Sterilization & disinfection
What is sterilization?

- **Def:**
  - process by which an article, surface or medium is freed of all living microorganisms either in **vegetative** or **spore** state.
What is disinfection?

• Def: The destruction or removal of all pathogenic organisms capable of giving rise to infection.
antisepsis

• The term is used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues.

• This is done by the antiseptics – Chemicals or disinfectants which can be safely applied on skin or mucous membrane to prevent infection by inhibiting the growth of bacteria.
Bactericidal agents

- Bactericidal agents / germicides
  - those who able to kill bacteria.

- Bacteriostatic agents
  - Only prevent multiplication of bacteria, but they remain alive.
What is cleaning?

• **Def:**
  - Important preparatory step before sterilisation or disinfection, by removing soil and other dirt.

• **DECONTAMINATION**
  - The process of rendering an article or area free of contaminants, including microbial, chemical, radioactive and other hazards.
Agents for sterilisation

• Physical agents
  – Sunlight
  – Drying
  – Dry heat: flaming, incineration, hot air
  – Moist heat: pasteurization, boiling, steam under pressure
  – Filtration: candles, asbestos pads, membranes
  – Radiation
  – Ultrasonic and sonic vibrations
Agents for sterilisation

- **Chemicals**
  - Alcohol
  - Ethyl, isopropyl, trichlorobutanol
- Aldehydes
  - Formaldehyde, glutaraldehyde
- Dyes
- Halogens
- Phenols
- Surface active agents
- Metallic salts
- Gases:
  - Ethylene oxide, formaldehyde (g), beta propiolactone
PA: sunlight

- Action primarily due to UV rays
- However, effects vary due to places
  - Eg: in tropical country, the germicidal effect is better than in 4 seasoned countries.
- Bacteria in water are readily destroyed by sunlight.
PA : drying

- Moisture is essential for growth of bacteria.
- Drying in air has deleterious effect on many bacteria.
- However, spores are unaffected. Therefore, it is not really reliable.
PA: heat

• Most reliable method of sterilization and should be the method of choice.
• The factors influencing sterilization by heat:
  – Nature of heat-dry or moist
  – Temperature and time
  – Number of microorganisms present
  – Characteristics of organisms—species, strain, sporing capacity
  – Type of material from which organism have to be eliminated.
Killing effect of dry heat

• Killing effect is due to protein denaturation, oxidative damage and toxic effect of elevated level of electrolytes.
Killing effect of moist heat

• Due to denaturation and coagulation of proteins.
Thermal death time

• Def: Minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

• Thermal death time is inversely proportional to temperature.

• TDT is increased in presence of organic substance, proteins, nucleic acid, starch, gelatin, sugar, fats, oils.
Dry heat

• Flaming:
  – Items: inoculating loop/wire, tip of forceps, searing spatulas
  – Using: bunsen burner
  – *inoculating loop is better dipped in disinfectant first before flaming to prevent spattering.
• Incineration
  – Items: contaminated cloth, animal carcasses and pathological material.
  – PVC, polythene can be dealt.
  – However, polystyrene will emit black smoke. Hence should be autoclaved in appropriate container.
• Hot air oven
  – Holding period: 160°C, 1 hour
  – Items: glassware, forceps, scissors, scalpels, all-glass syringes, swabs, liquid paraffin, dusting powder, fats, grease.
  – Materials should be properly arranged to allow free circulation of air.
Hot air oven
• Sterilization control
  – Usage of the spores of *Clostridium tetani*. The nontoxic bacteria will be cultured to see whether it is growing or not.
  – To make sure the sterilization is properly done.
Moist heat can be categorized into 3 groups:
- Temperature below 100°C
- Temperature at 100°C
- Temperature above 100°C
MH: temperature $< 100^\circ$C

- Pasteurisation of milk
  - Holding period: 63°C, 30 minutes (older method); or 72°C, 15-20 minutes.
  - Target: all nonsporing pathogens
    - Eg: mycobacteria, brucellae, salmonella.
    - Coxiella burnetti, relatively heat resistant, may survive the holding (older) method.
• Inpissator
  – Media like LJ and Loeffler’s serum
  – Holding period: 80-85°C, half an hour.
inpissator
Mh: temperature @100°C

- Boiling
  - Not recommended for sterilising but ok for disinfection.
  - Sterilisation may be promoted by addition of 2% sodium bicarbonate to the water.
  - Holding period: 10-30 minutes.
  - The lid of sterilizer should not be opened during the period.
• **Steam @ atmospheric pressure (100°C)**
  – Used to sterilize culture media.
  – Koch or Arnold steamer is usually used.
  – Inexpensive method
  – Holding period: 100°C, 20 minutes on three successive days (tyndallisation/intermittent sterilization)
  – Principle: first exposure kills vegetative bacteria and then the next exposure will kill vegetative bacteria that matures from the spore.
• Steam under pressure
  – Autoclave/steam sterilizer
  – Principle: water boils when its vapour pressure equals the surrounding atmosphere.
  – Thus, when pressure inside closed vessels increases, the temperature at which water boils increases too.
  – Holding period: varies.
  – Temperature: between 108°C and 147°C.
  – Items: dressings, instruments, laboratory ware, media and pharmaceutical products.
autoclave
Several types of steam sterilizer:
- Laboratory autoclaves
- Hospital dressings sterilizers
- Bowl and instrument sterilizers
- Rapid cooling sterilizers

Sterilization control—
- Spores of *Bacillus stearothermophilus* is used.
Steam sterilizer
filtration

• Helps to remove bacteria from heat labile liquids

• Items: sera and solutions of sugars or antibiotics.

• Principle: as viruses pass through the ordinary filters, filtration can be used to obtain bacteria-free filtrates of clinical samples for virus isolation.
• Types of filters
  – Candle filters
  – Asbestos filters
  – Sintered glass filters
  – Membrane filters
• Candle filter
  – Types-
    • Unglazed ceramic filters
    • Diatomaceous earth filters
• Asbestos filters
  – Disposable, single-used disc
  – Usage is discouraged because of its carcinogenic property.
  – Eg: Seitz and Sterimat filters
• **Sintered glass filters**
  - has low absorptive properties
  - Brittle and expensive

• **Membrane filters**
  - Made of cellulose esters or other polymers
  - Usually used for water purification and analysis, sterilization and sterility testing and preparation of solutions for parenteral use.
radiation

• 2 types of radiation
  — Ionising → gamma rays, high energy electrons
  — Non-ionising → infrared, UV

• Non-ionising radiation
  — Infrared
    • Used for rapid mass sterilization of prepacked items;
    • Syringe,
    • Cathaters
  — UV
    • Disinfect enclosed area such as entryways, operation theatres and labs.
• Ionising radiation
  – Gamma rays
• Items: plastics, syringes, swabs, catheters, animal feeds, cardboard, oils, greases, fabric and metal foils.
Chemical agents

• Ideal antiseptic/disinfectant should
  – Effective against all microorganisms
  – Be active in presence of organic matter
  – Effective in acid as well in alkaline media
  – Have speedy action
  – Have high penetrating power
  – Stable
  – Compatible with other antiseptics and disinfectant
  – Not corrode metals
  – Not cause local irritation or sensitisation
  – Not interfere with healing
  – Not be toxic if absorbed into circulation
  – Not Inexpensive and easily available.
Chemical agents

• Factors determine the potency of disinfectants:
  – Concentration of the substance
  – Time of action
  – pH of the medium
  – Temperature
  – Nature of the organisms
  – Presence of extraneous material
Chemical agents

- Action of chemical action
  - Protein coagulation
  - Disruption of cell membrane resulting in exposure, damage/loss of contents
  - Removal of sulfhydryl group essential for normal functioning of enzyme
  - Substrate competition
CA: alcohol

- Frequently used:
  - Ethyl alcohol
  - Isopropyl alcohol

- Must be used at concentration 60-90%

- Isopropyl alcohol used in disinfection of clinical thermometer.

- Methyl alcohol is effective against fungal spores, treating cabinets and incubators.

- Methyl alcohol is also toxic and inflammable.
CA: aldehyde

- **Formaldehyde:**
  - Bactericidal, sporicidal and has lethal effect on viruses.
  - Used to preserve anatomical specimens, destroying anthrax spores on hair and wool.
- **Glutaraldehyde:**
  - Effective against *tubercle bacilli*, fungi, viruses.
  - Less toxic and irritant to eyes, skin
  - Used to treat corrugated rubber anaesthetic rubber, face masks, plastic endotracheal tubes, metal instruments and polythene tubing.
CA: dyes

• 2 groups of dyes:
  – Aniline dye
  – Acridine dye

• Both are bacteriostatic in high dilution but are of low bactericidal activity.

• Aniline dye is more active against gram +ve than gram-ve organisms.

• Used in microbiology labs as selective agents in culture media.
• Acridine dye
  – Not selective as aniline dye.
  – Important dyes:
    • Proflavine
    • Acriflavine
    • Euflavine
    • Aminacrine
  – Impair the DNA complexes of the organisms and thus kill or destroy the reproductive capacity of the cell.
CA: halogens

- **Iodine**
  - Skin disinfectant
  - Active bactericidal, moderate action on spores.

- **Chlorine**
  - Water supplies, swimming pools and food and dairy industries.
  - Along with hypochlorides are bactericidal. Also act on viruses.
CA: phenols

- Obtained from distillation of coal tar between 170-270°C.

- Lethal effect:
  - Capacity to cause cell membrane damage, releasing cell contents and causing lysis.

- Low concentration will precipitate proteins.
CA: gases

- Types of gases
  - Ethylene oxide
  - Formaldehyde gas
  - Beta propiolactone (BPL)

- Ethylene oxide
  - Action is due to its alkylating the amino, carboxyl, hydroxyl and sulphydryl groups in protein molecules. Also on DNA and RNA.
  - Items: heart-lung machines, respirators, sutures, dental equipment, books, clothing.
• **Formaldehyde gas**
  - Employed for fumigation of OT and other rooms.
  - After fumigation, the doors should be sealed and left unopened for 48 hours.

• **BPL**
  - Product of ketane and formaldehyde with a boiling point of 163°C.
  - Rapid biocidal activity but carcinogenic.
  - Capable of killing all microorganisms and is very active against viruses.
CA: surface-active agents

• Def \(\Rightarrow\) substance that alter the energy relationship at interfaces, producing a reduction of surface or interfacial tension.

• Widely used as wetting agents, detergents and emulsifiers.

• 4 main groups:
  - anionic
  - Cationic
  - Nonionic
  - amphoteric
Ca: metallic salts

- Salts of heavy metals have a greater action.
  - Eg: salts of silver, copper and mercury

- Protein coagulant and have capacity to combine with free sulfhydryl group of cell enzymes.
For more information, please mail us:

support@tqsglobal.org

You may also reach us on our 24X7 Toll Free Food Safety Helpline

1800-102-2440

www.tqsglobal.org