temperatures.

Temperature: **140 to 210°C**, Time: **30 to 180 minutes**

✓ **Utilities** : Metal equipment, Porcelain (filters, mortars), Glassware (except precision glassware, which may deform due to expansion)

✗ **evoiding** : Rubber, Plastics, Culture media, Liquids

Glassware must be: Clean, Completely dry, Plugged with cotton if necessary, Wrapped in strong paper .

After sterilization, materials must remain in the oven until completely cooled and then stored away from dust.

B) Sterilization by Moist Heat

Denaturation and coagulation of microbial proteins

The combined action of water and temperature causes protein denaturation, preventing microbial reproduction and leading to cell death.

b-1) Autoclaving (Most widely used and most effective method)

The technique is performed in an **autoclave**, which is a sealed pressure vessel.

Principle:

- Water boils at 100°C under normal atmospheric pressure (760 mmHg).
- When pressure increases, the boiling temperature also increases.

Sterilization conditions: 115–121°C for 15–20 minutes using 1.5–2 atmospheres

To kill bacteria, Spores and Fungi.

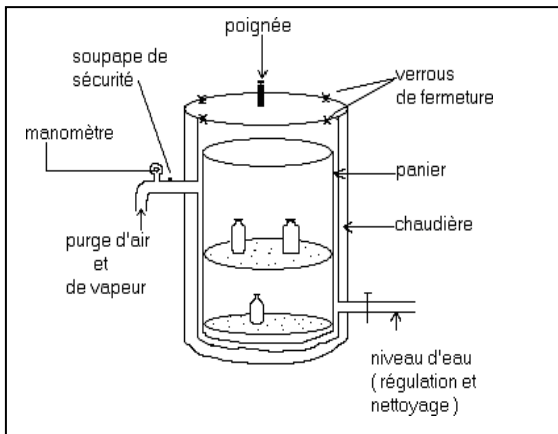
✓ **Used for:** Non-thermolabile culture media, Liquids **and** plastic materials, Rubber materials

Precautions:

-Check the water level before each operation, -Do not completely fill flasks and tubes

,-Do not tightly close containers

Main parts of an autoclave: Lid with wing bolts, Basket for materials, Pressure gauge, Valves (purge and safety valve), Heating system (electricity or gas)



- Lid secured with wing bolts
- Basket containing the items to be sterilized
- Pressure gauge (manometer)
- Valves: one for air purge and one safety valve (overpressure protection)
- Heating system: electric or gas-operated

THE AUTOCLAVE

b-2) Pasteurization :Not sterilization (spores are not destroyed) than always followed by rapid cooling.

The product is gradually heated with water spray up to **65–75°C**, held for **20–30 minutes**, then cooled progressively.

We can use :

Low-temperature pasteurization at **65°C** for 30 minutes or High-temperature pasteurization at **85–90°C** for 20–30 seconds.

Used for: Milk, Fruit juices, Cider

b-3) Tyndallization (Limited-use sterilization method)

Consists of discontinuous heating in a water bath at **60–70°C**, followed by cooling.

Currently performed as: Three heating cycles for 1 hour at **70–80°C**, Separated by 24-hour intervals at room temperature. This allows spores to germinate and then be destroyed without excessive heat.

Used for: Fragile media, Thermolabile and viscous substances, Serum, eggs

C) Sterilization by Filtration

A liquid is passed through a porous substance that retains suspended solid particles and bacteria.

Used for: Thermolabile substances, Vitamins, Certain medications

Types of filters: Porcelain candles (Chamberland type), Sintered glass filters (No. 5 retains many bacteria), Disposable membrane filters (Millipore, Sartorius)

For effective sterilization: Pore size must be $< 0.2 \mu\text{m}$, Viruses ($< 0.1 \mu\text{m}$) are not retained.

D) Sterilization by Radiation

d-1) Ionizing Radiation :X-rays, Radioactive radiation, Wavelength $\leq 10 \text{ nm}$

Used under special industrial conditions for: Plastic Petri dishes, Non-autoclavable and non-filterable medications

- Dangerous and strictly controlled.

d-2) Non-Ionizing Radiation (Ultraviolet – UV) : Wavelength: 15–385 nm
Commonly used range: **200–280 nm** (Maximum activity around 280 nm)

Used in laboratories for: Air decontamination, Work surfaces, Laminar flow hoods

Inconvenient : Limited penetration capacity.

LAB EXPERIMENT

Materials

- Milk
- Water bath
- Thermometer
- Stopwatch
- Flasks or beakers

Principle

Milk is heated at different temperatures for varying time periods in order to reduce or eliminate microorganisms.

Procedure

1. Heat the milk to **63°C for 30 minutes**.
2. Heat another sample to **72°C for 15 seconds**.

QUESTIONS

- 1• What is the difference between sterilization and pasteurization?
- 2• Why is pasteurization followed by rapid cooling?
- 3• Are bacterial spores destroyed during pasteurization? Why?
- 4• Why is temperature control important during milk heating?
- 5• Why do we compare heated milk with unheated milk?
- 6• What happens if milk is heated at too high a temperature for a long time?