STERILISATION &

DISINFECTION

Objective of sterilization

–Removal of microorganisms or destroy them from materials or from areas since they cause contamination, infection and decay.

In microbiology - to prevent contamination

Surgery - to maintain asepsis

Drug & food -for ensuring the safety

Definitions

- ***Sterilization*** – The process by which an article, surface, or medium is freed of all living microorganisms either in the vegetative or spore state
- ***Disinfection*** – The destruction or removal of all pathogenic organisms, or organisms capable of giving rise to infection
- ***Sanitization*** - This term is sometimes used as a synonym for disinfection, particularly used with reference to food processing & catering

Definitions

- ***Antisepsis*** – used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues

SEPS (A Greek word) – PUTRID

- Bactericidal agents
- Bacteriostatic agents
- Cleaning
- Degerming

Spaulding system (1972)

- Critical - penetrate/touch broken skin or mucous membrane
  - must be sterilized
- Semicritical -touch intact mucous membrane
  - sterilize, high level disinfection
- Noncritical - surfaces do not touch mucous membrane
  - disinfection

Heat

- Fast
- Reliable
- Inexpensive (relatively)

THERMAL DEATH TIME

THERMAL DEATH POINT

HEAT

Factors determining the time & temperature for sterilization

- ***Nature of heat*** – dry or moist
- ***Presence of organic matter***
- ***Number*** of microorganisms present
- ***Characteristics*** of the organism
- ***Type of material*** from which the organisms have to be eradicated

PHYSICAL CONTROL WITH HEAT

- ***SUNLIGHT*** – Ultraviolet rays

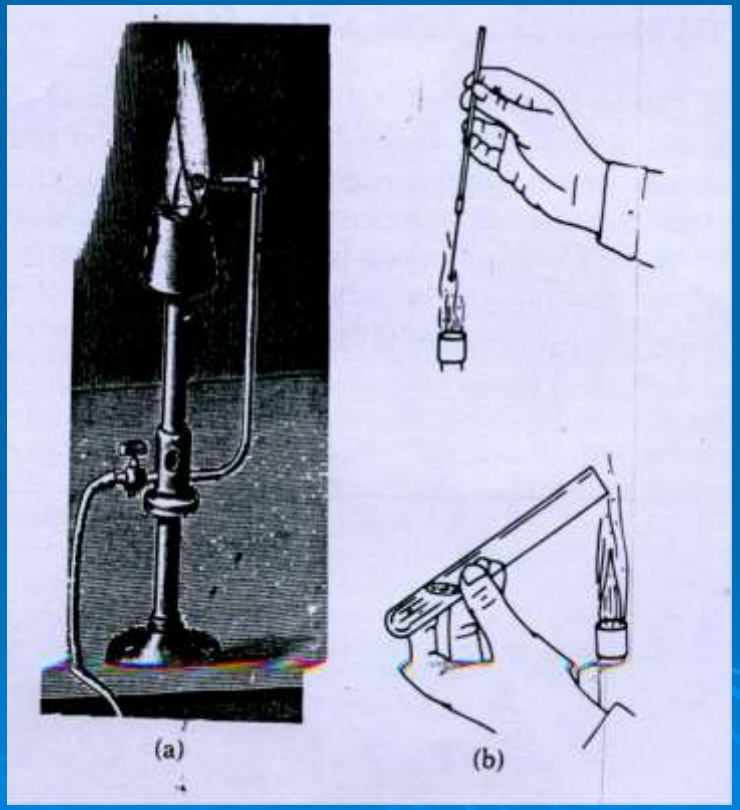
  Typhoid bacilli exposed to sun on pieces of cloth were killed in 2 hours, where the controls kept in dark were alive after 6 days

- ***DRYING*** - 4/5th of the bacterial cell is made-up of water

Dry heat

DIRECT FLAME

Bunsen burner



Incineration

HOT AIR OVEN

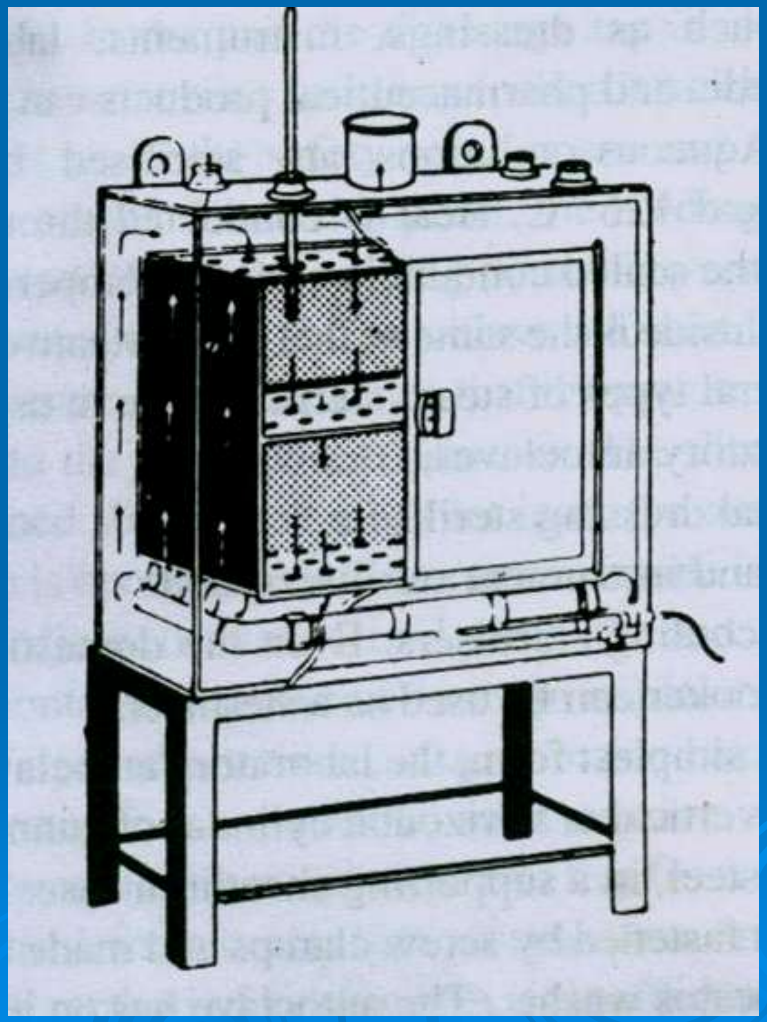
Radiating dry heat

160^0 C (320^0 F) & 2 Hours

useful for sterilizing dry powders, water free oily substances, many types of glass ware such as pipettes, flasks, and syringes.

Advantage – non corrosive method

Hot air oven



Moist heat

- Temperatures below 100^0C/ pasteurization
- Temperatures at 100^0C/ boiling
- Steam at atmospheric pressure

Pasteurization (below 100^0C)

Purpose – To reduce the bacterial population of a liquid such as milk

Spores are not affected by pasteurization

- **Holding method**

  62.9^0C for 30 minutes (Mycobacterium tuberculosis & Coxiella burnetti)

- **Flash pasteurization** – 71.6^0C for 15sec
- **Ultra pasteurization** – 82^0C for 3sec

BOILING WATER (100^0C)

Lower temperatures & less time of exposure are required

Denaturation of proteins

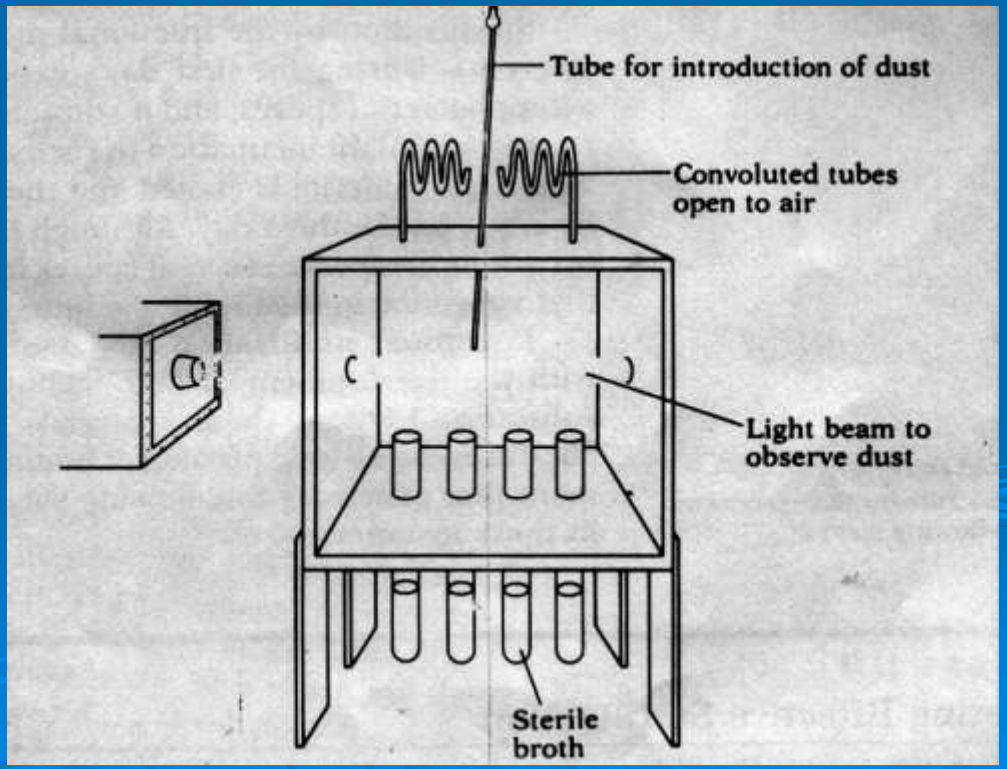
Minimum exposure time – 30 minutes

Less reliable

Washing soda (2% conc.) may be added

Fractional sterilization (steam at atmospheric pressure)

- Tyndallization
  (John Tyndall)
- Intermittent
  sterilization



Free flowing steam at 100^0C for 30 minutes on each of 3 successive days.

First day - steam kills all organisms except spores, and it stimulates spores to germinate vegetative cells

Second day –vegetative cells are killed

Third day – kills the remaining cells

AUTOCLAVE

Moist heat In the form of pressurized steam

increase in the pressure of the gas

increase in the temperature

As the water molecules in steam becomes more energized, their penetration increases

Used for glassware, metal ware, blankets, intravenous solutions and a broad variety of other objects

Pressure in autoclave

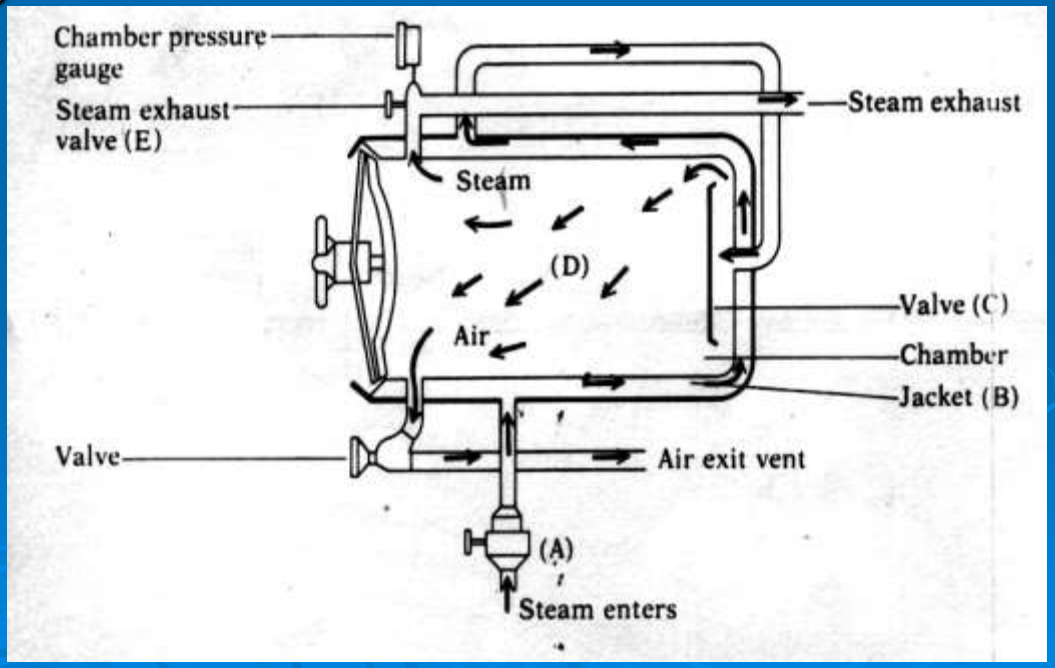
- 15pds/sq. inch

Temperature

– 121.5^0C

Time

– 3 to 30 min



Limitations

- Plastic ware melts in high heat
- sharp instruments become dull
- Oily substances cannot be treated

Prevacuum autoclave

Temperature - 132^0C to 134^0C

Pressure – 28 to 30 lb/$1n^2$

Time – 4minutes

Advantage – minimal exposure time for sterilization

- HOT OIL

160^0C for 1 hour

Advantages – no rusting of instruments

minimal corrosion

- SILICON

Physical control by other methods

FILTRATION (1980s)

filter technology – Charles Chamberland

Julius petri

Filter – a mechanical device used to remove microorganisms from a solution

Ex; IV solutions,bacteriological media, toxoids, pharmaceuticals etc.

Types of filters

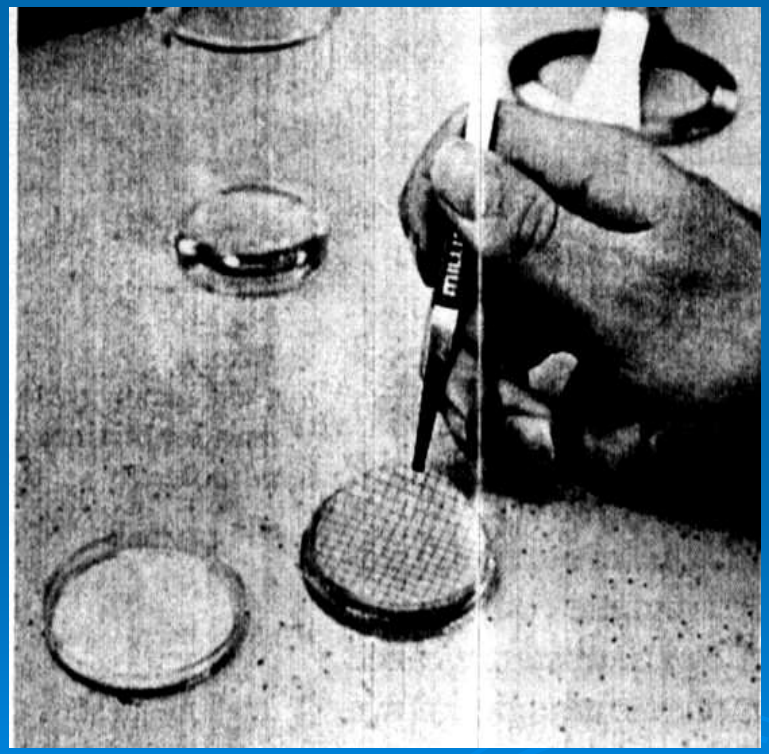
- **Candle filters** 1.Unglazed ceramic filters

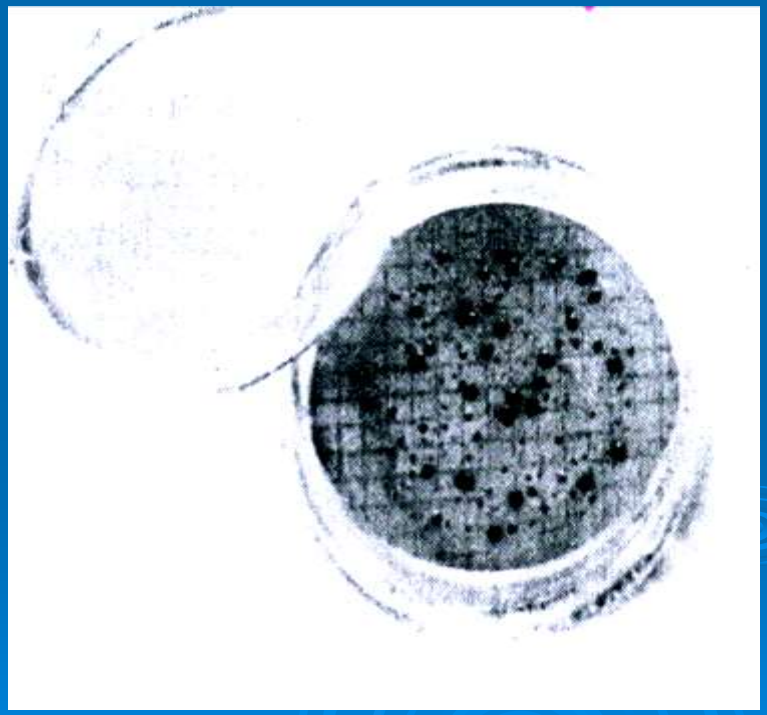
  Ex; Chamberland filter

  2.Diatomaceous earth filters

  Ex; Berkefeld filter
- **Asbestos filters**
- **Sintered glass filters**
- **Membrane filters**

Membrane filter technique



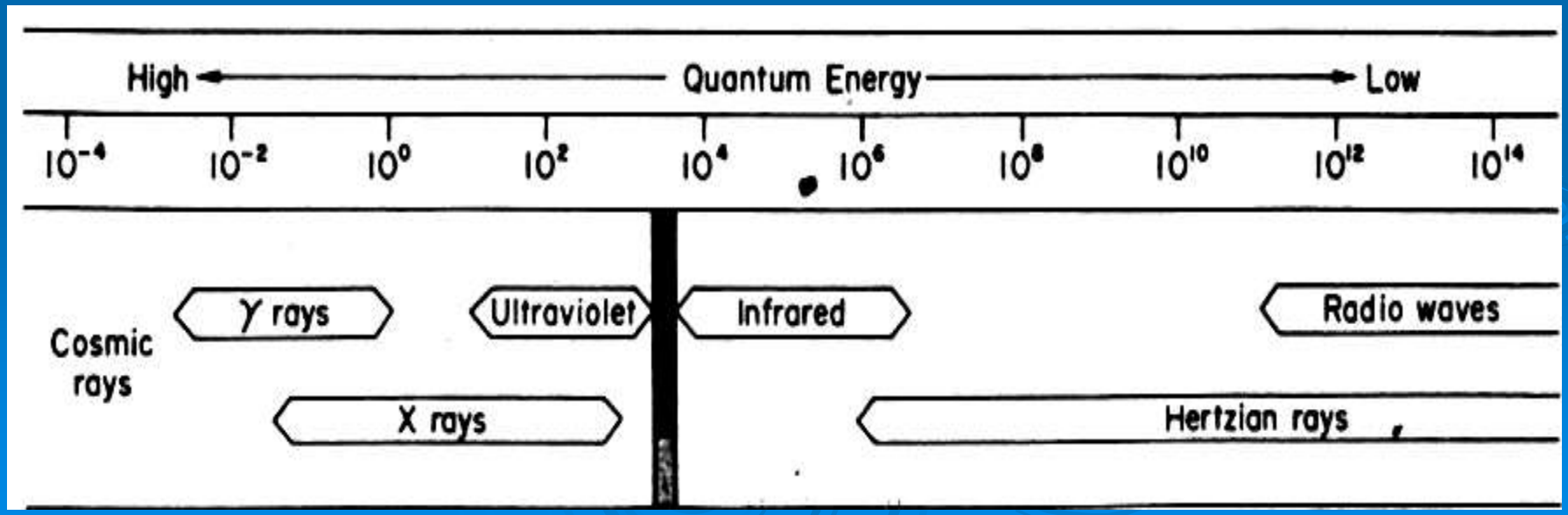


ULTRASONIC LIGHT

Wave length

Visible light is between 400 & 800nm

Ultraviolet light is between 100 & 400 nm



Mechanism of action

When microorganisms are subjected to UV light linking of thymine molecules occurs

Demerits;

- It is not effective against bacterial spores
- Does not penetrate liquids or solids
- It may cause damage to human cells

Other types of radiation

- **Ionizing radiation**

X-rays & gamma rays

Both have wavelengths shorter than the UV light

They force electrons out of their shells, thereby creating ions

- **Microwave**
  - Wave length is longer than that of UV light
  - Molecules are set into a high speed motion

- **Laser beam**

  Light ***A***mplification by ***S***timulated ***E***mission of ***R***adiation

ULTRASONIC VIBRATIONS

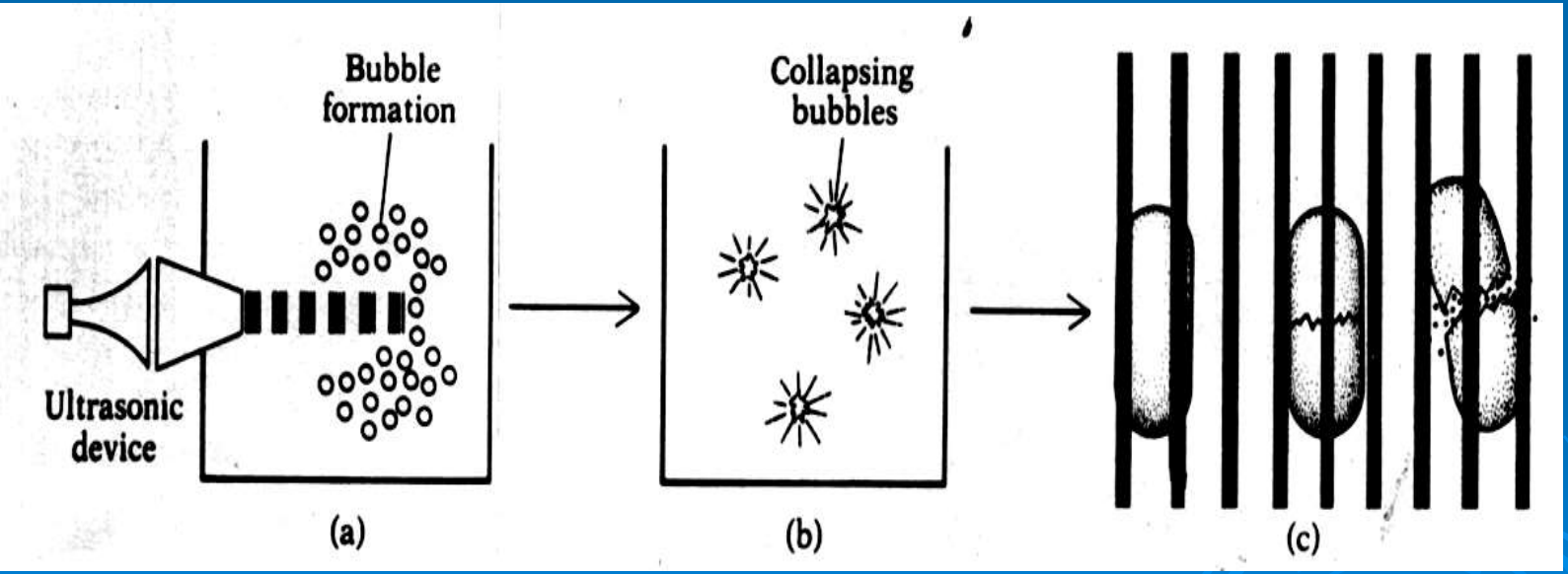
High frequency sound waves beyond the range of the human ear

‘Cold boiling’

They cause the formation of bubbles or cavities and the water appears to boil - ***cavitation***

Demerits

- Not very effective
- Liquid is required


Bubble formation
Collapsing bubbles
Ultrasonic device
(a)
(b)
(c)

Preservation methods (bacteriostatic)

Retard spoilage & prolong the ***shelflife*** of foods

- Drying
- Salting - osmosis
- Freezing – lowering temperature

Physical agents of infection control

Heat - Sunlight

- Drying
- Dry heat (flaming, incineration & hot air)
- Moist heat (pasteurization, boiling, steam under normal pressure, steam under pressure)

Methods other than heat

- Filtration
- Radiation
- Ultrasonic & sonic vibrations

Preservation methods

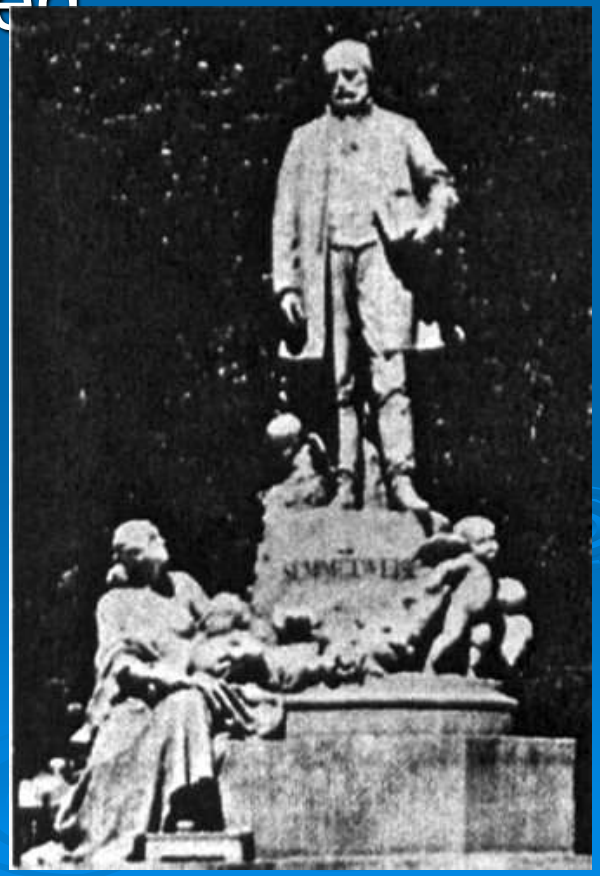
- Drying
- Salting
- Freezing

Chemical control of microorganisms

Chemical control of microorganisms

Puerperal fever (childbed fever)

– a blood disease accompanied by high fever and often transmitted during child birth



- Ignaz semmelweis
  - “savior of mothers”

General principles of chemical control

Egyptians – resins & aromatics

Ancient people burned sulfur for deodorizing and sanitary purposes

Spices –preservatives as well as masks for foul odors

Medicinal chemicals - 1800s

U.S.Pharmacopia (1830) – tincture of iodine

Copper sulphate – fungal infections

Mercury – syphilis

Joseph Lister (1860s) – principles of aseptic surgery(phenol)

Selection of antiseptics & disinfectants

Prerequisites

- It should have a wide spectrum of activity
- Fast acting
- Active in the presence of organic matter
- Nontoxic to animals or humans (antiseptic)
- Soluble in water
- It should not separate on standing
- Should have high penetrating power
- Surface compatibility
- Relatively inexpensive

Factors

- Concentration of the substance
- Time
- pH of the medium
- Temperature
- Nature of microorganism
- Surface to be treated
- Presence of extraneous material

Evaluation of antiseptics & disinfectants

Phenol Coefficient (PC)

- A measure of the effectiveness of an antiseptic or disinfectant as compared to phenol

sta. Aureus, sal. typhi

Drawback – it does not consider factors like tissue toxicity, presence of organic matter

- In-use test

Bacterial species **Resistance**

Bacterial endospores

Mycobacterium tuberculosis

Small nonlipid viruses

Fungi

Medium sized lipid viruses

Vegetative bacteria

3 Levels of disinfection

1. **High** - sterilizing agents
   ex; ethylene oxide gas
2. **Intermediate** - bactericidal agents
   ex; formaldehyde, alcohols
3. **Low** - narrowest anti-microbial activity
   ex; soaps, detergents

Mechanisms of anti-microbial action

- Agents that interfere with membrane function
- Agents that denatures proteins
- Agents that destroy or modify the functional groups of proteins

1. **Agents that interfere with membrane function**

Structural derangement or disorganisation of cell wall proteins and lipids

- Surface active agents
- Phenols
- Alcohols

Surface active agents

"Substances which alter energy relationship at interfaces producing a reduction of surface or interfacial tension"

- Anionic
- Cationic
- Nonionic
- Amphoteric

- **Cationic detergents** – quaternary ammonium compounds

Ex; Acetyl trymethyl ammonium bromide & Benzalkonium chloride

+vely charged hydrophylic portion reacts with membrane phospholipids

Disadvantages ; Inability to penetrate organic debris

Incompatibility with anionic agents

- **Anionic detergents** – Soaps &fatty acids
  Gross disruption of lipoprotein framework
- **Nonionic detergents** – Tween 80
  relatively non toxic
- **Amphoteric compounds** – ‘TEGO’ compounds

Soap

– A chemical compound of fatty acids combined with potassium or sodium hydroxide

- pH - 8.0
- Mechanical removal of organisms
- Wetting agents
- Reduce surface tension

PHENOL (Carbolic acid)

- Active against gram-positive bacteria
- Coagulating proteins esp. cell membrane
- Used in PC test

Disadv;

- Expensive
- Pungent odour
- Caustic to the skin

PHENOL DERIVATIVES

- ***CRESOLS*** - Greater germicidal activity & lower toxicity
- ***BISPHENOLS*** - 2 phenol molecules

  ex; Hexachlorophene, Chlorhexidine

  FDA (1976) approved as a surgical scrub, hand wash, superficial skin wound cleanser

  Hexylresorcinol – mouthwash, topical antiseptic & in throat lozenges

ALCOHOLS

Effective skin antiseptics

- **Ethyl alcohol** - Denatures proteins and dissolves lipids
  - Dehydrating agent

Readily reacts with organic matter

50-80% solution

- **Isopropyl alcohol**
- **Methylalcohol**

Agents that denatures proteins

Denaturation of polypeptide chain

Unfolding of polypeptide chain

Ex; Acids
Alkalies
Alcohols
Acetone
Organic solvents

ACIDS & ALKALIES

Free H^+ and OH^- ions

All organic acids – food preservatives

Ex; benzoic acid, propionic acid

Acids are valuable adjuncts to disinfection

Agents that destroy or modify the functional groups of proteins

- Mercuric compounds – sulphydryl groups
- Anionic detergents - amino & imidazole groups

Ex; heavy metals
halogens
hydrogen peroxide

Heavy metals

‘An electron donating element whose atoms are large, with complex electron arrangements’

‘Oligo-dynamic action’

Heavy metals are very reactive with proteins

Mercury ($Hgcl_2$)

- Skin diseases
- Toxic to the host
- antimicrobial activity is reduced in the presence of organic matter

Copper

- chlorophyll containing organisms
- $CuSO_4$ is a potent inhibitor of algae
- ***BORDEAUX*** mixture

Silver -

- ***$AgNO_3$*** - antiseptic & disinfectant
  - 1% AgNo3 solution is active against ***Neisseria Gonorrhoeae*** infection
  - to treat suturing threads
- ***Colloidal preparation***

- Not sporicidal

OXYDISING AGENTS

Halogens –

'A group of highly reactive elements whose atoms have 7 electrons in the outer shell'

- Chlorine – gasseous form, organic & inorganic compounds

chlorine is available in 3 other forms

1. Hypochlorites
2. Organic chloramines
3. Inoganic chloramines

Chlorine compounds

1. $Ca(Ocl)_2$ - Chlorinated lime

2. NaOCl - DAKIN's solution used to treat 'ATHLETE's foot

3. Clorax & Purex bleach

4. Chloramines – Chloramine-T

Iodine

More reactive than chlorine

Halogenating tyrosine portions of protein molecules

Tincture of iodine –2% iodine solution in ethyl alcohol

- ***Iodophors***

‘Iodine detergent complexes that release iodine over a long period of time’

Advantage – no staining of tissues or fabrics

Ex; wescodyne - preoperative skin preparation

Betadine - presurgical scrubbing

Ioprep - local wound antiseptic

Hydrogen peroxide (H_2O_2)

- A simple chemical compound digested by catalase to water and oxygen
- Mechanical removal of microorganisms
- New forms – super D H_2O_2
- Heat sensitive plastics

DYES

- **Tryphenylmethane dyes/Aniline dyes**

EX; Brilliant green, Malachite green, Crystal violet & Gention violet

Interference with cell wall synthesis

Gram +ve organisms

- **Acrydine dyes – Flavines**

Ex; Acriflavine, Proflavine

Combines with DNA, thereby halting RNA synthesis

Both gram +ve and –ve organisms

ALKYLATING AGENTS

- Formaldehyde
- Ethylene oxide
- Gluteraldehyde

Formaldehyde

- Gas at high temperatures & a solid at room temperatures
- 37% solution – Formalin
- In gaseous form - Sterilize surgical equipment & medical instruments
- 20% solution in 70% alcohol for 18hrs – to sterilize instruments
- Contact dermatitis

Ethylene oxide

- Toxic & Highly explosive
- Freon gas in cryoxide / CO_2 gas in carboxide
- Cold burns
- Paper, leather, wood, metal, rubber & plastics
- Gas autoclaves & chemiclave
- Beta propiolactone(BPL) – Vaccines, sera, & surgical ligatures

Gluteraldehyde

- 2 to 3.4% is effective
- Activity will not reduce in the presence of organic matter
- It does not damage delicate objects
- Irritating fumes
- Discoloration & corrosion of instruments

- Agents interfere with membrane function
  - surface active agents
  - phenols
  - alcohols
- Agents denatures proteins - acids & alkalies
- Agents destroy or modify the functional groups of proteins
  - heavy metals
  - oxidizing agents (halogens, H_2O_2)
  - dyes
  - alkylating agents (formaldehyde, ethylene oxide, gluteraldehyde)

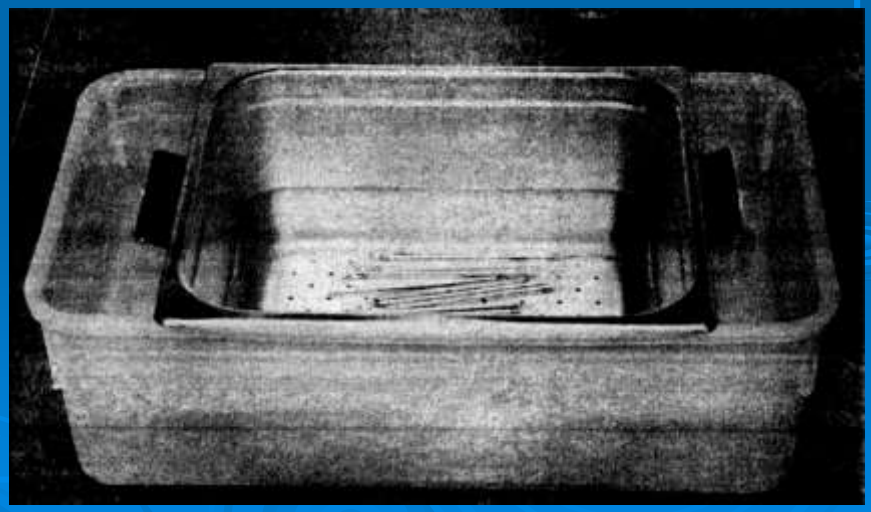
INSTRUMENT PROCESSING

The overall process consists of

- Holding (presoaking)
- Precleaning
- Corrosion control, drying, lubrication
- Packaging
- Sterilization
- Sterilization monitoring
- Handling processed instruments

HOLDING (PRESOAKING)

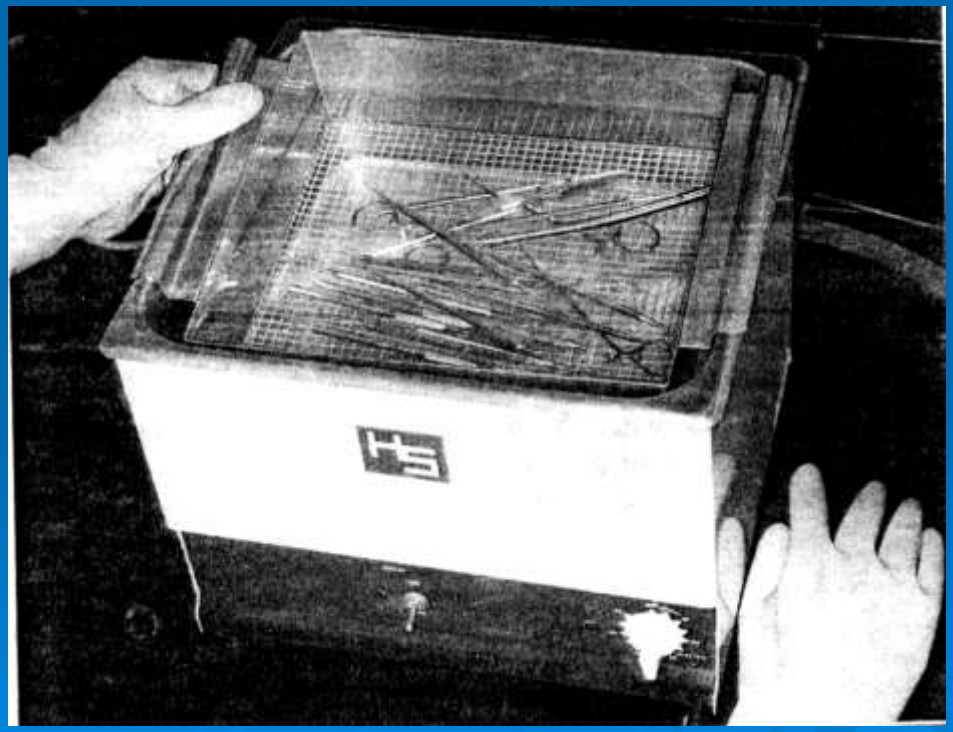
- **Holding solution–** detergent/water/enzyme solution
- To prevent drying
- Perforated basket
- Extended soaking



PRECLEANING

- **Ultrasonic cleaning** - reduces direct handling
  - time saving
- **Manual scrubbing** - dangerous
  - long handled brush
- **Instrument washers**

Ultrasonic cleaning of instruments



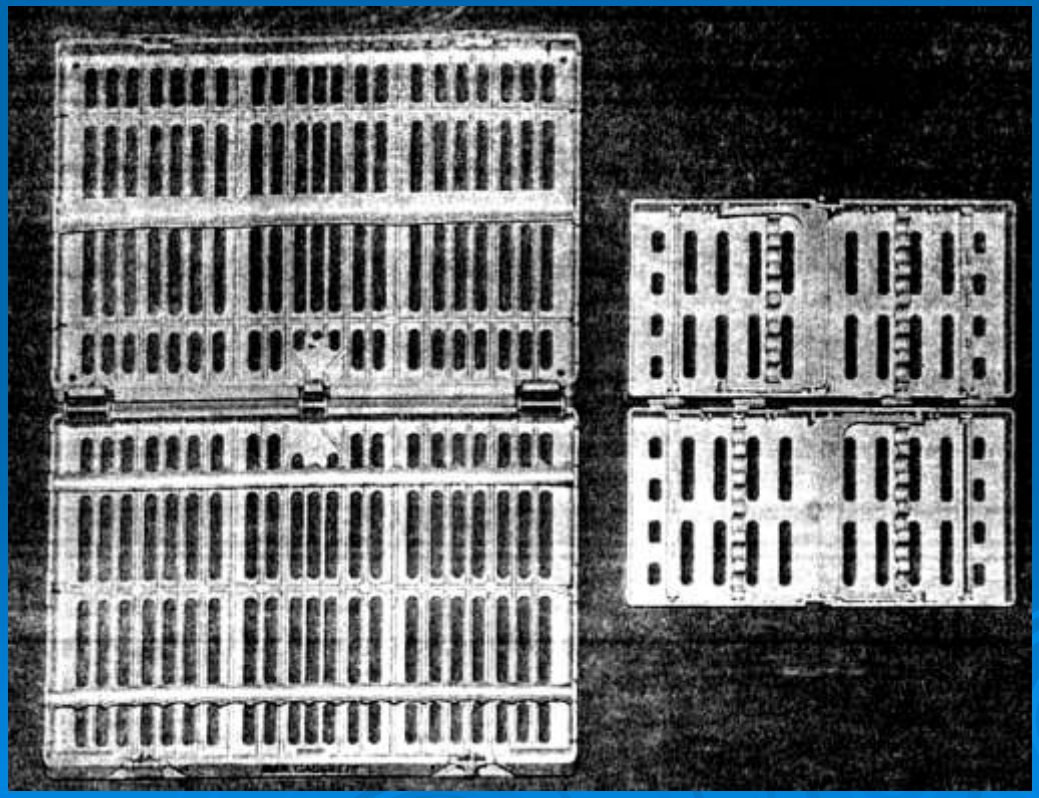
Corrosion control, drying, lubrication

- Steam sterilization causes corrosion
- Rust inhibitors – **silver nitrite**
- Drying remove excess water
- Hinged instruments – lubrication

Packaging

- Prevents contamination after sterilization, during storage or when being distributed to chair side
- Pouches, bags, cassettes (stainless steel, aluminum, and plastic/resin)
- Closed containers

Resin cassettes



SRERILIZATION

Universal sterilization

"if it can be sterilized, sterilize it"

Sterility assurance

- the correct performance of the proper instrument processing steps and monitoring the sterilization with biologic & chemical indicators

Types of sterilization

1. Heat sterilization – moist heat
   - dry heat
   - unsaturated chemical vapor
2. Liquid chemical sterilization
3. Gas sterilization

Steam sterilization

Heating water to generate steam in a closed chamber producing a moist heat that rapidly kills microorganisms

4 cycles – 1. Heat-up cycle

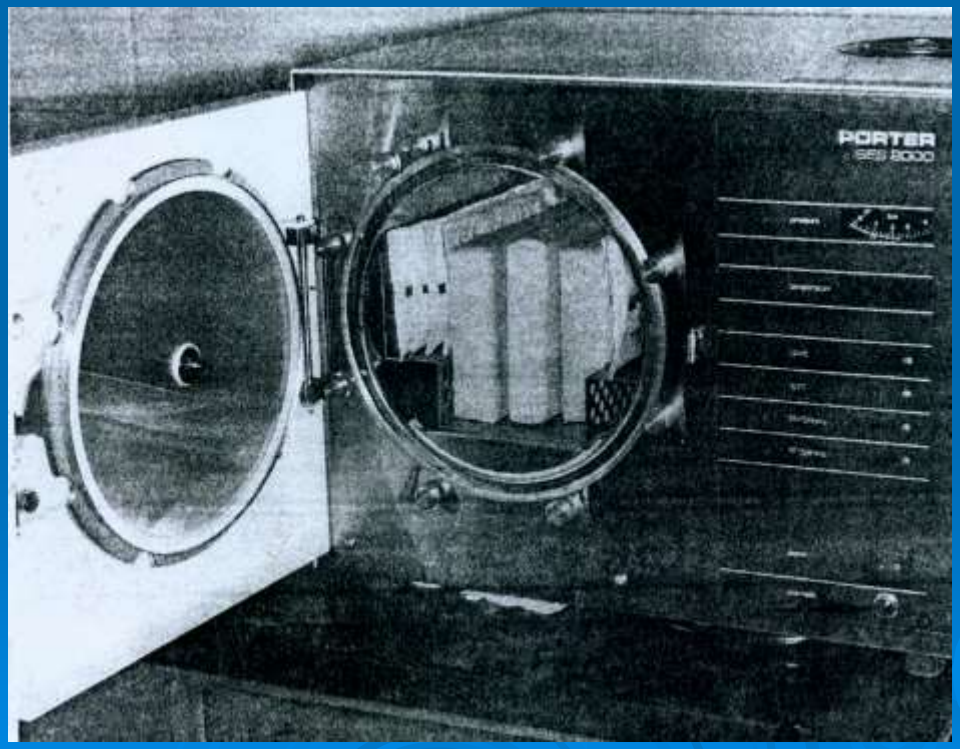
2. Sterilizing cycle

3. Depressurization cycle

4. Drying cycle

Flash sterilization cycle

Small office sterilizer



Unsaturated chemical vapor sterilization (chemiclave)

Heating a special chemical solution

Solution – 0.23% formaldehyde & 72.38% ethanol plus acetone, ketone, water & other alcohols

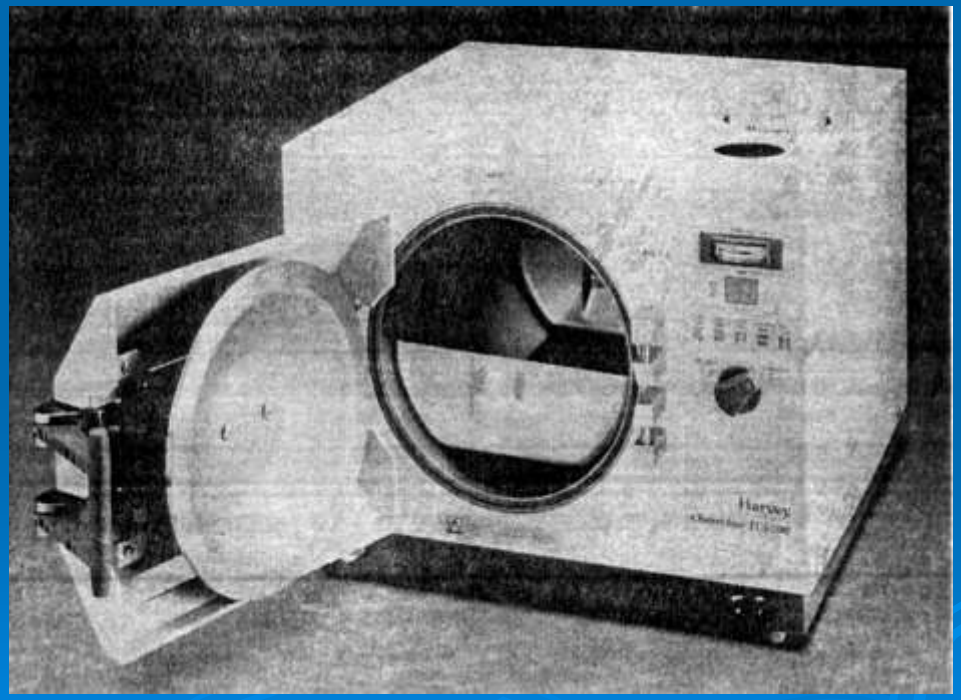
4 cycles 1. Heat-up/vaporization cycle

2. Sterilization cycle

3. Depressurization cycle

4. Optional purge cycle

CHEMICLAVE



Temperature - 270^0 F(132^0 C)

Pressure - 25 psi (172 Kpa.)

Time - 20 min

Positive feature – corrosion is reduced or completely eliminated

Negative feature – irritating fumes

Dry heat sterilization

Heating air with transfer of heat energy from air to the instruments

Requires high temperatures

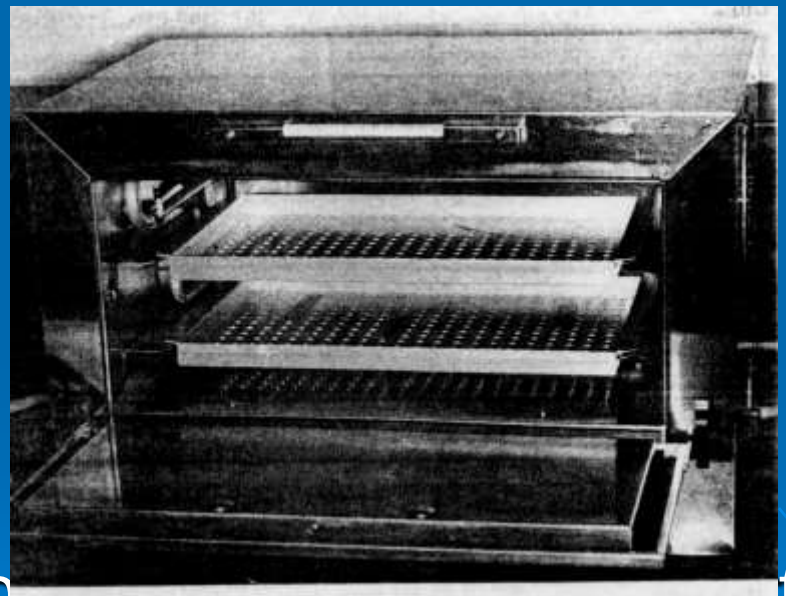
Temperature – 320^0F to 375^0F (160^0C to 190^0C)

Adv; No corrosion

No irritating fumes

Static air type dry heat sterilizer

Heat energy from static air is transferred to the instrument

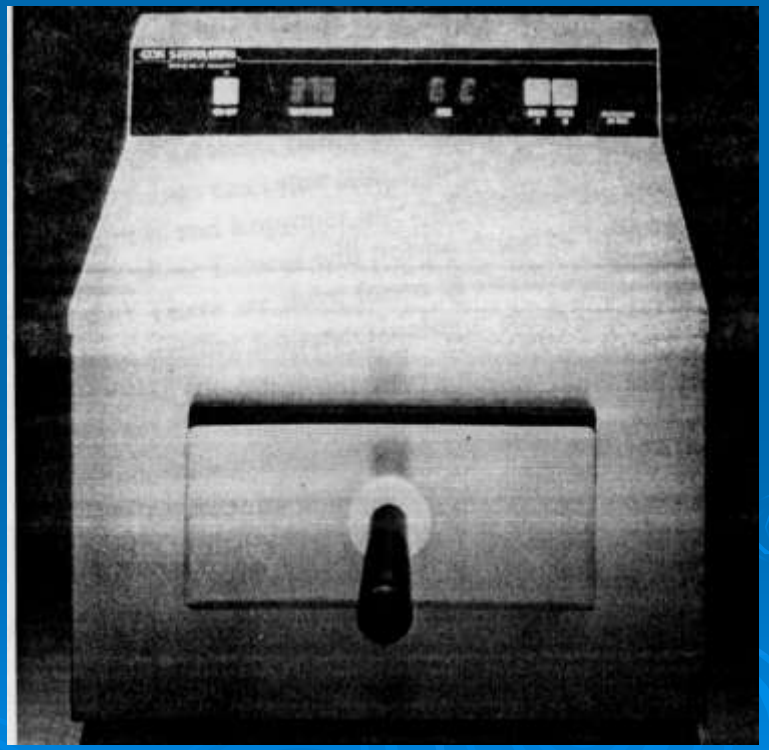


Heat-up cycle begins 15 to 30min from a cold start

Forced air type dry heat sterilizer

It circulates the heated air through out the chamber at a high velocity

Packaged items -12min
Unpackaged items - 6min



GAS STERILIZATION

Ethylene oxide

Adv; low temperatures (below room temp.)

Disadv; time consuming
explosive if mixed with air
toxicity

LIQUID CHEMICAL STERILIZATION

2 to 3.4% gluteraldehyde

RECENT ADVANCES

- **Low temperature sterilization involves vaporized H_2O_2**
- **Bead sterilizers**

Size of glass beads – 1.2 to 1.5mm

Temperature - 424^0 to 450^0F

Time - 3 to 5sec

Disadv ; uneven temperatures

- **Hot oil sterilization** - mineral oil

Sterilization monitoring

Sterilization failure**s** – improper cleaning, packaging, use of sterilizer

Helps to achieve high level of sterility

- Biologic
- Chemical
- Physical

- **Biologic monitoring**
  - Bac. Stearothermophilus (steam/chemical vapor)
  - Bac. Subtilis (dry heat/ethylene oxide gas)

- **Chemical monitoring**
  - rapid change indicator ex; autoclave tape, special markings on the bags
  - slow change or integrated indicator

- **Physical monitoring** –temperature, pressure, exposure time

Handling processed instruments

- Drying
- Cooling
- Storage

Shelf life – the period of time during which sterility is assumed to be maintained

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