Methods For Sterilization Process

Disclaimer

All the content material provided here is only for teaching purpose.

surface or medium is made free of all microorganisms either in vegetative or spore form.

Disinfection : Destruction of all pathogens or organisms capable of producing infections but not necessarily spores. All organisms may not be killed but the number is reduced to a level that is no longer harmful to health.

Antiseptics : Chemical disinfectants which can safely be applied to living tissues and are used to prevent infection by inhibiting the growth of microorganisms.

Asepsis : Technique by which the occurrence of infection into an uninfected tissue is prevented.

Why we need Sterilization

- Microorganisms capable of causing infection are constantly present in the external environment and on the human body. Microorganisms are responsible for contamination and infection.
- The aim of sterilization is to remove or destroy them from materials or from surfaces.

How can microorganisms be killed?

- Denaturation of proteins
- Interruption of DNA synthesis/repair
- Disruption of cell membranes

Classification

1. Physical sterilization includes:
   - heat
   - radiation
   - Filtration

2. Chemical sterilization includes:
   - Alcohols and Aldehydes
   - Phenols and Halogens
   - Oxidizing agents and Salts
   - Surface active agents and ethylene oxide gas
   - Dyes and Vapor phase disinfectants

Factors that influence efficacy of disinfection/sterilization

- Contact time
- Physico-chemical environment (e.g. pH)
- Presence of organic material
- Temperature
- Type of microorganism
- Number of microorganisms
- Material composition

Uses of sterilisation:

1. Sterilisation of materials, instruments used in surgical and diagnostic procedures, Media and reagents used in the microbiology laboratory.

2. Food and drug manufacturing to ensure safety from contaminating organisms.

What to sterilize?

– all instruments that penetrate soft tissues and bone.

– Instruments that are not intended to penetrate the tissues, but that may come into contact with oral tissues.

- If the **sterilization** procedure may damage the instruments, then, sterilization can be replaced by **Disinfection** procedure

Ideal sterilization/disinfection process

- Highly efficacious
- Fast
- Good penetrability
- Compatible with all materials
- Non-toxic
- Effective despite presence of organic material
- Difficult to make significant mistakes in process
- Easily monitored

Physical Methods How to Sterilize

	Materials	Method
1	Inoculating wires and loops	Red heat
2	Glass ware- syringes, petridishes, testtubes, flasks etc.	Hot –airoven
3	Disposable syringes, and otherdisposableitems	Gamma radiation
4	Culture media	Autoclaving
5	Culture media containing serum and egg	Tyndallisation
6	Toxin , serum, sugar, and antibioticsolutions	Filtration
7	Cystoscope and endoscope	Glutaraldehyde
8	Infected soiled dressings	Incineration
9	Skin	Iodine, alcohol
10	Milk	Pasteurisation

Heat-Related Methods: Dry-Heat Sterilization

- Involves heating at atmospheric pressure and often use a fan to obtain uniform temperature by circulation.

- Heat at 180º for half hour , 170º for 1 hr., or 160º C for 2 hrs.
- Times are the periods during which object is maintained at the respective temp.
- Dry heat:

1. Red heat
2. Flaming
3. Incineration
4. Hot air oven

Factors influencing dry heat sterilization:

- Nature of heat
- Temperature and duration
- Characteristic of organism and spores
- Type of material

Principle:

- Dry heat kills the organism by
  - denaturation of the bacterial proteins,
  - oxidative damage
  - toxic effect of elevated levels of electrolytes.

1. **Red heat**: Materials are held in the flame of a Bunsen burner till they become red hot.

   - Inoculating wires or loops
   - Tips of forceps and Needles

2. **Flaming**: Materials are passed through the flame of a Bunsen burner without allowing them to become red hot.

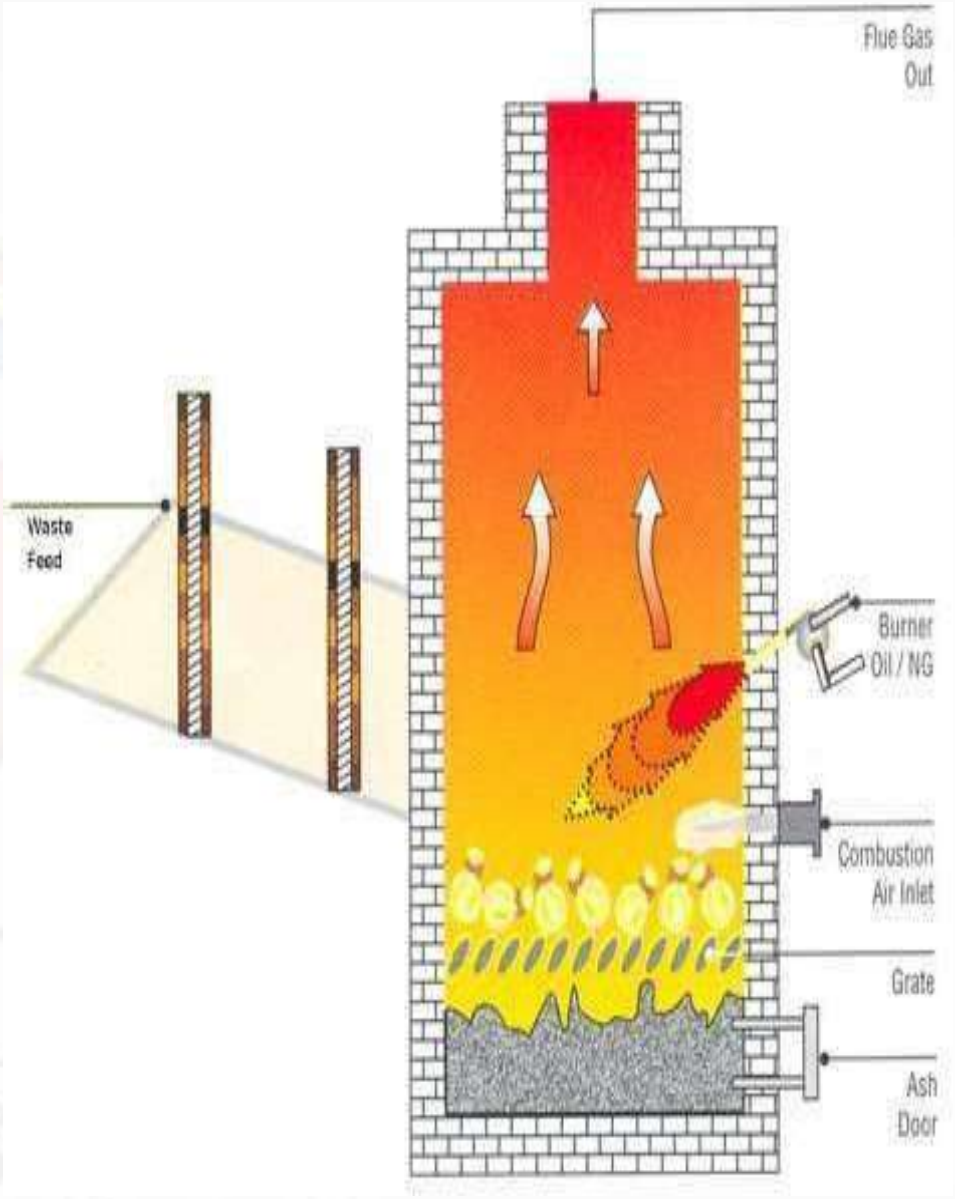
   - Glass slides and Scalpels
   - Mouths of culture tubes

3. Sun light:

- Active germicidal effect due to its content of ultraviolet rays .
- Natural method of sterilization of water in tanks, rivers and lakes.

Incineration

- Materials are reduced to ashes by burning.
- Instrument used was incinerator.
- Soiled dressings
- Animal carcasses
- Bedding
- Pathological material



Hot air oven:

- Electrically heated and fitted with a fan to even distribution of air in the chamber.
- Fitted with a thermostat that maintains the chamber air at a chosen Temperature and time:

» 160 °C for 2 hours.

» 170 °C for 1 hour

» 180 °C for 30 minutes.

- Glassware like glass syringes, Petri dishes, pipettes and test tubes, Surgical instruments like scalpels, scissors, forceps and Chemicals like liquid paraffin, fats etc. can be sterilized

Precautions :

1. Should not be overloaded
2. Arranged in a manner which allows free circulation of air
3. Material to be sterilized should be perfectly dry.
4. Test tubes, flasks etc. should be fitted with cotton plugs.
5. petridishes and pipetts should be wrapped in paper.
6. Rubber materials and inflammable materials should not be kept inside.
7. The oven must be allowed to cool for two hours before opening, since glass ware may crack by sudden cooling.

Disadvantages of Dry-Heat Sterilization

- Less reliable than autoclaving
- Many materials don't tolerate dry heat

Heat-Related Methods: Moist heat I : Pasteurization: below100°C

- ✓ Used for milk, ice cream, yogurt, and fruit juices
- ✓ Heat-tolerant microbes survive
- ✓ Batch method
- ✓ temperature below 100° **Pasteurization of milk**

✓ Developed by Louis Pasteur to prevent the spoilage of beverages. Used to reduce microbes responsible for spoilage of beer, milk, wine, juices, etc.

✓ Milk was exposed to 65^{o}C for 30 minutes.

✓ **High Temperature Short Time Pasteurization (HTST):** Used today. Milk is exposed to 72^{o}C for 15 seconds.

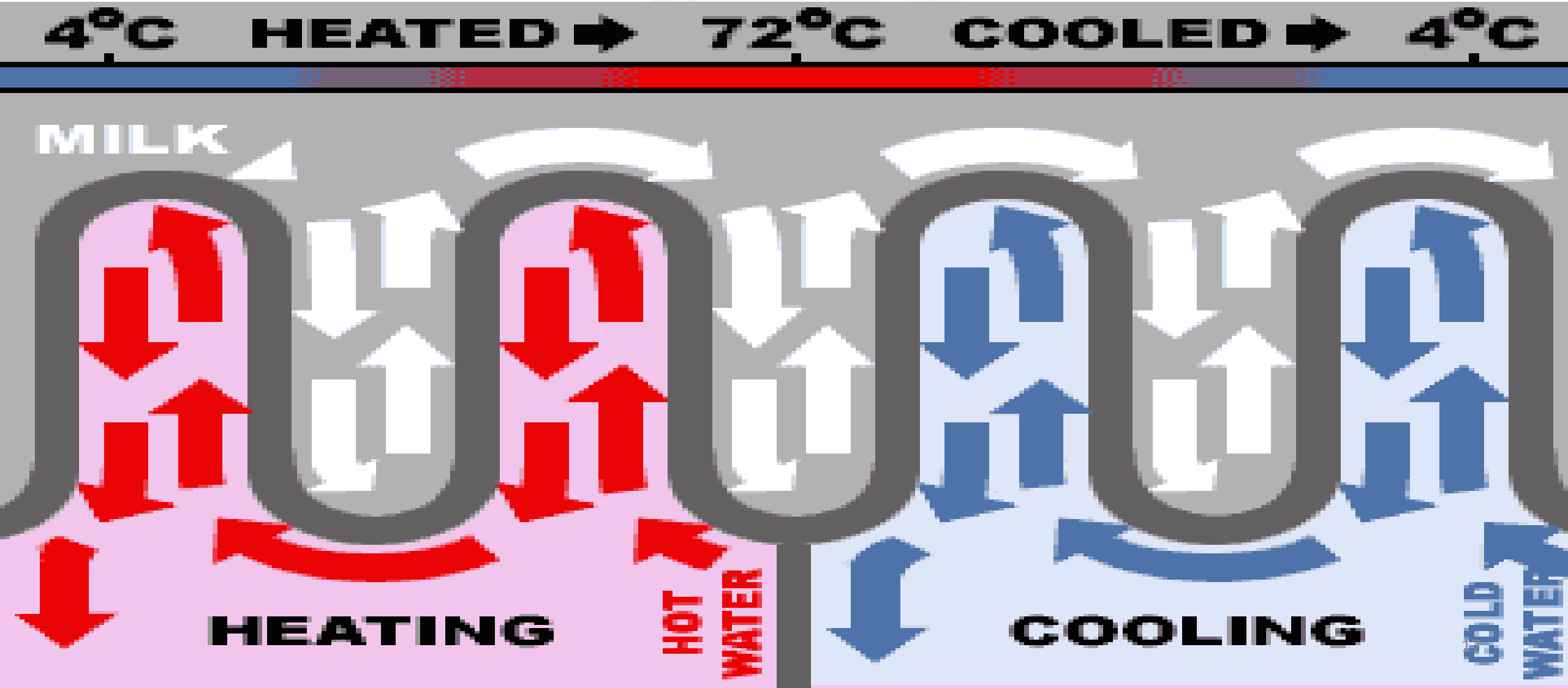
Inspissations:

- Materials are reduced to ashes by burning.
- Heating at 80-85°C for half an hour daily on three consecutive days
- Serum or egg media are sterilized

Vaccine bath:

- Heating at 60°C for an hour daily in vaccine bath for several successive days. Serum or body fluids can be sterilized by heating at 56°C for an hour daily for several successive days.

Principle of Pasteurization



100°C

1. Boiling: Boiling for 10 – 30 minutes may kill most of vegetative forms but spores with stand boiling.

2. Tyndallisation: Steam at 100C for 20 minutes on three successive days. Used for egg , serum and sugar containing media.

3. Steam sterilizer : Steam at 100°C for 90 minutes. Used for which media are decomposed at high temperature.

III. A temperature above 100°C

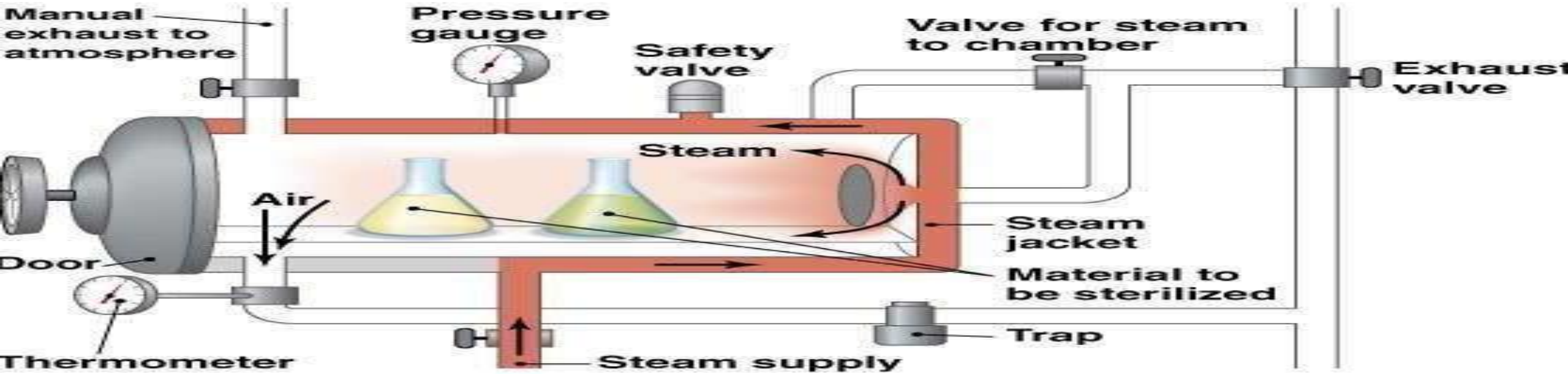
Autoclave:

- Steam above 100°C has a better killing power than dry heat.
- Bacteria are more susceptible to moist heat.

Components of autoclave:

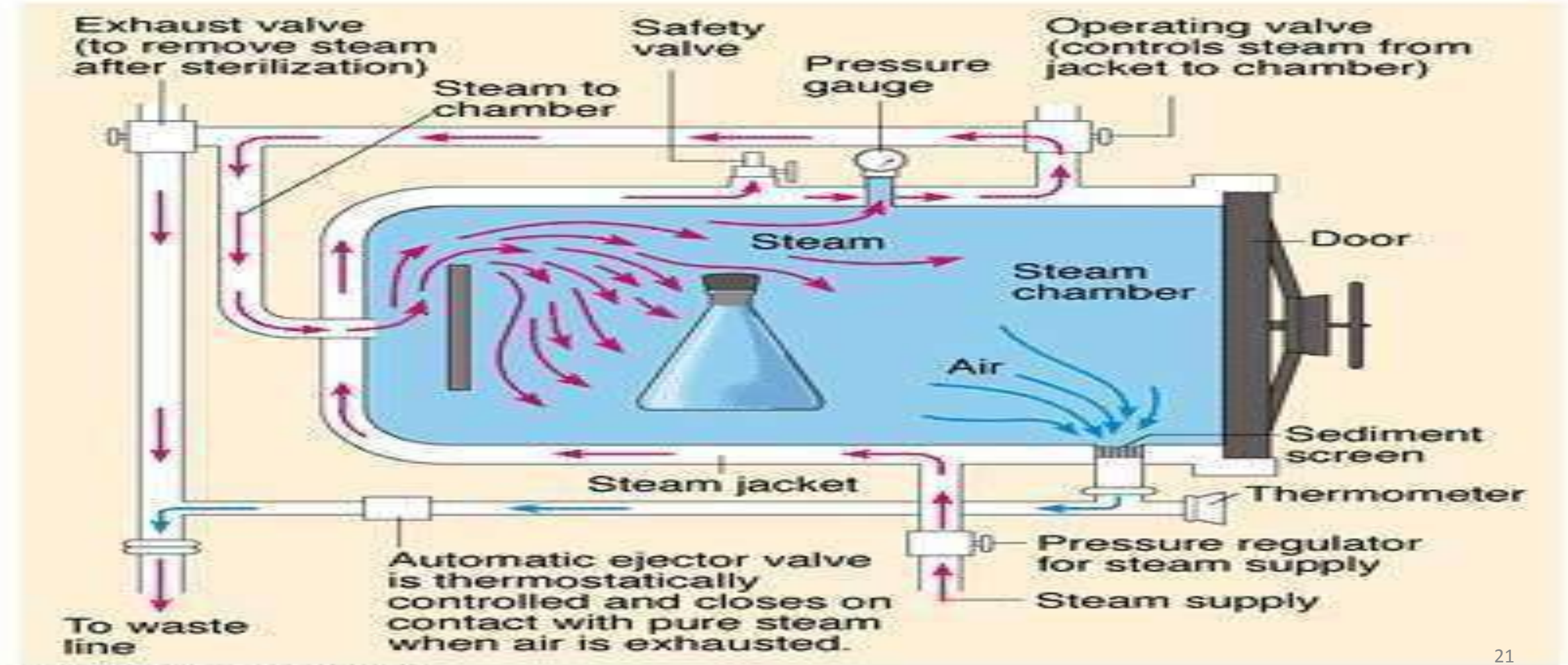
- Consists of vertical or horizontal cylinder of gunmetal or stainless steel.
- Lid is fastened by screw clamps and rendered air tight by an asbestos washer.
- Lid bears a discharge tap for air and steam, a pressure gauge and a safety valve.

(a)



(b)

Autoclave: Closed Chamber with High Temperature and Pressure



Transformation in design

BEFORE

AFTER

Sterilization conditions:

- Temperature – 121 °C
- Chamber pressure -15 lb per square inch.
- Holding time – 15 minutes
- Others :
  - 126°C for 10 minutes
  - 133°C for 3 minutes

Uses of Autoclaves:

- Useful for materials which can not withstand high temp.
- To sterilize culture media, rubber material, gowns, dressings, gloves etc.

Sterilization – instrument Packing

- Often instruments are packed for sterilization to be stored and handled without being contaminated.

- Packing depend on the intended shelf life after sterilization.
  - Textile has shelf life of 1 month
  - Paper has shelf life of 1 – 6 months
  - Nylon, glass, and metal have shelf life of 1 year if tightly closed

Sterilization controls:

- Thermocouples
- Bacterial spores- ***Bacillus stearothermophilus***
- Browne's tube and Autoclave tapes

Sterility Controls

Sterility Controls

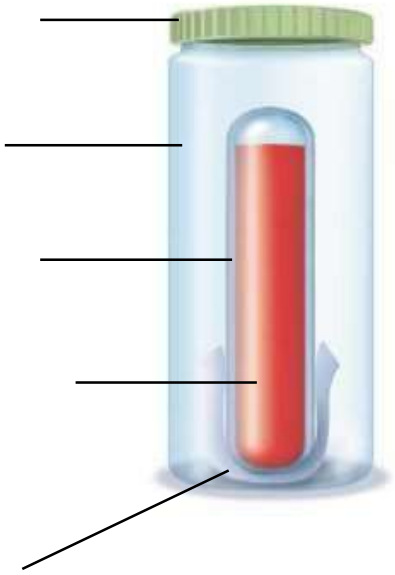
Cap that allows steam to penetrate

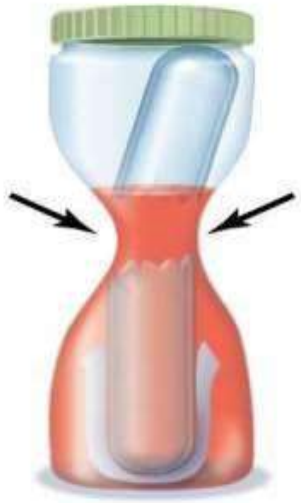
Flexible plastic vial

Crushable glass ampule

Nutrient medium containing pH color indicator

Endospore strip





Incubation

After autoclaving, flexible vial is squeezed to break ampule and release medium onto spore strip.

Yellow medium means spores are viable; autoclaved objects are not sterile.

Red medium means spores were killed; autoclaved objects are sterile.

Types of Load

Non-porous loads

- Also called Hard goods load.
- No pre- post vaccum required
- Sterilized by gravity displacement method or sterilizers.
- Liquid load for terminal sterilization
- Media cycles in microbiology lab.
- Glassware and unwrapped load in microbiology lab.

- Porous loads

- Also called wrapped goods load.
- Pre and post vaccum required
- Pre and post vaccum required

- Sterilization of garments, silicon tubing, filters, machine parts, rubber stoppers and seals.

Production Loads

Machine parts load

Garments load

Sterilization approach

OverkillApproach

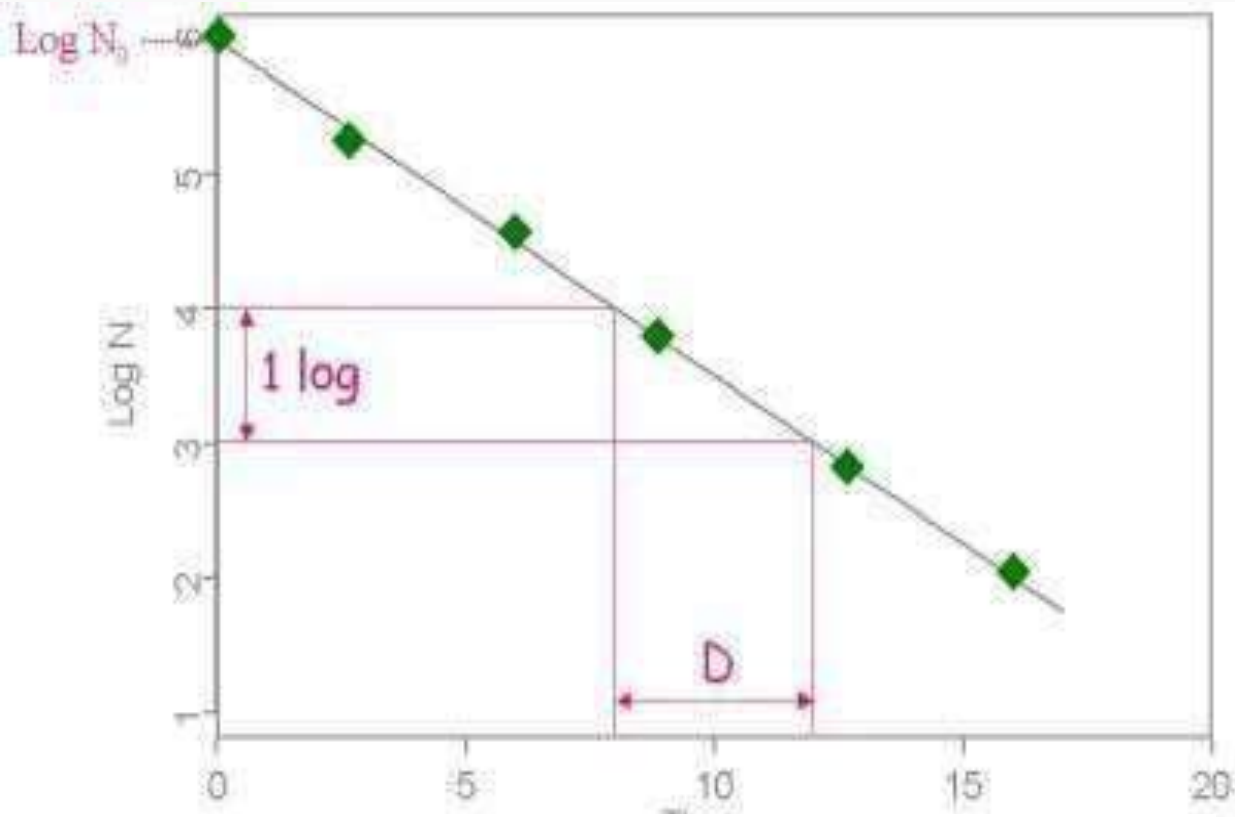
- Overkill sterilization primarily is applied to the moist-heat processing of materials, supplies, and other heat-stable goods.
- "This is usually achieved by providing a minimum 12-log reduction of microorganisms considering worst case of *D*- value at 121.1 °C.

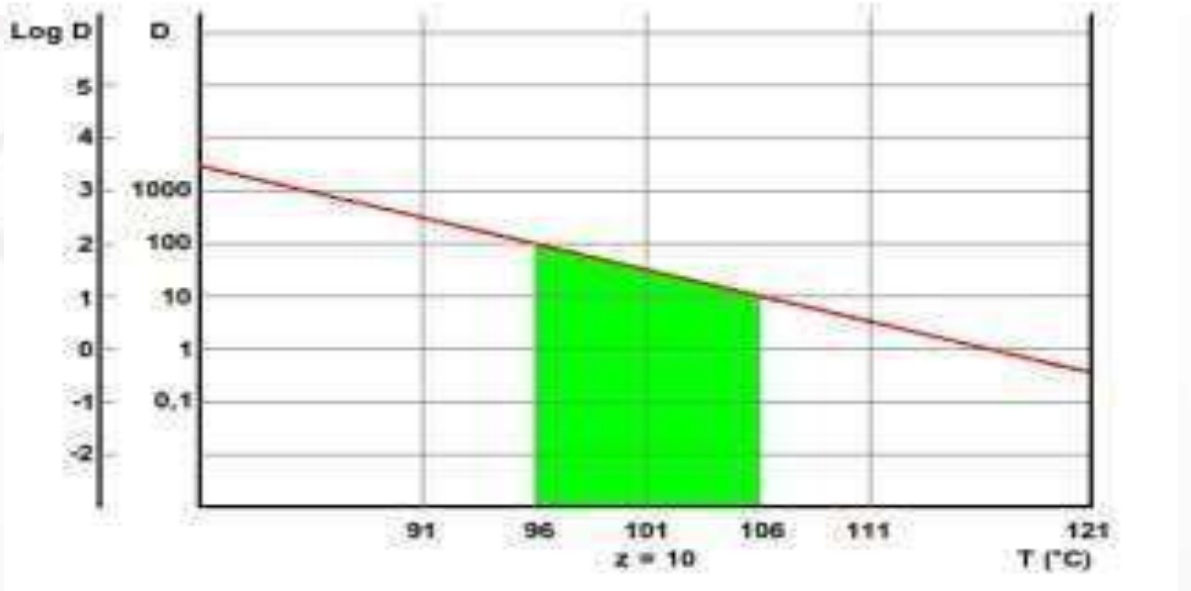
BioburdenApproach

- For items that are heat sensitive and can not withstand an overkill approach. It is necessary to collect the bioburden data and possibly D-value data.
- This will reduce the sterilization cycle time. For example: 134 CFU(bioburden). To reduce the bioburden from 134 to 01 = log (134) = 2.14 minutes.

D-value refers to decimal reduction time and is the time required at a given temperature to kill 90% of the exposed microorganisms or to reduce the population by 1 log reduction.

Z- value is the temperature required for one log10 reduction in the D-value.





Test to be carried out in performance Qualification

1. Chamber vacuum leak test

Objective: Objective of this test is to check the integrity of chamber and ensure that the rate of vacuum drop is within the acceptable limits.

Acceptance criteria: 1.3mbar/ minute

Use: To ensure microorganisms and air entrance into autoclave chamber.

Objective: Pre-vaccum pulses are sufficient to remove the entrapped air or non-condensable gases so as to facilitate rapid and even steam penetration into all parts of the load.

Acceptance criteria: Test- kit colour should change from yellow to black Use: To ensure complete removal of air from chamber through pre vaccum pulses

UNEXPOSED BOWIE-DICK KIT

EXPOSED BOWIE-DICK KIT

3. Empty chamber heat distribution

Objective: Objective of this test is to ensure that equipment is suitable for chamber heat distribution of heat in the sterilizer when the parameters.

- **Acceptance criteria:** Temperature: NLT

NLT 30 minute

HEAT DISTRIBUTION STUDY SENSOR LOCATION

4. Heat penetration study

Objective: Objective of this test is to ensure that equipment is suitable for sterilization of loaded articles in the chamber.

sterilizer

Acceptance criteria: Temperature: 121°C – 124°C Sterilization Time NLT 30 minute

Equilibration time:- NMT 30 Sec.

Use: To check and ensure sterilization of articles in load.

5. Assurance of sterilization :

Physical challenge : By temperature mapping.

Chemical challenge: Autoclable tape.

Biological challenge: By keeping the biological indicator (Geobacillus stearothermophilus, population = 10^6) inside the sterilizing articles.

For Porous loads

For Liquid loads

Filtration:

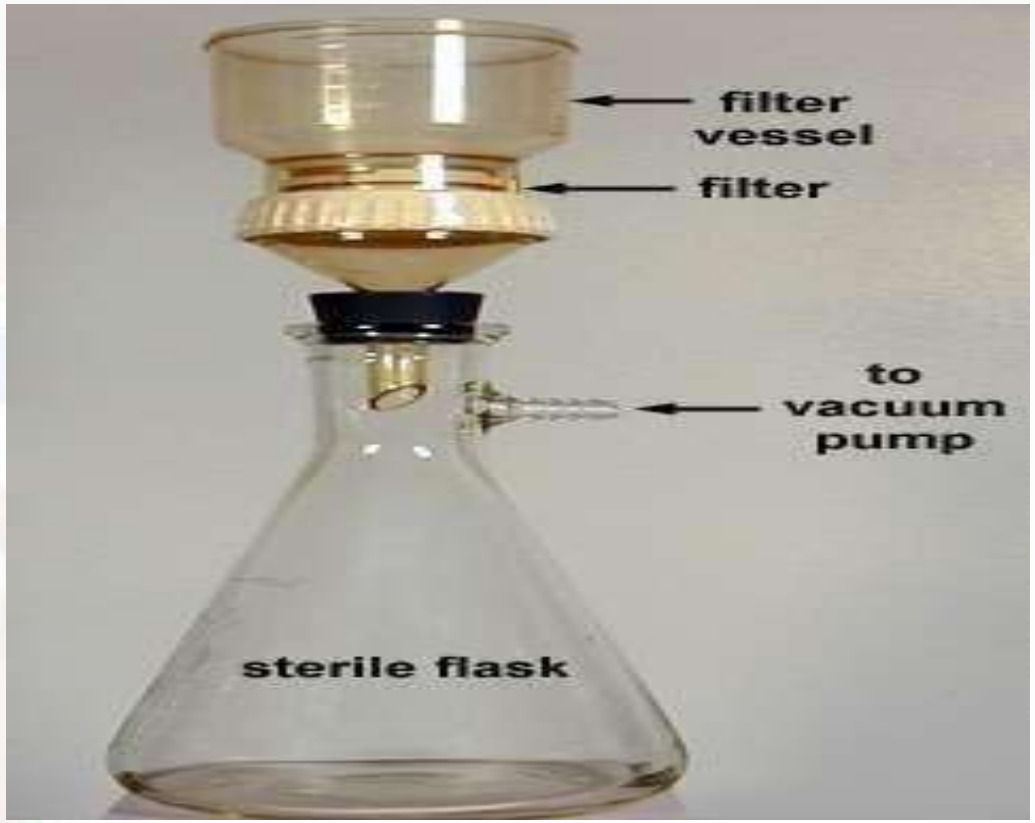
- This method is commonly used for sensitive pharmaceuticals and protein solutions in biological research.
- A filter with pore size 0.2 µm will effectively remove bacteria.
- If viruses must also be removed, a much smaller pore size around 20 nm is needed.
- Prions are not removed by filtration.
- The filtration equipment and the filters themselves may be purchased as presterilized disposable units in sealed packaging,
- or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes.

- To ensure sterility, the filtration system must be tested to ensure that the membranes have not been punctured prior to or during use.
- To ensure the best results, pharmaceutical sterile filtration is performed in a room with highly filtered air (HEPA filtration) or in a laminar flow cabinet or "flowbox", a device which produces a laminar stream of HEPA filtered air.
- HEPA filters are critical in the prevention of the spread of airborne bacterial and viral organisms and, therefore, infection. Typically, medical-use HEPA filtration systems also incorporate high-energy ultra-violet light units to kill off the live bacteria and viruses trapped by the filter media.

Types of filters:

1. Candle filters
2. Asbestos disc filters
3. Sintered glass filters
4. Membrane filters
5. Air filters
6. Syringe filters

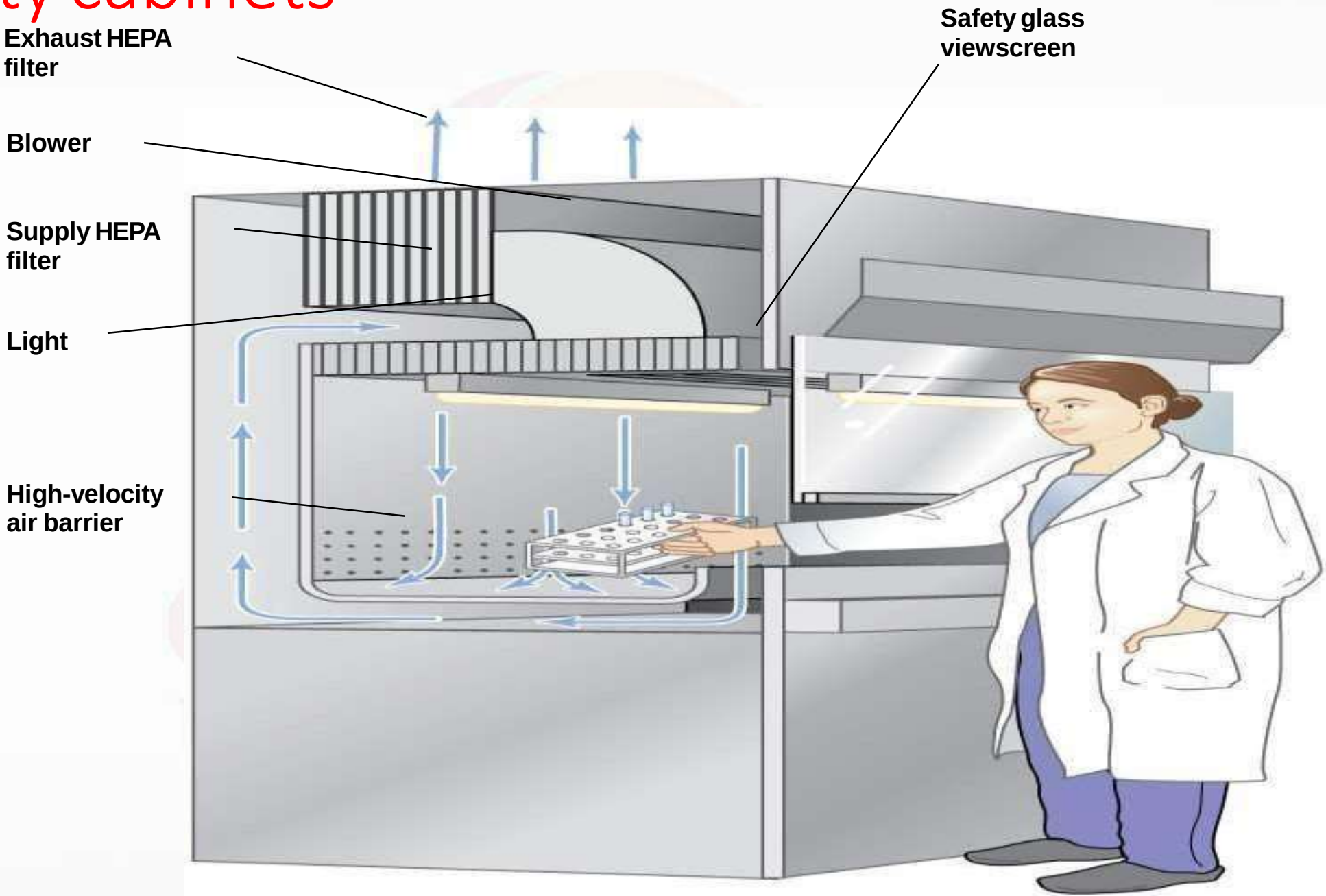
- Sterilize solutions that may be damaged or denatured by high temperatures or chemical agents.
- The pore size for filtering bacteria, yeasts, and fungi is in the range of 0.22-0.45 μm (filtration membranes are most popular for this purpose).



Candle filters

The roles of HEPA filters in biological flow safety cabinets

safety cabinets



Radiation
s :

1. Ionizing radiations:

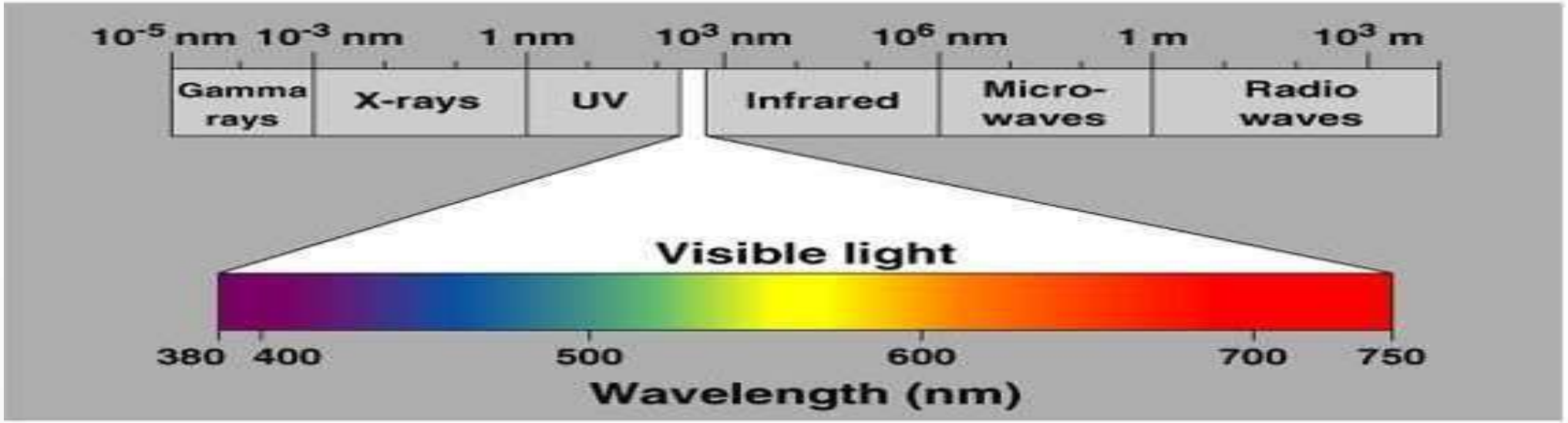
- X rays
- Gamma rays: commercially used for sterilization of disposable items. (cold sterilization)
- Cosmic rays

2. Nonionizing Radiation

- **Infra red rays:** Used for rapid mass sterilization of syringes and catheters.

Ultraviolet light :

- Wavelength is longer than 1 nanometer. Damages DNA by producing thymine dimers, which cause mutations.
- Used to disinfect operating rooms, nurseries, cafeterias. Ultraviolet radiation is used for disinfecting enclosed areas such as bacterial laboratory, inoculation hood, laminar flow and operation theatres. Damages skin, eyes.



Quality Assurance: Each Health Care Facility should have a system in place to provide quality patient care through the provision of sterile equipment and medical devices.

Quality Assurance Program Shouldinclude:

- Administrative Controls
- Chemical Indicator Monitoring
- Biological Indicator Monitoring
- Mechanical Indicators
- Continuing Education

Accepted Practice Guidelines

- CSA Canadian Standards Association International
- AAMI Association for the Advancement of Medical Instrumentation
- ASHCSP American Society for Healthcare Central Service Professionals
- AORN Association of Operating Room Nurses
- ORNAC Operating Room Nurses Association of Canada
- CDC Centers for Disease Control and Prevention
- LCDC Laboratory Centre for Disease Control

Objectives of Monitoring the Sterilization Process

- Assure high probability of absence of microbes on processed items
- Detect failures as soon as possible
- Remove medical devices involved in failures before patient use
- Improve patient outcomes
- Control costs
- Peace of mind

Methods of Monitoring

1. Mechanical Indicators: *Equipment control*
2. Chemical Indicators: *Exposure/Process control/ Pack control*
3. Biological Indicators: *Load control*


MECHANICAL
CHEMICAL
BIOLOGICAL
COMBINED RESULTS
STERILITY ASSURANCE

Mechanical Indicators show:

- what is happening in the chamber, whether conditions are being met
- cycle, time, temperature and pressure
- Recording thermometer – circle graph
- Computer printouts – paper strip
- Gauges – jacket and chamber pressure
- If conditions were not met:
  - Consider load un-sterile and do not use sterilizer until the problem is identified
  - monitor one location in sterilizer
  - do not monitor each pack or tray, do not indicate sterility

Chemical Indicators (CI)

- monitor one or more of requirements -time, temp, and sterilant
- can be external and Internal
- give instant results
- indicate proper conditions for sterilization were present

External Chemical Indicator

- process indicator - autoclave tape
- distinguishes processed from unprocessed medical devices
- secures pack
- labels pack

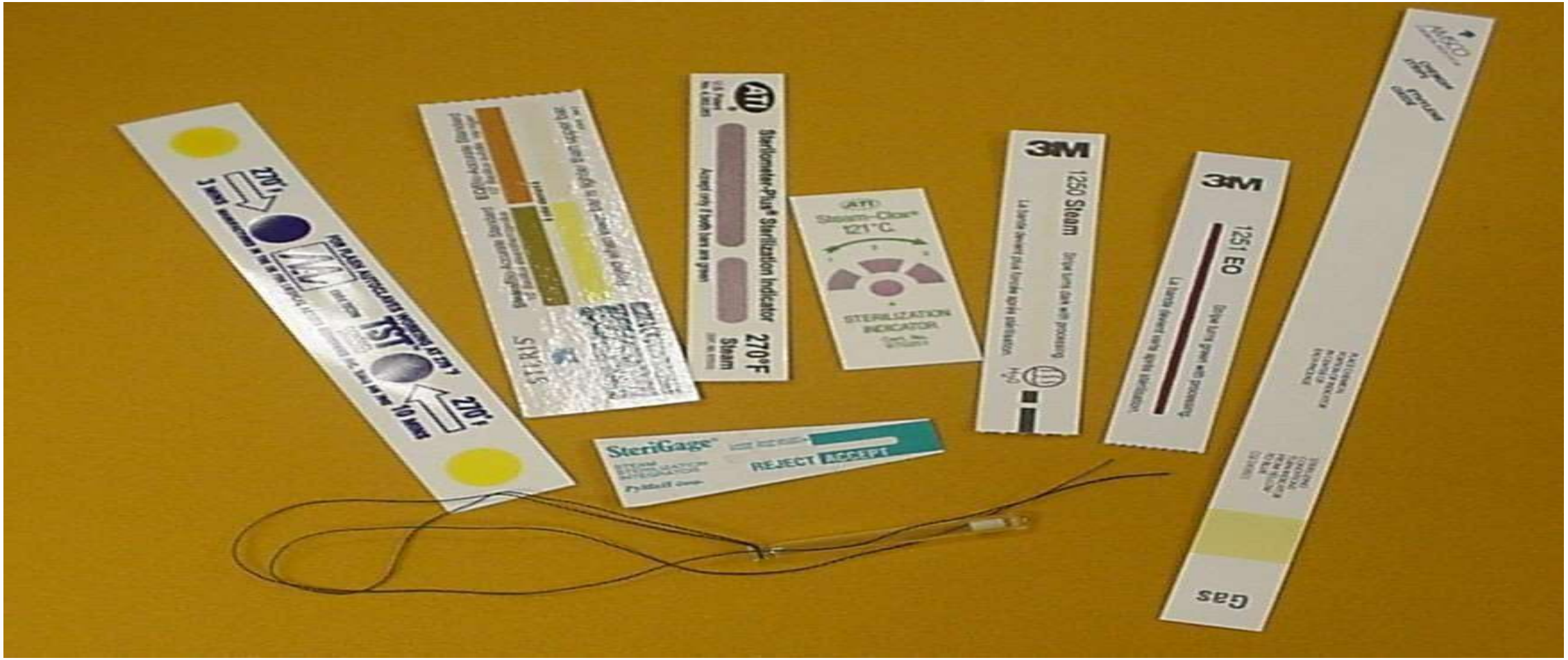
If indicator did not change, do not use

Internal Chemical Indicator

- Inside each package, tray or container
  - Paper strips or cards
  - Validates sterilant penetration
  - Colour change strip or moving front format
- Can measure all process parameters (integrators) CI - advantages
  - Detects incorrect packaging
  - Incorrect loading
  - Malfunction of sterilizer
  - Easy to retrieve and read

Do not tell you that spores are killed. Do not tell you that item is sterile

Chemical Indicators

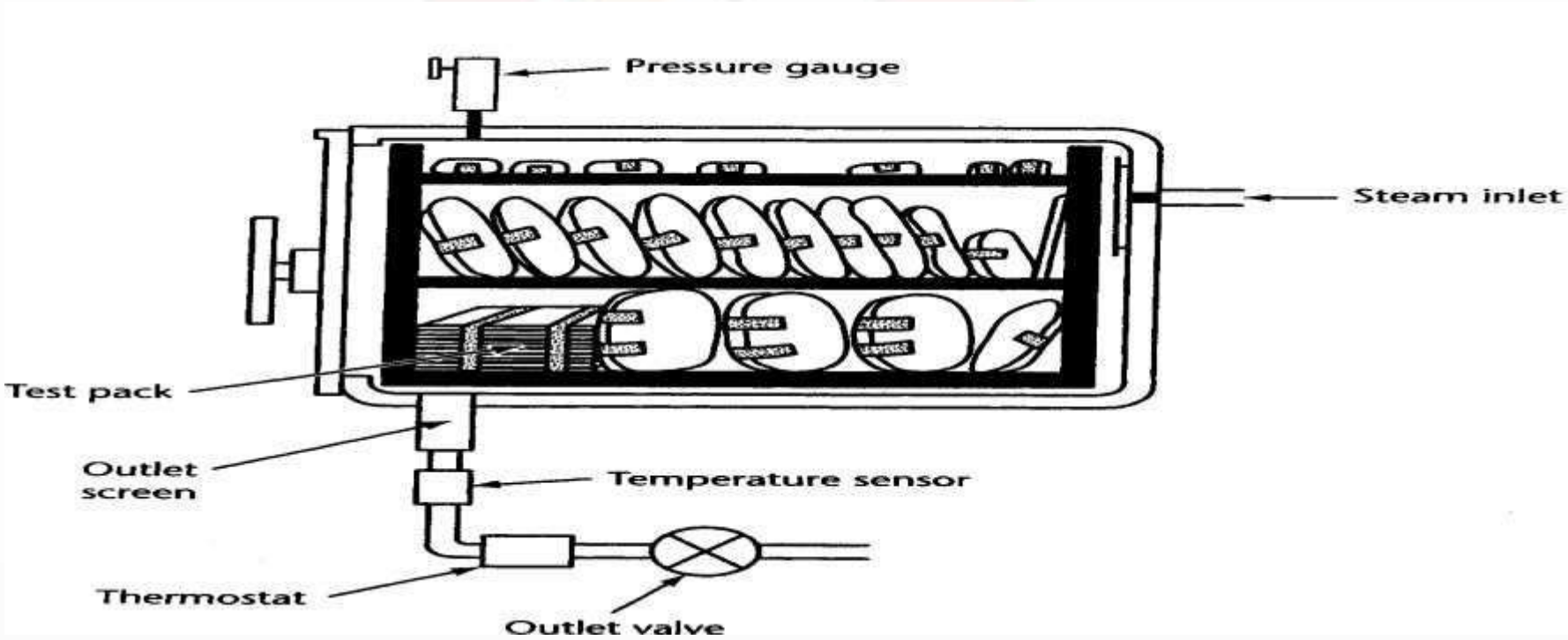


Biological Indicators

- Confirm the ability of the sterilization process to kill microbial spores
- large number of spores
- Integrate all the parameters of the sterilization process
- Most critical test of the sterilization process
- CSA requires routine monitoring daily

Routine Monitoring – Steam Sterilizers

- Test pack – includes BI containing *Bacillus stearothermophilus*
- Performed daily and in every load containing implantable device
- Placement - near drain in fully loaded sterilizer



Routine Monitoring – Ethylene Oxide Sterilizers

- EO Test pack – includes BI containing *Bacillus Subtilis*
- Performed every load
- Placement - centre of normally loaded sterilizer

Biological Indicator TestPacks

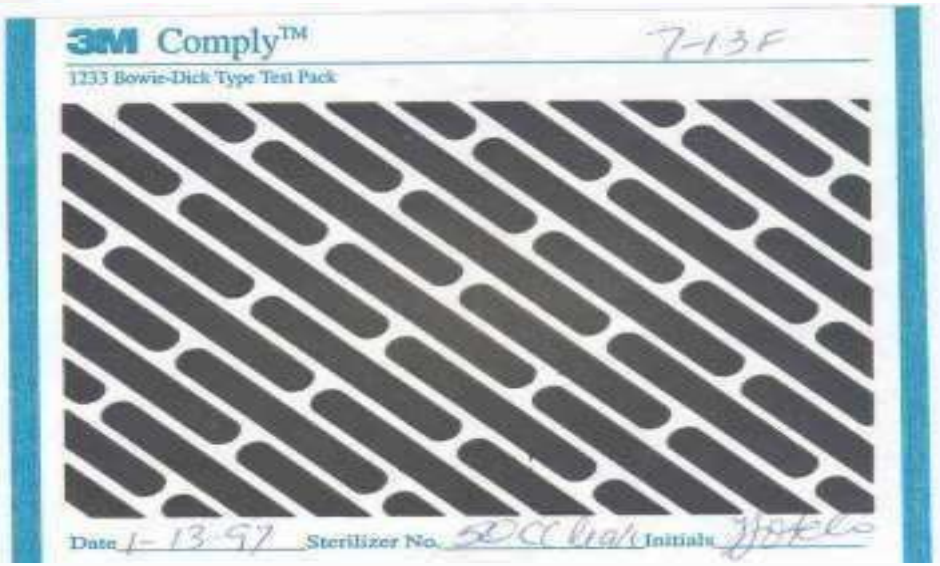
Bowie Dick Type Tests

- Detects entrapped air in Vacuum-assisted sterilizers, not for Gravity
- Measures steam penetration
- Run daily
- Test packs – can be in-house or commercially prepared
- Run a warm-up cycle first
- Place test pack in an empty sterilizer over the drain
- 132C (270F) for 3.5 - 4 minutes
- Uniform colour change
- Retain in records

Bowie Dick Test results

- If colour change not uniform
  - Repeat test
  - Shut down
  - Call repair person
  - Retest
- If uniform colour change
  - Use sterilizer

Unprocessed



Chemical Indicator	Recommends
Bowie Dick Type Test	– Daily
External	– Each package, tray, container
Internal	–Each package, tray, container

Biological Indicator	CSA Recommends
Steam	– Daily; every load with an implantable device
Flash	– Daily; every load with an implantable device
Ethylene Oxide	– Every Load

Installation & Repair Testing

Performed:

- **before sterilizer released for use**
- **after major repairs or relocation**
- **after unexplained sterility failures**
- **after changes in sterilant supply**
- **annually**

3 cycles using BI test pack – yielding 3 negative results

If vacuum – 3 cycles with Bowie-Dick test pack

Sterilization Process Monitors

Record Keeping

- Document all materials that have been processed and the results of the sterilization process monitoring

Product Labeling

- lot or load control number
- processing date
- sterilizer number
- cycle number
- Expiration statement
    - event-related and time-related

Load Records

- date and time of all cycles
- exposure time and temperature
- load contents
- initials of operator
- BI results, CI results
- Records of sterilizer maintenance, calibration, and repair

Product Recall

If microorganism is the spore, do further testing

- Initiate recall and request sterilizer service as needed
- Written recall order and Written report

Recall Procedure

If positive BI:

- review record, quarantine load
- notify maintenance personnel
- identify microorganism on + BI

If contamination occurred, and record is OK, release load

Continuing Education

- Quality patient care
- Review CSA standards
- Know your hospital policies
- Ask questions; Keep learning

Reference CSA Standards

- CAN/CSA-Z11140-1-98 Sterilization of Health Care Products - Requirements for Chemical Indicators - Part 1: General (Adopted ISO 11140-1:1995)
- CAN/CSA-Z314.2-01 Effective Sterilization in Health Care Facilities by the Ethylene Oxide Process
- CAN/CSA-Z314.3-01 Effective Sterilization in Health Care Facilities by the Steam Process

Thank You