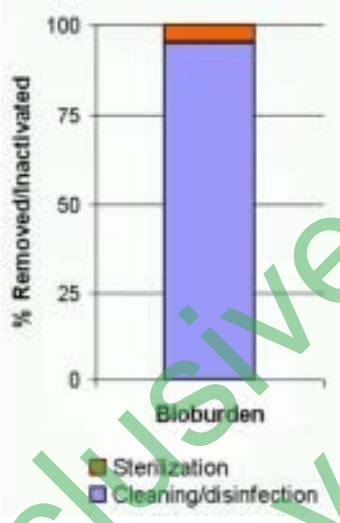
Sterilisation and Disinfection for EHOs

EHL1053

History

- established in the nineteenth century,
- These measures were designed to reduce the microbial load in an environment
- reduce the risk acquire an infection.

Cleaning



- Grime removal
- Absence of viable microbiota is not sufficient
- Endotoxins

- Failure
  - Product failure
  - Consumer effects (industry)
  - Patient morbidity/mortality (healthcare)

Justification for the labour incurred

- By cleaning, the vast majority of the bioburden is removed.
- Thus, **Cleaning is considered the most essential step in the sterile supply cycle**

Care of the material/equipment to be processed

- Cost
  - Financial
  - Moral
- Corrosion
- Process must be scrutinized

Summarizing the importance of cleaning of supplies

- removal of all visible dust and dirt
- removal of breeding ground for surviving microorganisms
- reducing of the bioburden
- protection against corrosion
- ensure more safe free movement of equipment and materials

Importance of quick cleaning after use

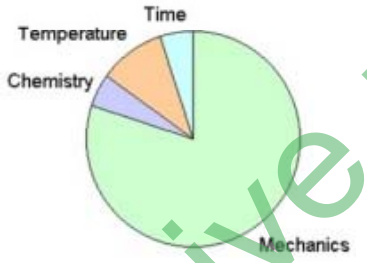
- Majority of soils coagulate
- Must be removed asap
- Organic material is more difficult to handle
- Dried material forms encrustations
- Initial cleaning process advisable

The cleaning process

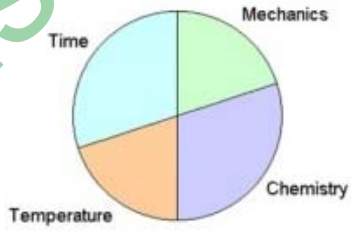
- Sorting
- **Cleaning/ disinfection**
- **Verification**

- **All factors essential**

The cleaning circle

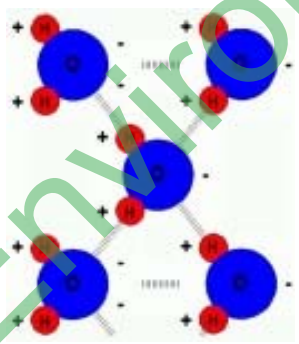


Manual or ultrasonic cleaning



Machine cleaning

Water and cleaning

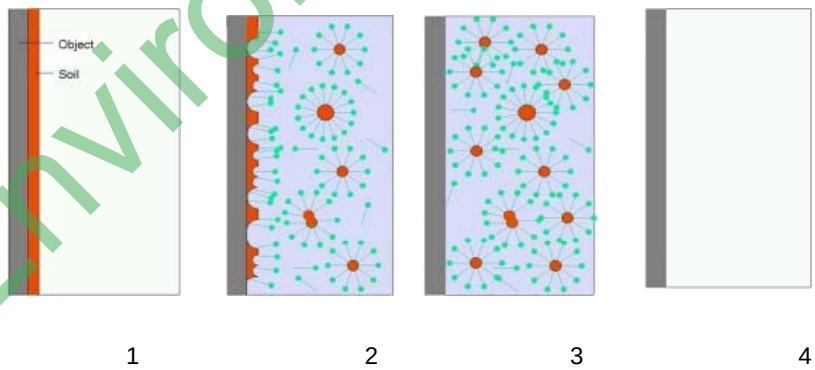


- Dissolution
- Disruption
- Flushing

- Aided chemically

Problems related to cleaning with water

- Wettability.
- fats and oils nonpolar
- Surfactants

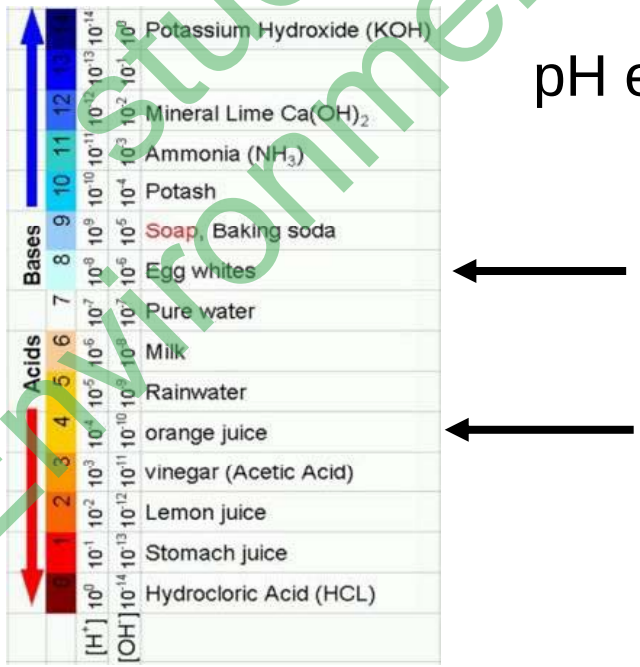


Removing of soil with water to which a surfactant was added

water quality

- Minerals
  - **Water is termed hard or soft**
  - **Modified or removed**
- Chlorides (corrosion)
- pH

pH effects



Improving water quality

- Water analysis and water treatment
- Depending on the stage of the cleaning process a more or less high quality of water is required

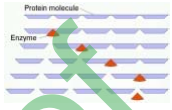
High quality ➔ minimum of particles and minerals dissolved

Chemicals used in the cleaning process

- One or more chemicals
- Manual
  - Detergent
- Machine
  - Various
  - Targeted
  - Eco-friendly

Chemicals used

- Surfactants
  - Decrease surface tension
- Alkalis
  - Optimise surfactant activity
  - Solubilise fats
- Builders
  - Bind hardness ions
- Corrosion Inhibitors
  - Protective coat
- **Biocides**
- Enzymes



Chemicals used

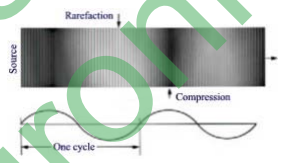
- Neutralizers
  - Reduction of alkalinity
- Lubricants
  - Reduce friction
  - Reduce corrosion
- Rinse aids
  - Surfactant, will enhance rate of drying

Importance of intermediate rinsing after cleaning

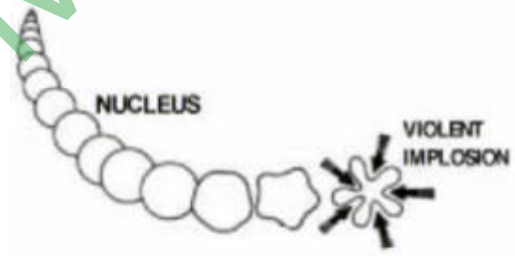
- Chemical residues
- Harmful to equipment
- Harmful to product

- **Harmful to the end user**

Ultrasonic cleaning

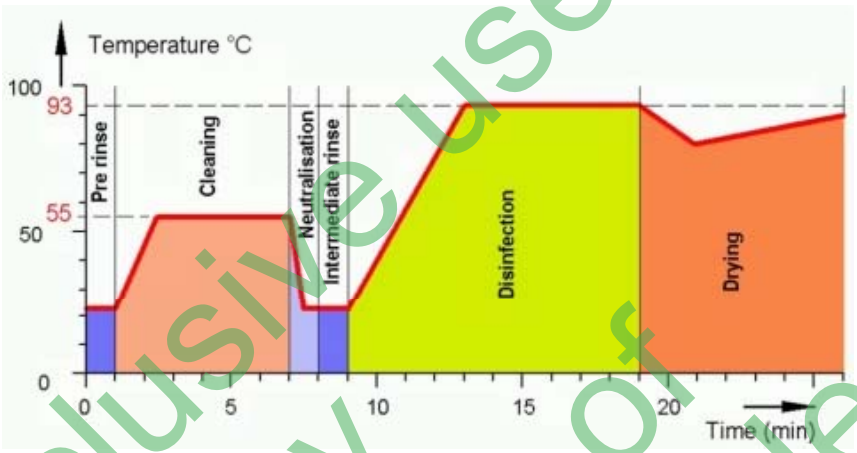


Ultrasonic waves

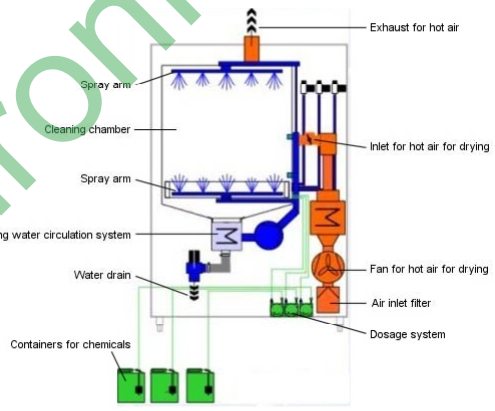


Cavitation

Wash and disinfection cycle



Batch type washer/disinfectors



Why package sterile goods?

- Maintain sterility
- Care for the product
- Identificatiion

Requirements for the primary packaging

- Enablies sterilization.
- Compatible with the sterilization process
- Compatible with the sterilization process
- Maintaining Sterility.
- Strong
- Ensure product integrity and patient safety
- Indicator

Good Packaging Practice

- The packaging should have an indication showing clearly for which sterilization method it should be used
- All materials should be checked
- The content of a pack, which was opened by mistake, should be considered as non-sterile.

Packaging

- Primary
  - prevents recontamination of the product after sterilization
- Secondary
  - to facilitate proper storage and internal transport to user
- Transport
  - used for external transporting of sterile goods

Packaging materials

- wide range of materials
- Traditionally, were reusable (Healthcare facilities etc; most do not pass rigid modern quality standards for use as 1^{o} packaging)
- 1^{o} packaging
  - non-wovens,
  - laminated film pouches,
  - paper bags and containers

Packaging

- Ordinary packaging
- Smart packaging

Food packaging

- the enclosing of food that requires protection from tampering,
  - whether by physical, chemicals, or biological needs.
- It also shows the product that is labelled to show any nutrition information on the food being consumed

Food packaging's functions

- Containment
- Protection:
- Communication:
- Functionality:
- Environmental issues (usual 3**R**s)
- Package and food safetysafety

Packaging type	Type of container	Food examples
Aseptic packages	Primary	Liquid whole eggs
Bags	Primary	Potato chips
Boxes	Secondary	Box of Coca-Cola
Cans	Primary	Can of Campbell's Tomato soup.
Cartons	Primary	Carton of eggs
Flexible packaging	Primary	Bagged salad
Pallets	Tertiary	A series of boxes on a single pallet used to transport from the manufacturing plant to a distribution center.
Wrappers	Tertiary	Used to wrap the boxes on the pallet for transport.

Primary packaging is the main package that holds the food that is being processed. Secondary packaging combines the primary packages into one box being made. Tertiary packaging combines all of the secondary packages into one pallet.

There are also special containers that combine different technologies for maximum durability:

- Bags-In-Boxes (used for soft drink syrup, other liquid products, and meat products)
- Wine box (used for wine)

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WHAT IS SMART PACKAGING?

- A conventional package made smart by an RFID tag or label.
  - (track & trace)

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RFID

- **Radio-frequency identification** (**RFID**)
- automatic identification method.
- An RFID tag is an object that can be applied to or incorporated into a product, animal, or person for the purpose of identification using radiowaves.
- Some tags can be read from several meters away and beyond the line of sight of the reader.

WHAT IS SMART PACKAGING?

- A package made smart by functional attributes that add benefits to the consumers.
  - design elements,
  - else mechanical, chemical, electrical or electronically-driven functions
  - The major beneficiary is the consumer.

What's new?

- Self cooling beer kegs
- Self-heating soup/coffee
- Active humidity control (Smart pouch for cigars!!!)

MAP

- **Modified atmosphere**
  - modifying the composition of the internal atmosphere of a package
  - shelf life.
- The modification process
  - lower the amount of oxygen (O_2), moving it from 20% to 0
  - replaced with nitrogen (N_2), or carbon dioxide (CO_2), which can lower the pH or inhibit the growth of bacteria.

Modified Atmosphere Packaging (MAP)

- Prolonging shelf life
- Preservation technique
- Meat, fish & veg
  - mixture of gases In the package depends on the type of product, packaging materials and storage temperature.
  - Meat and fish need very low gas permeability films so for non-respiring products (meat, fish, cheese etc.) high barrier films are used.

Mixture of gases

- depends on the type of product, packaging materials and storage temperature.
  - Meat and fish need very low gas permeability films so for non-respiring products (meat, fish, cheese etc.) high barrier films are used.
  - The initial flushed gas-mixture will be maintained inside the MA package.
  - But fruits and vegetables are respiring products adapted permeability materials to the products respiration, an equilibrium modified atmosphere will establish In the package and the shelf-life of the product will increase.

EMAP

- Fresh cut produce
- When packaging vegetables and fruits the gas atmosphere of package is not air (O_2 21%; CO_2 0.038%; N_2 78%) but consists usually of a lowered level of O_2 and a heightened level of CO_2.
- Slows normal respiration, prolongs shelf life

Technology

- Gas flushing
  - Cheaper option
  - Flushed with desired mix
- compensated vacuum
  - Air removal, followed by gas mixture introduction
- The label "packaged in a protective atmosphere" can refer to either of these;

Gases

- MA package gases consists of N_2, O_2, CO_2
- Ratio will affect self life
- Oxygen reduction will affect
  - respiration and ethylene production rates can be reduced,
  - softening can be retarded and various compositional changes associated with ripening can Be slowed down.

Packaging films

- Gas permeability
- Water vapour transmission rates
- Mechanical properties
- Traditionally like
  - LDPE (low density polyethylene),
  - PVC (polyvinyl chloride),
  - EVA (ethylene-vinyl acetate)
  - OPP (oriented polypropylene)
- are not permeable enough for highly respiring products
- Specially designed permeable films

Introduction to sterilisation and Disinfection

- Sterilisation and disinfection processes are widely used in health care facilities, pharmaceutical and general industry
- The processes deal with either the partial or total eradication of microrganisms
- Each process has its use
- It is important to know the processes in order to know when a particular process is needed.

Use & Misuse

- Each process has its:
  - Function
  - Benefits and disadvantages
  - Danger factor

Definitions (1)

- **Sterilisation** (or **Sterilization**)

  refers to any process that effectively kills or eliminates all transmissible agents (such as fungi, bacteria, viruses, prions and spore forms etc) from a surface, equipment, foods, phatmaceuticals, or biological culture medium.

Definitions (2)

- **Disinfection**

  refers to any process that effectively kills or eliminates most transmissible agents (but not spores) from a surface, equipment, foods and pharmaceuticals.

Understanding Common Jargon

- There is a lot of jargon associated with these processes
- It pays to familiarise yourself with as many of them as soon as possible

- The following are a collection of the more common terms

Activation	Initiation of a chemical or biological process (e.g. germination of bacterial spores).
Airborne infection	Infection caused by inhaling airborne dust particles or droplet nuclei carrying microbial contaminants.
Antimicrobial	Adjective describing an agent or action that kills or inhibits the growth of microorganisms.
Antisepsis	Prevention of infection by topical application of biocidal or biostatic agents to injured tissues.
Antiseptic	A chemical agent used for antisepsis.
Asepsis	Prevention of microbial contamination of living tissues or sterile materials by excluding, removing or killing microorganisms.
Autoclave	A vessel fitted with a self-sealing door; not descriptive of modern pressure steam sterilizers but continued usage (as noun or verb) is convenient.
Bactericide	A chemical or physical agent that rapidly kills vegetative (non-sporing) bacteria.
Bacteriostat (Bacteristat)	An agent that prevents multiplication of bacteria.
Biocide	A physical or chemical agent that kills some or all types of microorganisms (often used in the inexact sense).
Biological safety cabinet	A completely or partly enclosed work station with laminar air flow through high-efficiency filters for personnel and product protection against infection and contamination.
Buffered solution	An aqueous solution containing chemicals which maintain a specified pH value.
Commensals	Non-pathogenic microorganisms that are living and reproducing as human or animal parasites.
Contact infection	Infection transmitted by direct personal contact or indirectly by contaminated droplets or fomites (inanimate objects).
Contamination	Introduction of microorganisms to sterile articles, materials or tissues.

Contamination level (Bioburden)	The number, or density, of microorganisms on a particular object or surface, or in a specified volume of liquid or air.
Culture medium	A nutrient solution or agar gel for isolating and identifying microorganisms.
Decontamination	Disinfection of used articles to make them safe to handle.
Detergent-sanitizer	A cleaning solution containing an antibacterial agent.
Disinfection	A process that is intended to kill or remove pathogenic microorganisms but which cannot usually kill bacterial spores.
Disinfectant	An agent that is used for disinfection.
DNA	Deoxyribonucleic acid; nuclear material which determines inherited characteristics and controls metabolism of living organisms.
Droplets	Rapidly sedimenting particles of liquid (>5 μm) expelled from the respiratory tract or water systems.
Droplet nuclei	Particles (≤5 μm) which arise from dehydration of small airborne droplets and are capable of wide airborne dispersal.
D value	The time of exposure to heat or chemicals, or the dose of ionizing radiation, that effects a tenfold (decimal) or 90 per cent reduction in the number of viable cells in a microbial population.
Fomites	Inanimate objects, other than food, that may harbour and transmit microorganisms.
Fungicide	An agent that kills fungi and their spores.
Fungistat	An agent that inhibits fungal growth.
Germicide	A colloquial term, usually referring to chemical disinfectants; biocide or bactericide are recommended alternatives.
Heat penetration time	The additional time required for all of the articles in a steam or dry heat sterilizer to reach the selected sterilizing temperature after it has been reached in the chamber.
Heat shock	A sublethal heat treatment that may be applied to bacterial spores to kill residual vegetative forms or induce spore germination.
Holding time	The time for which all of the articles in a steam or dry heat sterilizer must be held at the selected sterilizing temperature.
Inactivation	Death of microorganisms, destruction of enzyme activity or 'neutralization' of the antimicrobial activity of a disinfectant.
Infection	Growth of microorganisms in the tissues of a host, with or without detectable signs of injury.
Infectious disease	The harmful result of infection by microorganisms.

Laminar air flow (unidirectional)	System in which the entire body of air in a confined area moves with uniform velocity along parallel flow lines.
Pasteurization	A process that kills non-sporing microorganisms by hot water or steam at 65–100°C.
Pathogenic microorganism	A species that is capable of causing disease in a susceptible host.
Plenum	A chamber upstream from the air filters in a ventilation system.
Preservation	Prevention of microbial spoilage of foods, pharmaceuticals or industrial materials.
Preservative	A chemical agent used for preservation.
Pyrogens	Heat-stable substances in the cell walls of Gram-negative bacteria that cause a febrile reaction if introduced into the blood or tissues.
Relative humidity (RH)	The amount of water vapour in air, steam or other gaseous atmospheres, expressed as a percentage of the maximum amount that is possible at the existing temperature.
RNA	Ribonucleic acid; controls protein synthesis.
Sanitization	A process that reduces microbial contamination to a low level by the use of cleaning solutions, hot water or chemical disinfectants.
Spores (bacterial)	Thick-walled resting cells formed by certain Gram-positive bacteria (e.g. *Bacillus* and *Clostridium*), capable of survival in unfavourable natural environments and often highly resistant to heat and chemicals.
Spores (fungal)	Unicellular or multicellular reproductive cells, capable of survival in dry conditions with some resistance to chemicals but not highly resistant to heat.
Sporicide	An agent that kills bacterial spores.
Sterilant	An agent that kills all types of microorganisms.
Sterile	Term applied to organisms that are incapable of multiplication or articles that are free from living microorganisms.
Sterilization	A process that is intended to kill or remove all types of microorganisms, with an acceptably low probability of an organism surviving on any article.
Sterilization time	The time for which sterilizing conditions are maintained in a steam, hot air or gas sterilizer.
Tuberculocide	An agent that kills *Mycobacterium tuberculosis* and related acid-fast bacteria.
Vegetative bacterium	A bacterium that is in the growth and reproductive phase.
Viable microorganism	A microorganism that is capable of multiplication in favourable conditions.
Virucide	An agent that renders viruses non-infective.

Essentials for Sterilisation and Disinfection

- Biocidal Action
- Effective Contact
- Appropriate Biocidal Action
- Appropriate Agents and Apparatus
- Severity

Biocidal Action

- Implies microbial death
  - Failure to multiply
- Essential for sterilisation and disinfection.
- The agents chosen have to be capable of facilitating such a situation.
- Biocidal Action terms used:

  bactericidal, sporicidal, virucidal or fungicidal

Effective Contact

- Penetration of the physical process or chemical agent to all surfaces occupied and colonised by microorganisms

Appropriate Agents and Apparatus

- **Specific apparatus** designed for the purpose, together with …..
- **Predetermined** physical and Chemical **Conditions**.

- Such combinations are vital for process success

Severity

- Sterilisation and Disinfection process severity are characterised by
  - Temperature
  - Pressure
  - Concentration
  - Absorbed dose
  - Relative humidity
- Such predetermined process parameters have to be rigorously followed.

Definitions

Sterilisation

Disinfection

Antisepsis

Sanitisation

Decontamination using physical methods

The Mode of action of Heat

DECONTAMINATION

- **Definition**

A process which destroys or removes contamination and thereby prevents micro-organisms or other contaminants reaching a susceptible site in sufficient quantities to initiate infection or any other harmful response

DECONTAMINATION

Cleaning – A process which physically removes contamination but does not necessarily destroy micro-organisms

Disinfection – A process used to reduce the number of viable organisms, which may not necessarily inactivate some viruses and spores

Sterilization – A process used to render the object free from viable micro-organisms, including bacterial spores

DECONTAMINATION

Environmental contamination

- Clostridium difficile
- MRSA
- Vancomycin resistant Enterococci
- SRSV

DECONTAMINATION

Cleaning – mechanical

- **Thermal washer disinfectors**
- **Chemical washer disinfectors**
- **Ultrasonics**

DECONTAMINATION

Cleaning – manual

- **Single use wipes**
- **Detergent and water**
- **Drying**

DECONTAMINATION

Disinfection – physical

- **Low temperature steam**
- **Boiling water**
- **Washer disinfectors**

Miscellaneous

- SOUND WAVES
  - Ultrasonic-High frequency waves
  - Disrupts cells
  - Sonicator--removes microbes by cavitation
- FILTRATION
  - Sterilize liquids that can't be heated
  - HEPA filter for air
  - Pore size determines removal

Disinfection – chemical

- **Inactivated by organic matter**
- **Use appropriate disinfectant for given situation**
- **Correct concentrations**
- **Mode of action**

Chemical Agents in Microbial Control

- Desirable chemical for disinfection
- Rapid action in low concentrations
- Soluble in water or alcohol (tincture)
- Broad-spectrum and nontoxic
- Penetrates surface with persistence
- Resists inactivation
- Non-corrosive, non-staining
- Sanitize, deodorize, cheap, available

Factors that Affect Germicidal Activity of Chemicals

- Concentration of agent
- • Time of exposure
- • Presence of organic material
- • Nature of organisms to be removed

Chemical means of achieving decontamination

- ...phenol and its derivatives;
  ...alcohols;
  ...halides;
  ...aldehydes;
  ...quaternary ammonium compounds;
  ...chloroform;
  ...ethylene oxide;
  ...heavy metal ions;
  ...dyes.

Phenolic disinfectants

- Black and white fluids – Jeyes fluid
- Clear soluble phenolics – Stericol
- Broad spectrum
- Good fungicidal activity
- Poor viral and spore activity
- inexpensive
- Good use for environmental decontamination

Not readily inactivated by organic matter
Hard water inactivation

Alcohols

- Methanol Ethanol Isopropanol
- Good bacterial and fungal activity
- Variable viracidal activity
- Rapid action
- Readily inactivated by organic matter
- Flammable

Chlorine based disinfectants

- **Hypochlorites – Chloros, Domestos, Milton**
- **NaDCC compounds (Dichloroisocyanurates) – Presept and Actichlor**

Chlorine based disinfectantsare:

- **Active against viruses and spores**
- **Good fungicidal and bactericidal activity**
- **Poor Mycobacterial activity**
- **Inactivated by organic matter**
- **Corrosive**
- **Unstable**

Halides/Halogens

- Halides are very powerful oxidising agents
- **chlorine** and **iodine**.
  - rapid germicidal action,
  - inactivated in the presence of organic matter.
  - highly irritant to humans.
- **Chlorine** is used in low concentrations to prevent
  - **sodium hypochlorite - household bleach**
- **Iodine**
  - **iodophores**

- • Iodines - betadine
- • QAC's –
- • Aldehydes - gluteraldehyde

Skin disinfection

- • Liquid soap and water
- • Alcohol, chlorhexidine, iodine

- Chemical Groups
- Halogens
- Phenolics
- Chlorhexidine
- Alcohols
- Hydrogen peroxide
- Detergents
- Heavy metals
- Aldehydes
- Gases
- Dyes
- Acids and alkalis

- Denature proteins
- Chlorine
- Sanitizers
- Iodine
- Tincture, skin antiseptic
- Iodophors
- Iodine and PVA (polyvinyl alcohol)
- Time release effect
- Eg Betadine →Povidone Iodine

PHENOLIC COMPOUNDS

- Damages cell membrane, protein
  - Phenol
  - Carbolic acid--poisonous
  - Bisphenols
  - Hexachlorophene
- Skin antiseptic--time release effect
- Amphyl, Triclosan

Phenol and its derivatives:

- '**gold standard**'
- Phenol acts
  - by causing **cell disruption**
  - **denaturing proteins**.
- It is highly **corrosive** and **toxic** to humans

ALCOHOLS

- Dissolves membranes
- Ethyl and isopropyl
- Good against vegetative cells
- Evaporation diminishes contact time

Alcohols:

- Alcohols such as methanol, ethanol and isopropanol dehydrate cells, disrupt membranes and cause coagulation of protein.
- A **70% (v/v) aqueous solution** is **more effective** at killing microbes than absolute alcohols.
- Because the primary cidal effect of alcohols is membrane disruption, bacterial **endospores** and many viruses are **unaffected by alcohols**.

Aldehydes:

- Aldehydes denature **nucleic acids** and **proteins**
  - lethal
  - **glutaraldehyde** and **formaldehyde**,
  - inactivated in the presence of organic matter.

ALDEHYDES

- Denature proteins
- Glutaraldehyde
  - Cidex--used to disinfect endoscopes, fiber optics, dental
- Formaldehyde
  - Used to disinfect some surgical instruments and dialysis machines
- TOXIC

Quaternary ammonium compounds

- Organically substituted ammonium compounds such as **cetrimide** and **benzalkonium chloride**
- cationic detergents.
- widely used as disinfectants for domestic use and in hospitals.

GASES

- Affect DNA
- Ethylene oxide (ETO)
  - Alkylating agent--carcinogenic
  - Used in chemiclave (gas autoclave)
- Chlorine dioxide
  - Treatment of drinking water,
  - wastewater, food processing
  - equipment, medical waste
  - Decontaminant—anthrax 2001

GASES 2

- Propylene oxide
  - Less toxic—breaks down
- Betapropiolactone
  - Highly toxic--rooms, bone grafts,
  - inactivate viruses in vaccines
- GASES
  - Better than ETO for foods (nuts,powders, starches, spices

Chloroform

- **Chloroform** is an organic solvent that **disrupts membranes**. It has **no direct clinical applications** as a disinfectant, but can be used in the laboratory to sterilise items and some solutions.

Ethylene oxide

- Very few gases are able to kill microbes
- It is a **highly effective disinfectant**, capable of **killing spores** rapidly.
- Its cidal activity is enhanced by the presence of moisture.
- It can be used to sterilise bulky items and very delicate instruments
- cost & flammability preclude widespread use.

HEAVY METALS

- (Hg, Ag, Au, Cu, As, Zn)
- Denature proteins
- Toxic, allergens, inactivated by
- inorganic substances
- Mercury compounds
- Thimersol--preservative
- Mercurochrome, merthiolate

Heavy metal ions

- Most heavy metal ion preparations are now considered **too toxic** for routine use.
- **Silver sulphadiazine** is used topically to help to prevent colonisation and infection of **burn tissues**.

HYDROGEN PEROXIDE (OXIDIZING AGENTS)

- Highly reactive—damaging to cells
- Somewhat unstable
- Inactivated by catalase
- 35% H_2O_2 and 35% peracetic acid
- Powerful sterilants, endoscopes
- Ozone (O_3)—sterilize air, water,
- industrial air conditioners

CHLORHEXIDINE

- Surfactant, denatures protein
- Chlorine + 2 phenolic rings
- Hibiclens, Hibitane
- Skin antiseptic--time release effect
- Mild, low toxicity, rapid action

DETERGENTS (SURFACTANTS)

- Damages cell membrane
- Cationic most effective
- Quaternary ammonium compounds
  - (quats)—sanitizes
- Anionic have limited microbicidal activity
- Soaps
- Mechanical removal of microbes

DYES

- Aniline dyes
- Crystal violet
- Malachite green
- Used in solutions for skin infections
- Also used in veterinary ointments

ACIDS AND ALKALIS

- Denature proteins
- Caustic, corrosive, hazardous
- Acids primarily used as food
- preservatives
- Ammonium hydroxide used in
- detergents and cleansers as
- deodorizing agent

Dyes used for disinfection

- **Acridine dyes**
  - bactericidal because of their interaction with bacterial nucleic acids.
  - They may be used topically as antiseptics to treat mild burns

STERILISATION

- **Steam – autoclaving**
- **Hot air – dry heat 160ºC for 2 hours**
- **Chemical – ethylene oxide**
- **Irradiation**
- **Incineration**

STERILISATION

- Steam
- Autoclaving – physical method utilising steam
- Steam under pressure attains a higher temperature than boiling water
- Viruses and bacteria with heat resistant spores are rendered non-viable at 121°C for 15 mins

Steam STERILISATION

- Non toxic
- Non corrosive
- Inexpensive
- Automated – quick turn around time

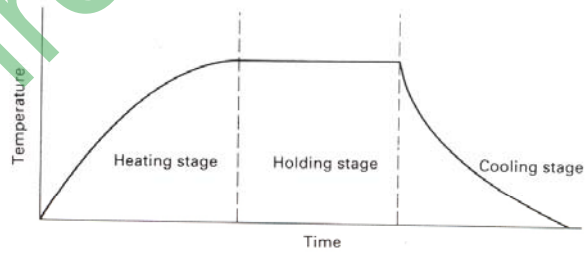
Steam STERILISATION

- Can only be used on heat and moisture
- resistant items

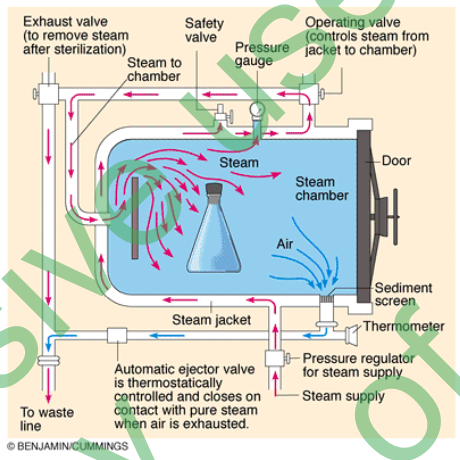
Uses

- • Infected materials that need recycling
- • Surgical instruments

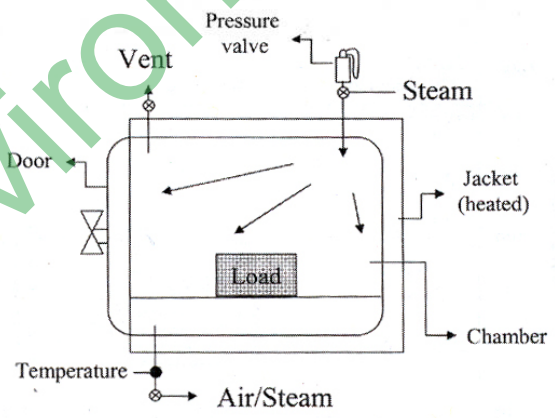
Heat cycle



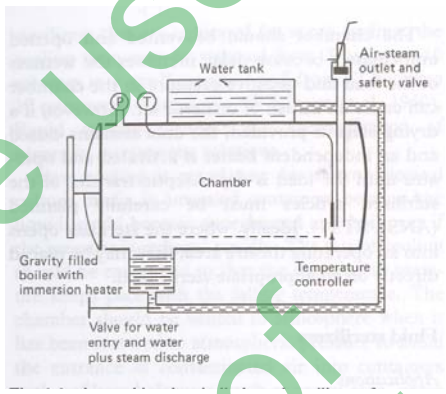
Moist Heat at Raised Pressure: The Autoclave (2)



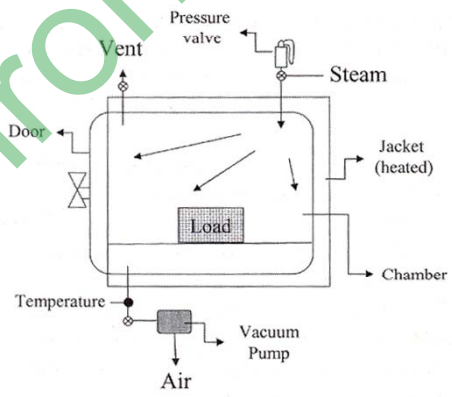
Downward Displacement autoclave



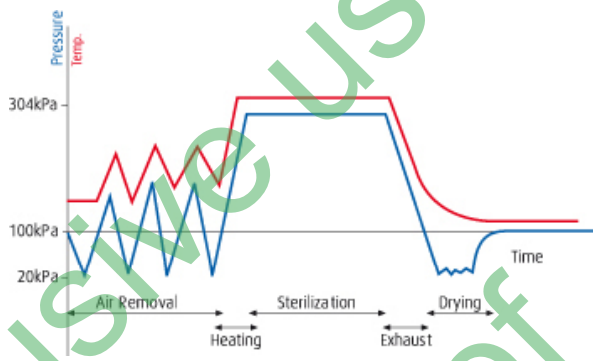
Bench top autoclave



Pre-vacuum autoclave



Pre-vacuum autoclave cycle



Hot air STERILISATION

- **Hot air oven**
- **• 160°C for 2 hours**
- **• 170°C for 1 hours**
- **• 180°C for 30 mins**

Ethylene oxide

- Used for heat sensitive items and
- sterilisation of single use items on an
- industrial scale
- Expensive, requires specialist
- equipment as it is highly explosive

INCINERATION

- 850ºC with secondary burning
- Preferred method for all clinical waste

Factors Affecting Death Rate

- • Bioburden--number of organisms
- • Kind of organisms--spores, cells
- • Environment--temperature and pH
- • Concentration of agent
- • Mode of action of agent used
- • Presence of other substances

How do antimicrobial agents work?

- • Damage cell wall
  - – Lysozyme and penicillin
- • Damage cell membrane
  - – Surfactants
- • Affect protein and nucleic acid synthesis
  - – Chloramphenicol, radiation
- • Alter protein function
  - – Heat, alcohol, pH (denature)

Practical concerns in microbial control

- How clean does it have to be?
- Is it reusable?
- What can I do to it and not harm it?
- Is the method I want to use suitable?
- Will the agent penetrate sufficiently?
- Safe?
- Cost?
- Labour effective?

Methods of Physical Control

- Heat
- Cold
- Drying
- Radiation
- Sound waves
- Filtration

Methods of Sterilisation

HEAT

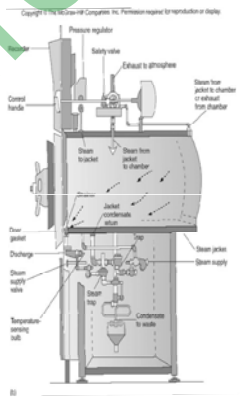
- Action
  - Moist heat coagulates proteins
  - Dry heat dehydrates
- Thermal Death Time
  - Shortest time necessary to kill a specific organism at a specific temperature

TABLE 11.3

Thermal Death Times of Various Endospores

Organism	Temperature	Time of Exposure to Kill Spores
Moist Heat		
Bacillus subtilis	121°C	1 min
B. stearothermophilis	121°C	12 min
Clostridium botulinum	120°C	10 min
Cl. tetani	105°C	10 min
Dry Heat		
Bacillus subtilis	121°C	120 min
B. stearothermophilis	140°C	5 min
Clostridium botulinum	120°C	120 min
Cl. tetani	100°C	60 min

Moist heat-



Pasteurization

- Disinfection of beverages—removal of unwanted organisms
  - (not sterilization)
- Batch pasteurization--63o C, 30 min
- Flash pasteurization--72o C, 15 sec
- Ultra high temperature treatments
  - 134º C, 1-2 sec (sterile milk)

Boiling water

- 100º C, 30 minutes
- Kills everything but endospores
  - Items recontaminated when removed from water
- Recommended method for disinfecting unsafe drinking water Food, utensils, bedding

Dry heat

160º C to several thousand degrees

Dry heat: Hot air

- 160-180º C, 2-4 hours
- Used for items that do not sterilize well with moist heat
- • glassware
- • metallic instruments
- • powder and oils
- • plastics, cotton, paper

COLD AND DRYING

- Effects erratic and uncertain
- Cold
- Some organisms killed
- Used as preservation technique
- Drying
- Some organisms killed
- Used as preservation technique
- Lyophilization
- Freeze-drying, Preservation

RADIATION

•Ionizing radiation

•Food, prepackaged plastic,

•vaccines, drugs

RADIATION

- Ultraviolet (nonionizing) radiation
  - Bacteria have repair mechanisms
  - Germicidal lamps
  - Surfaces only--does not penetrate Short term
  - DNA damage

The mode of action of heat

The mode of action of heat

- disrupting membranes
- denaturing proteins and nucleic acids

- estimating how effective heat sterilisation can be.
  - The **thermal death time**
  - **thermal death point** .
  - The **D-value** or **Decimal Reduction Time**

Dry heat

- **Dry heat** is **less effective** than moist heat
- **Dry heat** ➔ **corrode** if exposed to steam.

- A typical dry air oven sterilisation regime would be **two hours at 160 degrees Celcius**

Moist heat

- To be effective,
- **steam under increased pressure** is used in sterilisation.

Autoclaves

- Use the same principles as the domestic pressure cooker
- **15 minutes at 121 ºC**
- Efficiency Testing

Tyndallisation and pasteurisation

- Autoclaving may be too harsh a process
- **Tyndallisation** may be used
- Heat /cool/store/heat/ cool/
- The process is completed with a second overnight incubation and heating.

Decontamination using physical methods

- The principal physical methods of sterilisation are using...
  ...heat;
  ...irradiation;
  ...filtration.

Irradiation:

- **Ultraviolet irradiation:**
  - Ultraviolet light at 260 nm → to genetic damage to cells and their ultimate death.
  - Ultraviolet irradiation is an effective method of sterilising work surfaces and air but it **does not penetrate** glass and thus is used for sterilising surfaces.
- **X-rays:**
  - **X-rays** are too **unpredictable**
  - .
- **Gamma-irradiation:**
  - **Gamma-irradiation** **'cold sterilisation'**.

Filtration:

- Heat-labile materials
- Today **nitrocellulose filters** have replaced the older filtration methods

Dry Heat

- Kills by **oxidation**.
  - **Direct Flaming:** Used to sterilize inoculating loops and needles. Heat metal until it has a red glow.
  - **Incineration**: Effective way to sterilize disposable items (paper cups, dressings) and biological waste.
  - **Hot Air Sterilization:** Place objects in an oven. Requires 2 hours at 170ºC for sterilization. (Other time –temperature combinations exist)
  - Dry heat is transfers heat less effectively to a cool body, than moist heat.

Advantages

- depends upon the penetration of adequate heat to the article as a whole;
- possible to sterilize such objects already assembled and pre-sealed in their container,
- whereas steam and gases, such as ethylene oxide, **can only be relied upon to kill organisms if the steam or gas comes into direct contact with the surface of the objects**
- **Can be used for** objects which are damaged by water or steam

Disadvantages

- Heating time
- the temperature and time necessary for sterilization must be considerably higher and longer than for steam
- Lack of equipment reliability
- Oxidation of components being sterilised

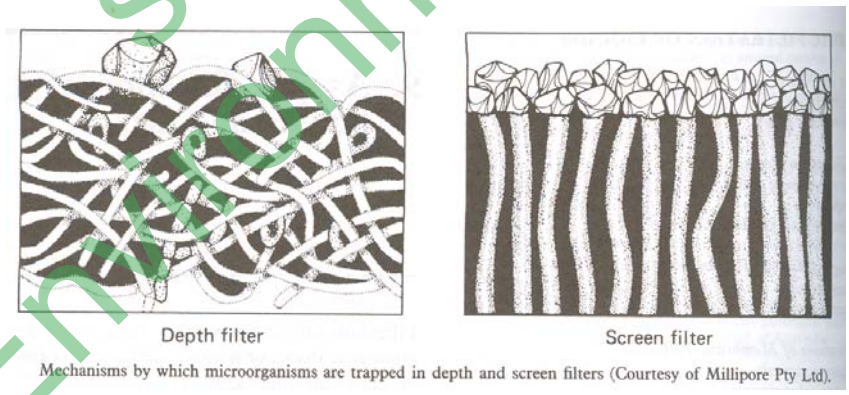
Filtration

- Removal of microbes by passage of a liquid or gas through a screen like material with small pores.
- Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.
- **High Efficiency Particulate Air Filters (HEPA):** Used in operating rooms and burn units to remove bacteria from air.
- **Membrane Filters**: Uniform pore size. Used in industry and research.
- Different sizes:
  - **0.22 and 0.45um Pores**: Used to filter most bacteria. Don't retain spirochetes, mycoplasmas and viruses.
  - **0.01 um Pores**: Retain all viruses and some large proteins.

ASEPTIC FILTRATION OF LIQUIDS

Liquids are filtered for the following purposes:

1. 'Sterile' filtration (removal of microorganisms from heat-sensitive aqueous solutions, organic solvents or oils)
2. Sterility testing of pharmaceutical products and medical devices
3. Collection of bacteria from water samples or other dilute suspensions for enumeration and identification.



Mechanisms by which microorganisms are trapped in depth and screen filters (Courtesy of Millipore Pty Ltd).

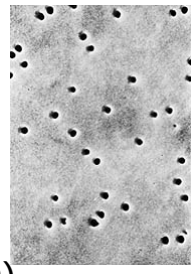
Filter Types (1)

- Depth
  - Pads
  - Compacted polypropylene, cellulose or glass fibres
  - Retention efficiency depends on nominal pore rating and selective adsorption of microorganisms to material
  - Pores larger than particles, long channels retain

Filter Types (2)

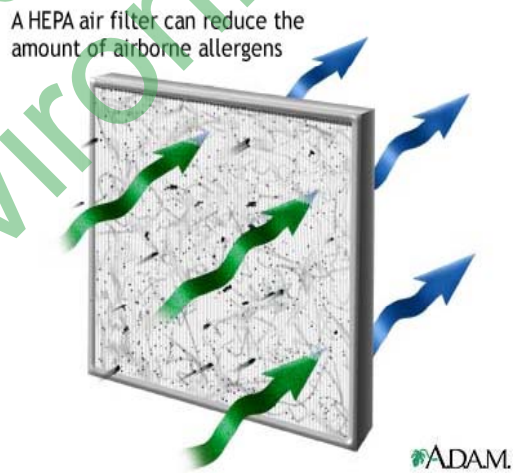
- Membrane filters
  - Polymers
  - Hydrophilic and hydrophobic types
  - Regularly spaced pores; rated in μm
  - Easily clogged
  - Retain particles larger than pore rating
  - Good flow rates
  - Filtrate not affected by the filter material

(Polycarbonate) Membrane Filters



- Precisely controlled cylindrical pores for maximum absolute particulate capture
- Smooth flat surface (less then 0.1µm) for microscopic work
- Narrow pore size distribution, 0 to -20%
- No sloughing or particle shedding, one integral plastic film
- Negligible adsorption or absorption
- Low extractables

HEPA filters



Applications of high-efficiency air filters

Locations	Applications
Hospitals	Ventilation of operating theatres and protective isolation facilities Decontamination of inspired or expired air in mechanical ventilators and suction apparatus Filtration of compressed gases (e.g. O_2) administered to patients Filtration of air admitted to steam or gas sterilizers
Microbiological laboratories	Ventilation of safety cabinets and 'sterile' rooms Decontamination of exhaust air from cabinets or rooms where aerosols of pathogenic microorganisms have been generated Decontamination of effluent air from aerated bacterial or fungal cultures
Industrial premises	Ventilation of rooms or enclosures for aseptic filling of liquids sterilized by filtration Ventilation of rooms for sterility testing Ventilation of rooms for assembly of micro-electronic equipment