# Sterilization and Disinfection

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# Introduction

- **Sterilization** is the killing or removal of **all microorganisms** in a material or on an object
  - There are no degrees of sterility; sterility means that there are no living organisms in or on a material (i.e. an item is either sterile or it is not)
- **Disinfection** means **reducing the number** of microorganisms on inanimate objects to a safe level; so that they pose no threat of infection
  - Disinfection involves the use of a cidal (killing) agent (called disinfectant) but does not necessarily kill all microorganisms
- **Antisepsis** is the **killing or inhibition of microorganisms** on living tissues; having the effect of preventing or limiting the harmful effects of infection
  - Both cidal (killing) and static (growth inhibiting) agents (called antiseptics) can be used in antisepsis
- **Preservation** is the addition of antimicrobial agents to prevent microbial spoilage of certain products (i.e. food and drugs) and to minimize the risk of causing infection to the consumer during the use of the product

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# Disinfection Policy

- The aim of such policy is to control the use of chemicals for disinfection and antisepsis and give guidelines on their use.
- The control of microorganisms is of prime importance in hospital and industrial environments. Usually there is a committee (**Infection Control Committee**) who is responsible to set & implement the policy (i.e. what disinfectants to use, conc, where to use them (floors, ceiling, walls, air, instruments), contact time)
- In hospitals, categories of risk to patients may be assigned to equipment that come in contact with him dictating the level of decontamination needed.
  - **High risk items**: have close contact with broken skin or mucous membrane or are those introduced into a sterile area of the body. These should be sterile. e.g. surgical equipment, gloves, catheters, syringes and needles.
  - **Intermediate-risk items**: are in close contact with intact skin or mucous membranes and disinfection will normally be applied. e.g. respiratory and anaesthetic equipment and bed-pans.
  - **Low-risk items or areas**: which are not in close contact with the patient. e.g. walls, floors, ceiling, etc.



# Chemical Antimicrobial Agents

- Disinfection, antisepsis and preservation are mainly achieved by the use of chemical biocides which are chemical agents that have the ability to kill or inhibit the growth of microorganisms
- Chemical disinfectants exert 3 different levels of disinfection:
  - **High level disinfection**: destruction of all m.o. but not necessarily all bacterial spores. Some have good sporicidal activity and hence they are called **'liquid chemical sterilants'** to indicate that they completely kill all microorganisms as in sterilization
  - **Intermediate level disinfection**: destruction of all vegetative bacteria (including *Mycobacterium tuberculosis*), most viruses & most fungi. They have little or no sporicidal activity
  - **Low level disinfection**: can destroy most vegetative bacteria (excluding *Mycobacterium tuberculosis*), some fungi and some viruses

## Factors Affecting the Activity of Chemical Biocides

- **Exposure time**: increasing the exposure time to a biocide will result in higher extent of microbial death
- **Concentration**: the more concentrated a disinfectant, the greater its efficacy and the shorter the time necessary to completely kill a certain number of m.o.
- **Temperature**: increasing temperature by 10°C roughly doubles the rate of chemical reactions and thereby increases the potency of chemical agents
- **pH**: pH can affect the potency of chemical agents by affecting their degree of ionization and hence their permeability into microbial cells. pH can also alter the properties of the microbial cell itself
- **Presence of organic matter**: organic matter like faeces, blood and pus may reduce the activity of different chemical agents to variable degrees



## Organic Acids

- Mainly used as preservatives for food and pharmaceutical products
- The undissociated form (HA) is usually the active antimicrobial agent, and hence a balance between their pKa and product pH should be considered
- Benzoic acid (pKa 4.2), sorbic acid (pKa 4.8) and lactic acid (pKa 3.9) are the main examples
  - Due to their relatively low pKa, these agents are only useful for acidic products (i.e. with pH less than 5)
- Parabens have higher pKa (between 8-8.5) and hence can be used for products with higher pH (up to 7-8)

# Alcohols

- Short aliphatic alcohols (i.e. ethanol and isopropanol) are mainly used as disinfectants and antiseptics
- They exert intermediate level disinfection and don't kill bacterial spores
- Used in concentrations of 60-95% for decontamination of skin and instruments
- Presence of water is essential for their activity but their activity drops sharply at concentrations below 50%
- Presence of organic matter significantly reduces the antimicrobial activity of aliphatic alcohols and hence proper cleaning is required prior to their use as disinfectants



# Alkylating Agents

- Aldehydes such as formaldehyde, gluteraldehyde and ortho-phthalaldehyde are highly effective biocides with sporicidal activity (liquid chemical sterilants)
- Aldehydes are mainly used in aqueous solutions for cold liquid chemical sterilization of medical and surgical equipment that cannot be sterilized by other methods (e.g. endoscopes)
- Gluteraldehyde solution should be activated by addition of alkaline solution prior to its use
- 1:10 diluted formalin (~4% formaldehyde) is also used to disinfect surfaces
- Ethylene oxide is another alkylating agent that is mainly used in its gaseous form for the sterilization of re-usable instruments in addition to thermolabile disposable medical equipment (e.g. gloves, syringes, cannulas and IV sets)
  - Ethylene oxide is explosive and highly toxic and hence it requires special handling procedures

# Biguanides

- Biguanides such as chlorhexidine and alexidine are well-tolerated bactericidal agents but with little or no activity against mycobacteria and spores
- Mainly used as antiseptics but can also be used as disinfectants and preservatives
- Biguanides are most active in their di-cation form which exist at pH 7-8
- Their activity is reduced by the presence of organic matter and many anionic compounds. The use of hard water with these compounds also reduces their activity



# Halogens

- Chlorine compounds:
  - Hypochlorite is the main chlorine compound used in disinfection which is considered a high level disinfectant with sporicidal activity (at relatively high concentrations)
  - Commercially available hypochlorites (e.g. household bleach) are prepared in alkaline solutions and therefore should be diluted in water before their use to give hypochlorous acid; the more active form of hypochlorite
  - Hypochlorite's activity is highly affected by organic matter is not used in antisepsis because it is highly irritant and corrosive
  - Organic chlorine compounds such as chloramine and halazone are much less irritant than hypochlorite and hence can be used for wound cleansing and treatment of drinking water

# Halogens

- Iodine:
  - Iodine has a wide spectrum of antimicrobial activity including G-ve & G+ve bacteria, mycobacteria, fungi, viruses and even bacterial spores.
    - However some G-ve bacteria (e.g. *Pseudomonas* spp.) have been reported to be resistant to some iodine preparations
  - Iodine tincture (hydroalcoholic solution) is one of the first skin antiseptics to be used, but it stains skin and fabric and causes allergic reactions in some people
  - Iodophors are more commonly used nowadays which, upon dilution, slowly release iodine and hence cause less skin staining and sensitization
  - Povidone-iodine (Betadine®) is a prominent example of iodophors that is used for surgical scrubs and as preoperative skin antiseptic



# Oxidizing Agents

- Hydrogen peroxide is an oxidizing agent that is considered a high level disinfectant.
- It can be used as antiseptic for open wounds and ulcers
- Also used for disinfection of contact lenses as it is active against *Acanthamoeba* which causes keratitis in contact lens wearers
- 3-6% solution can be used as general disinfectant
- Vapour-phase hydrogen peroxide is used for sterilization of small areas such as glove boxes and isolators.

# Phenols

- Phenol is one of the oldest used biocides but no longer plays any significant role as an antibacterial agent due to its caustic effect
- Other phenolic compounds such as cresols and xylenols are less corrosive derivatives and more commonly used nowadays as general purpose disinfectants
- Chlorinated phenols such as chlorocresol and chloroxylenol (Dettol®) can be used as skin antiseptics
- Triclosan is a bisphenol that is widely used in consumer products such as antibacterial soaps, hand lotions and tooth pastes
- Cresols and xylenols are intermediate level disinfectants while chlorinated phenols and bisphenols are generally considered as low level agents



# Surface Active Agents

- Quaternary ammonium compounds (quats) are cationic surfactants with good antimicrobial activity against a wide range of vegetative microorganisms; but not bacterial spores
- Quats (e.g. cetrimide and benzalkonium chloride ) are generally well tolerated and non-toxic when applied to skin and mucous membranes and hence frequently used as antiseptics.
- They are most active at pH 7-8 and their activity is reduced by the presence of organic matter and many anionic compounds.
- Some G-ve bacteria such as *Pseudomonas* have developed resistance to these agents.

# Physical Antimicrobial Agents

- Physical methods are much more preferred than chemical agents when performing sterilization.
- Among the most commonly used physical sterilization techniques are heat, radiation and filtration sterilization.
- Sterilization by heat or radiation involves the killing of all forms of microbial life; where bacterial endospores are known to be most resistant and hence used as 'biological indicators' to test the efficiency of these methods.
- While filtration sterilization relies on the mechanical removal of microorganisms based on their size.



# Heat Sterilization

- Heat is the most reliable and widely used means of sterilization but can only be used with thermostable items.
- Heat sterilization can be achieved by using moist heat (steam at elevated temperature and pressure) or dry heat (hot air)
- Moist heat is more efficient in destroying microorganisms and therefore it is performed at relatively lower temperatures (121-134 °C) than dry heat (160-180 °C). Heated steam is also characterized by better penetrating properties than dry heat. Therefore moist heat is preferred whenever the items to be sterilized are moisture-resistant.
- Killing efficiency of heat sterilization at a specified temperature is usually measured by the D-value (decimal reduction time) which is defined as the time needed to kill 90% of the microorganisms in a given population

# Heat Sterilization

- Moist heat sterilization is accomplished by using an autoclave and is commonly used for the sterilization of culture media, dressings, sheets, surgical equipment, containers and closures, aqueous injections, ophthalmic preparations, irrigation fluids in addition to the processing (decontamination) of soiled and contaminated items.
- Dry heat sterilization is usually performed in hot air ovens with perforated shelves and is usually used for the sterilization of glassware, metal surgical instruments, oils and powders.



# Radiation

- Radiation sterilization can be achieved using ionizing radiation (e.g. gamma-rays and X-rays) or non-ionizing radiation (e.g. UV light).
- The major target for radiation is microbial DNA, with damage occurring as a consequence of ionization and free radical production by ionizing radiation or thymine dimer formation by non-ionizing radiation
- Ionizing radiation is more damaging to m.o. and has better penetrating power so it is more reliable and has wider range of applications in sterilization

# Radiation

- Ionizing radiation is the preferred method for the sterilization of thermolabile disposable medical equipment (e.g. gloves, syringes, cannulas and IV sets). It can also be used for powders and non-aqueous liquid and semi-solid preparations (e.g. ointments)
- UV light can be used for air and surface sterilization in controlled areas (e.g. biosafety cabinets and operating rooms) and for sewage water treatment.



# Filtration

- Filtration sterilization is unique in that it removes rather than destroys microorganisms
- 0.2 – 0.45 µm membrane filters (defined pore size) are usually used for the sterilization of heat-sensitive liquids
- HEPA filters (depth filters) can remove more than 99.97% of particles greater than 0.3 mm in diameter and are commonly used for sterilization of air and other gases; especially for the provision of sterile air to:
  - Aseptic manufacturing sites
  - Hospital isolation units
  - Operating theatres
- HEPA filters are also used for:
  - Decontamination of air in mechanical ventilators
  - Clarification and sterilization of medical gases