Disinfection and sterilisation

Antiseptics

- (Greek αντι, *against*, and σηπτικος, *putrefactive*) are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. They should generally be distinguished from *antibiotics* that destroy microorganisms within the body, and from *disinfectants*, which destroy microorganisms found on non-living objects. Some antiseptics are true *germicides*, capable of destroying microbes (bacteriocidal), whilst others are bacteriostatic and only prevent or inhibit their growth. *Antibacterials* are antiseptics that only act against bacteria.

Some common antiseptics

- Alcohols
- Quaternary ammonium compounds
- Boric acid
- Chlorhexidine Gluconate
- Hydrogen peroxide
- Iodine
- Mercurochrome
- Octenidine dihydrochloride
- Phenol (carbolic acid) compounds
- Sodium chloride
- Sodium hypochlorite

Disinfectants

- are antimicrobial agents that are applied to non-living objects to destroy microorganisms, the process of which is known as **disinfection**. Disinfectants should generally be distinguished from *antibiotics* that destroy microorganisms within the body, and from *antiseptics*, which destroy microorganisms on living tissue. *Sanitisers* are high level disinfectants that kill over 99.9% of a target microorganism in applicable situations. Very few disinfectants and sanitisers can sterilise (the complete elimination of all microorganisms), and those that can depend entirely on their mode of application. Bacterial endospores are most resistant to disinfectants, however some viruses and bacteria also possess some tolerance.

Types of disinfectants

- Alcohols
- Aldehydes
- Halogens
- Oxidising agents
- Quaternary ammonium compounds
- Other

Alcohols

- Alcohols, usually ethanol or isopropanol, are wiped over benches and skin and allowed to evaporate for quick disinfection. They have wide microbiocidal activity, are non corrosive, but can be a fire hazard. They also have limited residual activity due to evaporation, and have a limited activity in the presence of organic material. Alcohols are more effective combined with water—70% alcohol is more effective than 95% alcohol. Alcohol is not effective against fungal or bacterial spores.

Aldehydes

- Aldehydes, such as Glutaraldehyde, have a wide microbiocidal activity and are sporocidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity

Halogens

- **Chloramine** is used in drinking water treatment instead of chlorine because it produces less disinfection byproducts.
- **Chlorine** is used to disinfect swimming pools, and is added in small quantities to drinking water to reduce waterborne diseases.
- **Hypochlorites** (Sodium hypochlorite), often in the form of common household bleach, are used in the home to disinfect drains, and toilets. Other hypochlorites such as calcium hypochlorite are also used, especially as a swimming pool additive. Hypochlorites yield an aqueous solution of hypochlorous acid that is the true disinfectant. Hypobromite solutions are also sometimes used.
- **Iodine** is usually dissolved in an organic solvent or as Lugol's iodine solution. It is used in the poultry industry. It is added to the birds' drinking water. Although no longer recommended because it increases scar tissue formation and increases healing time, tincture of iodine has also been used as an antiseptic for skin cuts and scrapes.

Oxidising agents

- act by oxidising the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death.
- **Chlorine dioxide** is used as an advanced disinfectant for drinking water to reduce waterborne diseases. In certain parts of the world, it has largely replaced chlorine because it forms fewer byproducts. Sodium chlorite, sodium chlorate, and potassium chlorate are used as precursors for generating chlorine dioxide.
- **Hydrogen peroxide** is used in hospitals to disinfect surfaces. It is sometimes mixed with colloidal silver. It is often preferred because it causes far fewer allergic reactions than alternative disinfectants. Also used in the food packaging industry to disinfect foil containers. A 3% solution is also used as an antiseptic. When hydrogen peroxide comes into contact with the catalase enzyme in cells it is broken down into water and a hydroxyl free radical. It is the damage caused by the oxygen free radical that kills bacteria. However, recent studies have shown hydrogen peroxide to be toxic to growing cells as well as bacteria; its use as an antiseptic is no longer recommended.[*citation needed*]
- **Ozone** is a gas that can be added to water for sanitation.
- **Acidic Electrolyzed Water** is a strong oxidising solution made from the electrolysis of ordinary tap water in the presence of a specific amount of salt, generally sodium chloride. Anolyte has a typical pH range of 3.5 to 8.5 and an Oxidation-Reduction Potential (ORP) of +600 to +1200 mV. The most powerful anolyte disinfecting solution is that produced at a controlled 5.0 to 6.3 pH where the predominant oxchlorine species is hypochlorous acid. This environmentally-responsible disinfectant is highly efficacious against bacteria, fungus, mold, spores and other micro-organisms, in very short contact times. It may be applied as liquid, fog or ice.
- **Peracetic acid** is a disinfectant produced by reacting hydrogen peroxide with acetic acid. It is broadly effective against microorganisms and is not deactivated by catalase and peroxidase, the enzymes which break down hydrogen peroxide. It also breaks down to food safe and environmentally friendly residues (acetic acid and hydrogen peroxide), and therefore can be used in non-rinse applications. It can be used over a wide temperature range (0-40°C), wide pH range (3.0-7.5), in clean-in-place (CIP) processes, in hard water conditions, and is not affected by protein residues.
- **Potassium permanganate** ($KMnO_4$) is a red crystalline powder that colours everything it touches, and is used to disinfect aquariums. It is also used widely in community swimming pools to disinfect ones feet before entering the pool. Typically, a large shallow basin of $KMnO_4$/water solution is kept near the pool ladder. Participants are required to step in the basin and then go into the pool. Additionally, it is widely used to disinfect community water ponds and wells in tropical countries, as well as to disinfect the mouth before pulling out teeth. It can be applied to wounds in dilute solution; potassium permanganate is a very useful disinfectant.

Phenolics

- Phenolics are the active ingredient in most bottles of "household disinfectant". They are also found in some mouthwashes and in disinfectant soap and handwashes. Phenol is probably the oldest known disinfectant as it was first used by Lister, when it was called carbolic acid. It is rather corrosive to the skin and sometimes toxic to sensitive people, so the somewhat less corrosive phenolic *o*-phenylphenol is often used in favour. Hexachlorophene is a phenolic which was once used as a germicidal additive to some household products but was banned due to suspected harmful effects.

Quaternary ammonium compounds

- **Quaternary ammonium compounds** (Quats), such as benzalkonium chloride, are a large group of related compounds. Some have been used as low level disinfectants. They are effective against bacteria, but not against some species of Pseudomonas bacteria or bacterial spores. Quats are biocides which also kill algae and are used as an additive in large-scale industrial water systems to minimize undesired biological growth. Quaternary ammonium compounds can also be effective disinfectants against enveloped viruses.

Other

- Dettol is used to disinfect surfaces at home. It kills the majority of bacteria. It is one of the few disinfectants useful against viruses.[*citation needed*]
- Virkon is a wide-spectrum disinfectant used in labs. It kills bacteria, viruses, and fungi. It is used as a 1% solution in water, and keeps for one week once it is made up. It is expensive, but very effective, its pink colour fades as it is used up so it is possible to see at a glance if it is still fresh.
- **High-intensity ultraviolet light** can be used for disinfecting smooth surfaces such as dental tools, but not porous materials that are opaque to the light such as wood or foam. Ultraviolet light fixtures are often present in microbiology labs, and are activated only when there are no occupants in a room (e.g., at night).

Disinfection

- a reduction in the number of viable organisms
- Can be achieved by:
- Low-temperature steam
- Boiling water
- Chemical disinfectants

Low-temperature steam

- Most bacteria and viruses are killed by exposure to moist heat
- Usually achieved with dry saturated steam at 73 C for greater than 10 minutes
- Effective and reliable and suitable for instrument with a lumen
- Unsuitable for heat-sensitive items

Chemical disinfectants

- Destroys microorganisms by chemical or physicochemical means
- Different organisms vary in their sensitivity
  - Gram-positive - highly sensitive
  - Gram-negative - relatively resistant
  - Clostridial & mycobacterial species - very resistant
  - Slow viruses - highly resistant
- Disinfectants are suitable for heat-sensitive items
- Less effective than heat
- Chemicals used include:
  - Clear soluble phenolics
  - Hypochlorites
  - Alcohols
  - Quaternary ammonium compounds

Sterilisation

- Removal of viable microorganisms including spores and viruses
- Can be achieved by:
  - Autoclaves
  - Hot air ovens
  - Ethylene oxide
  - Low-temperature steam and formaldehyde
  - Sporicidal chemicals
  - Irradiation
  - Gas plasma

Autoclaves

- Steam under pressure has a higher temperature than 100 °C
- To be effective against viruses and spore forming bacteria need to
- Have steam in direct contact with material
- Vacuum has to be created
- Need to autoclave for 3 min at 134 °C or 15 min at 121 °C
- Check performance by colour changes on indicator tape
- Autoclaves are highly effective and inexpensive
- Unsuitable for heat-sensitive objects

Hot ovens

- Inefficient compared to autoclaves
- Requires temperatures of 160 °C for 2 hours or 180 °C for 30 min

Ethylene oxide

- Highly-penetrative and active against bacteria, spores and viruses
- Also flammable, toxic and expensive
- Leaves toxic residue on sterilised items
- Instruments therefore need to be stored for prolonged period before use
- Suitable for heat-sensitive items

Sporicidal chemicals

- Often used as disinfectants but can also sterilise instruments if used for prolonged period
- Inexpensive and suitable for heat-sensitive items
- Toxic and irritants
- 2% Gluteraldehyde is most widely used liquid sporicidal chemical
- Most bacteria and viruses killed within 10 minutes
- Spores can survive several hours

Irradiation

- Gamma rays and accelerated electrons are excellent at sterilisation
- Used as an industrial rather than hospital based method

Sterilization (or **sterilisation**)

- is the elimination of all transmissible agents (such as bacteria, prions and viruses) from a surface, a piece of equipment, food or biological culture medium. This is different from disinfection, where only organisms that can cause disease are removed by a disinfectant

- In general, any instrument that enters an already sterile part of the body (such as the blood, or beneath the skin) should be sterilized. This includes equipment like scalpels, hypodermic needles and artificial pacemakers. This is also essential in the manufacture of many sterile pharmaceuticals.
- Heat sterilization is known to have been in used in Ancient Rome, but it mostly disappeared throughout the Middle Ages where sanitation was not usually a concern.
- The preferred principle for sterilization is through heat. There are also chemical methods of sterilization.

Heat sterilization

- **Autoclaves**
- A widely-used method for heat sterilization is the autoclave. Autoclaves commonly use steam heated to 121°C (250°F), at 103 kPa (15 psi) above atmospheric pressure, for 15 minutes. The steam and pressure transfer sufficient heat into organisms to kill them.
- Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. It will not necessarily eliminate all prions (discussed later).

Autoclaves


MIDMARK
M7 SpeedClave
Chamber Temperature
Temperature Control
Timer
Fill/Vent
Drain

- **Other Methods**
- Other heat methods include flaming, incineration, boiling, tindalization, and using dry heat.

Flaming

- is done to loops and straight-wires in microbiology labs. Leaving the loop in a Bunsen burner flame until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects, but not for large objects (see Incineration below).

Incineration

- will also burn any organism to ash. It is used to sanitize medical and other biohazardous waste before its ash goes to the tip.

- **Incineration** is a waste treatment technology that involves the combustion of waste at high temperatures[1]. Incineration and other high temperature waste treatment systems are described as "thermal treatment". In effect, incineration of waste materials converts the waste into heat (that can be used to generate electricity), sends gaseous emissions to the atmosphere, and makes residual ash.

Boiling in water

- for 15 minutes will kill most bacteria and viruses, but boiling is ineffective against prions and many bacterial spores; therefore boiling is unsuitable for sterilization. However, since boiling does kill most bacteria and viruses, it is useful if no better method is available. Boiling is a simple and familiar enough process, and is an option available to most anyone most anywhere, requiring only water, enough heat, and a container that can withstand the heat; however, boiling can be hazardous and cumbersome.

Tindalization

- is a cumbersome process designed to reduce the level of activity of sporolating bacteria that are left by a simple boiling-in-water method. The process involves boiling for 20 minutes, cooling, incubating for a day, boiling for 20 minutes, cooling, incubating for a day, boiling for 20 minutes, cooling, incubating for a day, and finally boiling for 20 minutes again. The three incubation periods are to allow spores formed by bacteria in the previous boiling period to produce the heat-sensitive bacterial stage, which are killed by the next boiling step. Tindalization is ineffective against prions.

Dry heat

- can be used to sterilize items, but as the heat takes much longer to be transferred to the organism, both the time and the temperature must usually be increased, unless forced ventilation of the hot air is used. The standard setting for a hot air oven is at least two hours at 160°C (320°F). A rapid method heats air to 190°C (374°F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects. Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (for instance, it does not cause rusting of steel objects).

Chemical sterilization

- Chemicals are also used for sterilization. Although heating provides the most effective way to rid an object of all transmissible agents, it is not always appropriate, because it destroys objects such as most fiber optics, most electronics, and some plastics.

Ethylene oxid (EO)

- gas is commonly used to sterilize objects that cannot survive temperatures greater than 60°C such as plastics, optics and electrics. Ethylene oxide treatment is generally carried out between 30°C and 60°C with relative humidity above 30% and a gas concentration between 200mg/l and 800mg/l for at least 3 hours. Ethylene oxide penetrates very well, moving through paper, cloth, and some plastic films and is highly effective. Ethylene oxide however is highly flammable, and requires a longer time to sterilize than any heat treatment. The process also requires time for aeration post sterilization to remove toxic residues. Ethylene oxide is widely used and sterilizes around 50% of all disposable medical devices.

Ozone

- is used in industrial settings to sterilize water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.

Bleach

- is another accepted liquid sterilizing agent. Household bleach, also used in hospitals and biological research laboratories, consists of 5.25% sodium hypochlorite. At this concentration it is most stable for storage, but not most active. According to the Beth Israel Deaconess Medical Center Biosafety Manual (2004 edition), in most cases, it should be diluted to 1/10 of its storage concentration immediately before use; however, it should be diluted only to 1/5 of the storage concentration to kill *Mycobacterium tuberculosis*. This dilution factor must take into account the volume of any liquid waste that it is being used to sterilize. Bleach will kill many organisms immediately, but should be allowed to incubate for 20 minutes for full sterilization. Bleach will kill many spores, but is ineffective against certain extremely resistant spores. It is highly corrosive, even causing rust of stainless steel surgical implements.

Glutaraldehyde, formaldehyde

- Glutaraldehyde and formaldehyde solutions (also used as fixatives) are additional accepted liquid sterilizing agents, provided that the immersion time is long enough – it can take up to 12 hours for glutaraldehyde to kill all spores, and even longer for formaldehyde. (This assumes that a liquid not containing large solid particles is being sterilized. Sterilization of large blocks of tissue can take much longer, due to the time required for the fixative to penetrate.) Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has quite a short shelf life (<2 weeks), and is expensive. Formaldehyde is less expensive and has a much longer shelf life if some methanol is added to inhibit polymerization to paraformaldehyde, but is much more volatile. Formaldehyde is also used as a gaseous sterilizing agent; in this case, it is prepared on-site by depolymerization of solid paraformaldehyde.

Ortho-phthalaldehyde (OPA)

- is a sterilizing chemical which received Food and Drug Administration (FDA) clearance in late 1999. Typically used in a 0.55% solution, OPA shows better myco-bactericidal activity than glutaraldehyde. It also is effective against glutaraldehyde-resistant spores. OPA has superior stability, is less volatile, and does not irritate skin or eyes, and it acts more quickly than glutaraldehyde. On the other hand, it is more expensive, and will stain proteins (including skin) gray in color.

Hydrogen peroxid

- It is relatively non-toxic once diluted to low concentrations (although a dangerous oxidizer at high concentrations), and leaves no residue.
- The Sterrad 50 and other Sterrad sterilization chambers use hydrogen peroxide vapor to sterilize heat-sensitive equipment such as rigid endoscopes. The Sterrad 50 sterilizes in 45 minutes and also penetrates some lumen devices. The most recent Sterrad model, Sterrad NX, can sterilize most hospital loads in as little as 20 minutes and has greatly expanded lumen claims compared to earlier models. The Sterrad has limitations with processing certain materials such as paper/linens and long thin lumens. Paper products cannot be sterilized in the Sterrad system because of a process called cellulostics, in which the hydrogen peroxide would be completely absorbed by the paper product.

Dry sterilization process (DSP)

- is a process originally designed for the sterilization of plastic bottles in the beverage industry. It uses hydrogen peroxide with a concentration of 30-35% and runs under vacuum conditions. Using the common reference germs for hydrogen peroxide sterilization processes, endospores of different strains of bacillus subtilis and bacillus stearothermophilus, the Dry Sterilization Process achieves a germ reduction of 106...108. The complete cycle time of the process is 6 seconds. The surface temperature of the sterilized items is only slightly increased during the process by 10°-15°. Particularly due to the high germ reduction and the slight temperature increase the Dry Sterilization Process is also useful for medical and pharmaceutical applications

Radiation sterilization

- Methods exist to sterilize using radiation such as X-rays, gamma rays, or subatomic particles. Gamma rays are very penetrating, but as a result require bulky shielding for the safety of the operators of the gamma irradiation facility; they also require storage of a radioisotope, which continuously emits gamma rays (it cannot be turned off, and therefore always presents a hazard in the area of the facility). X-rays are less penetrating and tend to require longer exposure times, but require less shielding, and are generated by an X-ray machine that can be turned off for servicing. Subatomic particles may be more or less penetrating, and may be generated by a radioisotope or a device, depending upon the type of particle. Irradiation with X-rays or gamma rays does not make materials radioactive. Irradiation with particles may make materials radioactive, depending upon the type of particles and their energy, and the type of target material: neutrons and very high-energy particles can make materials radioactive, but have good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.
- Devices to irradiate objects are used, for example, by the United States Postal Service to sterilize mail in the Washington, DC area. Also, some foods are irradiated for sterilization.

Ultraviolet light

- (UV, from a germicidal lamp) can also be used for irradiation, but only on surfaces and some transparent objects (note that many objects that are transparent to visible light actually absorb UV). It is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation, so that it is very difficult to remove). It also damages many plastics, as can be seen if one forgets a polystyrene foam object in the cabinet with the germicidal lamp turned on overnight.