STERILIZATION & DISINFECTION

Definition

According to the CDC (Centers for Disease Control and Prevention), ***"Sterilization means the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores***."

Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.

Although the chemical or physical process used to destroy all pathogenic microorganisms including spores is not absolute, when all parameters of the sterilization process have been met, instruments, supplies and equipment are thought to be sterile.

Sterilization is categorised into:

1. Heat Sterilization
2. Filtration
3. Radiation sterilization
4. Chemical sterilization

The common sterilization methods within each category:

I. Heat Sterilization
- i. Dry Heat- Incineration
  Hot Air Oven
- ii. Moist Heat
  - Temperature below 100^{O}C
  - Temperature at 100^{O}C
  - Temperature above 100^{O}C

II. Filtration

III. Chemical
- i. Ethylene Oxide (EtO)
- ii. Low Temperature Plasma Vapor
- iii. Glutaraldehyde
- iv. Formaldehyde
- v. Ethanol
- vi. Chlorine Dioxide
- vii. Ozone

IV.Radiation
- i.Gamma
- ii. Electron Beam (E-Beam)
- iii. X-rays
- iv. Infrared rays
- v. UV rays

Whatever method of sterilization is chosen, the procedure must be validated for each type of material, both with respect to the assurance of sterility and to ensure that no adverse change should take place.

Biological indicators are used to validate sterilization methods and sometimes for routine control of individual cycles. Periodic revalidation is recommended.

I. Heat Sterilization

i. Dry-heat sterilization

In dry-heat processes, the primary lethal process is considered to be oxidation of cell constituents. Dry-heat sterilization requires a higher temperature than moist heat and a longer exposure time.

Incineration

870^0C - 980^0C
Complete burning to ashes
Used for soiled dressings, animal carcasses, pathological material, disposables, non-reusable soiled bedding

Flaming

250^0C – 300^0C
Points of forceps & Inoculation loops – heat in bunsen flame till red hot
Slow passage through flame to destroy vegetative bacteria on surface of scalpel blade, glass slides, mouth of test tubes

Hot Air Oven

Holding temp. & time: 160^0C for 1 hr.

Used for glassware, forceps, swabs, water impermeable oils, waxes & powders.

Before placing in hot air oven
- Dry glassware completely
- Plug test tubes with cotton wool
- Wrap glassware in Kraft papers
- Don't over load the oven.
- Allow free circulation of air between the material.

The method is, therefore, more convenient for heat-stable, non-aqueous materials that cannot be sterilized by steam because of its deleterious effects or failure to penetrate.

Preparations to be sterilized by dry heat are filled in units that are either sealed or temporarily closed for sterilization. The entire content of each container is maintained in the oven for the time and at the temperature given in the table below. Other conditions may be necessary for different preparations to ensure the effective elimination of all undesirable microorganisms.

Temperature (°C)	Minimum sterilization time (min)
160	60 mins
180	30 mins

Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The oven should normally be equipped with a forced air system to ensure even distribution of heat throughout all the materials processed. This should be controlled by monitoring the temperature. Containers that have been temporarily closed during the sterilization procedure are sealed after sterilization using aseptic techniques to prevent microbial recontamination.

The bio-indicator strain proposed for validation of the sterilization process is: spores of ***Bacillus subtilis*** (e.g. *var. niger* ATCC 9372) for which the D-value is 5-10 minutes at 160 °C using about 10^6 spores per indicator.

ii. Moist Heat

Moist heat - Temp below 100^0C

Pasteurization

- 63^0C – 30 min (Holder method)
- 72^0C – 15-20 sec (Flash method)
- 132^0C – 1 sec (Ultra high temp)

Vaccine baths - 60^0C – 60 min

- For vaccines of non-sporing bacteria

Water bath - 56^0C – 60 min – 3 days

- For serum / body fluids containing coagulable proteins

Inspissation – 80-85^0C – 30 min – 3 days

- For media containing egg or serum – LJ

Moist Heat - Temp at 100^0C

Boiling - 100^0C for 10 min.
Kills all vegetative bacteria.
Water should be soft, deionized or distilled.
2% sodium bicarbonate promotes the process.
Kills vegetative bacteria, hepatitis virus & some spores
Steaming (free steam) – 30-60 min in Arnold /Koch steamer
For heat labile media – DCA, TCBS
Tyndallisation (intermittent sterilization) - 100^0C, 30 min, 3 days. On I^{st} day all vegetative bacteria are killed. On II^{nd} & III^{rd} day spores that germinate are killed.
Nutrient media & media containing sugars or gelatin.

Moist heat - Temperature above 100^{O}C

Autoclave (steam under pressure) - 121^{0}C, 15-20 min, 15 lbs
Used for rubber articles, dressings, sharp instruments, infectious medical waste, culture media

Sterilization control

- **Browne's tube (red-green), Bowie & Dick tape (white-brown)**
- **10^{6} spore of *Bacillus stearothermophilus*. Incubate at 55^{0}C for 5 days**

Steam autoclave is the oldest, safest, and most cost effective method of sterilization. The steam reaches 121-148^{O}C (250-300^{O}F) in the pressure chamber at 15 P.S.I. The sterilization period is dependent on the temperature and size of load and can range from 10-60 minutes. This method is not well suited for heat sensitive materials and instruments.
The common types of steam sterilization cycles are *gravity-displacement*, which removes air from the chamber by gravity displacement as steam-entering chamber exerts pressure on air; and the *pre-vacuum* cycle, which removes air by a vacuum pump while steam is simultaneously injected into the chamber.

Exposure of microorganisms to saturated steam under pressure in an autoclave achieves their destruction by the irreversible denaturation of enzymes and structural proteins. The temperature at which denaturation occurs varies inversely with the amount of water present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. As displacement of the air by steam is unlikely to be readily achieved, the air should be evacuated from the autoclave before admission of steam. This method should be used whenever possible for aqueous preparations and for surgical dressings and medical devices.

In the steam autoclave process, microorganisms are killed by heat, and this is accelerated by the addition of moisture. Steam by itself is not sufficient for sterilization, and pressure that is greater than atmospheric is needed to increase the temperature of steam for thermal destruction of microbial life.

Steam, for a specified time at required temperature, must penetrate every fiber and reach every surface of items to be sterilized. When steam enters the sterilization chamber under pressure;

- It condenses upon contact with cold items.
- This condensation frees heat, simultaneously heating and wetting all items in the load, thus providing heat and moisture.

Any living thing will be killed when exposed to saturated steam at 120^{O}C (250^{O}F) longer than 15 minutes. As temperature is increased, time may be decreased.

The recommendations for sterilization in an autoclave are **20 minutes at 121-124 °C (200 kPa)/15lb pressure.** The temperature should be used to control and monitor the process; the pressure is mainly used to obtain the required steam temperature.

Minimum sterilization time should be measured from the moment when all the materials to be sterilized have reached the required temperature throughout. Monitoring the physical conditions within the autoclave during sterilization is essential. To provide the required information, temperature-monitoring probes should be inserted into representative containers, with additional probes placed in the load at the potentially coolest parts of the loaded chamber (as established in the course of the validation programme). Each cycle should be recorded on a time-temperature chart or by other suitable means.

Application:

1. Steam autoclave is used mostly for surgical instruments & sharp instruments.
2. In certain cases, glass, porcelain or metal articles, liquids in vented containers are sterilized at 121 - 124 °C for 20 minutes.
3. Fats and oils may be sterilized at 121 °C for 2 hours but, whenever possible, should be sterilized by dry heat.
4. Used for rubber articles, dressings, infectious medical waste, culture media

In certain cases (e.g. thermolabile substances), sterilization may be carried out at temperatures below 121 °C, provided that the chosen combination of time and temperature has been validated. Lower temperatures offer a different level of sterilization; if this is evaluated in combination with the known microbial burden of the material before sterilization, the lower temperatures may be satisfactory. Specific conditions of temperature and time for certain preparations are stated in individual monographs.

The bioindicator strain proposed for validation of this sterilization process is: spores of ***Bacillus stearothermophilus*** (e.g. ATCC 7953) for which the D-value (i.e. 90% reduction of the microbial population) is 1.5-2 minutes at 121 °C, using about 10^6 spores per indicator.

Filtration

Sterilization by filtration is employed mainly for **thermolabile solutions**. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Asbestos-containing filters should not be used.

Appropriate measures should be taken to avoid loss of solute by adsorption onto the filter and to prevent the release of contaminants from the filter. Suitable filters will prevent the passage of microorganisms, but the filtration must be followed by an aseptic transfer of the sterilized solution to the final containers which are then immediately sealed with great care to exclude any recontamination.

Usually, membranes of not greater than 0.22 µm nominal pore size should be used. The effectiveness of the filtration method must be validated if larger pore sizes are employed.

To confirm the integrity of filters, both before and after filtration, a bubble point or similar test should be used, in accordance with the filter manufacturer's instructions. This test employs a prescribed pressure to force air bubbles through the intact membrane previously wetted with the product, with water, or with a hydrocarbon liquid.

All filters, tubes, and equipment used "downstream" must be sterile. Filters capable of withstanding heat may be sterilized in the assembly before use by autoclaving at 121^{O}C for 15 - 45 minutes depending on the size of the filter assembly. The effectiveness of this sterilization should be validated. For filtration of a liquid in which microbial growth is possible, the same filter should not be used for procedures lasting longer than one working day.

Sterilization of of tissue culture media / thermolabile liquids :

The various types of filters used for clarifying or to remove the bacteria, fungi from the thermolabile liquids,media,solutions & buffers are as follows:

1. Earthenware Candles e.g., Berkfeld, Chamberland filters.
2. Asbestos Paper Disks e.g., Seitz Filter.
3. Sintered Glass Filters.
4. Membrane Filters.

1.Earthenware Candles

Berkfeld Filters:

Made from kieselguhr, a fossil diatomaceous earth found in deposits in Germany.Filters are of coarse type owing to the size of the granules forming the substance the substance of filter.

Made in three grades of porosity:

V: Veil (the coarsest) do not allow the *Serratia marscens,* the test bacteria to pass through).

W: Wenig (the finest).

N: Normal (the intermediate)

Filters can be sterilized by steaming/autoclaving. Filters should be brushed with a stiff nailbrush and than boiled in distilled water. When clogged with organic matter heated to redness in a muffle furnace and allowed to cool slowly.

Chamberland Filters:

Made up of unglazed porcelain and are produced in various grades of porosity, the finest grade allows only small viruses such as FMD virus, Circovirus.

Most porous grades **L_{1a}, L_2,** and **L_3** are comparable with **V, N,** and **W** candles respectively.

2. Asbestos Paper Disk Filters. Seitz Filters:

Disk of Asbestos is inserted into a metal holder (14 cm in diameter-Large size). Various sizes are available.

Made in three grades of porosity:

K : Clarifying.

N : Normal.

EK: Special grade.

Do not allow *Serratia marscens,* the test bacteria to pass.

For sterilization the filter is loosely assembled wit h the asbestos disk in position and the delivery tube passed through a rubber bang when filtering flask if used. The whole assembly is wrapped in Kraft paper and sterilized in autoclave. Plug the filtration flask and the side arm is fitted with an air filter.

Before using flush the disk with sterile saline and then screw down tightly the metal holder.

3.Sintered Glass Filters:

Made up of finely ground glass fused sufficiently to make small particles adhere, giving uniform average pore diameter (APD).

Manufactured in three grades of porosity:

Grade 5 : Finest.

Grade 3 : Coarsest.

Grade5/3: Special grade

After use sintered glass filters are washed with running water in the reverse direction. They should be cleaned with warm sulphuric acid + potassium nitrate.

4. Membrane Filters:

Two types of cellulose acetate membrane filters are available:

-Older type (Gradocol membrane) is composed of cellulose nitrate whereas the

-Modern membrane filters in use nowadays are made up of cellulose acetate.

Gradocol membranes: Made in different grades with average pore diameter ranging from 3um to 10 nm. Used to determine the size of many viruses.

Modern membrane filters (Cellulose acetate): Developed by Millipore Filter Corporation in America.

Common Chemical Sterilization

A. Ethylene Oxide (EtO) Gas

Ethylene Oxide gas was introduced in the 1950's, and it is an effective, low temperature chemical sterilization method. It also takes *longer* than steam sterilization, typically, 16-18 hours for a complete cycle. Temperatures reached during sterilization are usually in the 50-60□Crange.

Ethylene Oxide (EtO) is an industrial chemical used in sterilizing medical items, fumigating spices, and manufacturing other chemicals.

Pure EtO is a colorless gas at room temperature and a mobile, colorless liquid at –47^{O}C. Sold as a mixture with either carbon dioxide or fluorocarbon 12.

Ethylene oxide kills microorganisms by denaturing their proteins and subsequently modifying their molecular structure.

The active agent of the gas sterilization process can be ethylene oxide or another highly volatile substance. The highly flammable and potentially explosive nature of such agents is a disadvantage unless they are mixed with suitable inert gases to reduce their highly toxic properties and the possibility of toxic residues remaining in treated materials. The whole process is difficult to control and should only be considered if no other sterilization procedure can be used. It must only be carried out under the supervision of highly skilled staff.

The sterilizing efficiency of ethylene oxide depends on the concentration of the gas, the humidity, the time of exposure, the temperature, and the nature of the load. In particular, it is necessary to ensure that the nature of the packaging is such that the gas exchange can take place. It is also important to maintain sufficient humidity during sterilization. Records of gas concentration and of temperature and humidity should be made for each cycle. Appropriate sterilization conditions must be determined experimentally for each type of load.

After sterilization, time should be allowed for the elimination of residual sterilizing agents and other volatile residues, which should be confirmed by specific tests.

Bioindicator strains: spores of *Bacillus subtilis* (e.g. *var. niger* ATCC 9372) or of *Bacillus stearothermophilus,* (e.g. ATCC 7953).

There are some hazards associated with EtO use. Acute inhalation of high levels of EtO has resulted in nausea, vomiting, neurological disorders, bronchitis, pulmonary edema, and emphysema. Skin and eye contact with solutions of EtO has caused irritation of the eyes and skin in humans. Tests involving acute exposure of animals have shown EtO to have relatively high toxicity from oral and inhalation exposures.

A short-term effect of EtO in humans is mainly central nervous system depression and irritation of the eyes and mucous membranes. Chronic (long-term) exposure to ethylene oxide in humans can cause irritation of the eyes, skin, and mucous membranes, and problems in the functioning of the brain and nerves. Some human data show an increase in the incidence of leukemia, stomach cancer, cancer of the pancreas, and Hodgkin's disease in

workers exposed to EtO. EPA has classified EtO as a Group B1 hazard (probable human carcinogen).

EtO is not only present in sterilizers but also (in small concentrations) in the environment. Sources of environmental EtO include automobile exhaust and tobacco smoke.

Ethylene oxide (EtO) is a chemical agent that kills microorganisms, including spores. EtO gas must have direct contact with microorganisms on the items to be sterilized. Due to EtO being highly flammable and explosive in air, it must be used in an explosion-proof sterilizing chamber in a controlled environment.
Items sterilized by this process must be packaged with wraps and be aerated. The aeration time may be long and is needed to make sterilized items safe for
handling and patient use.

Note: There are also gas sterilizers available that use a mixture of EtO with carbon dioxide or chlorofluorocarbon (CFC) to represent it as nonflammable for use in healthcare facilities. In addition to safety concerns, this type of sterilization process requires an even *longer* aeration process compared to pure EtO sterilization.

In general, EtO gas is a reliable and safe agent for sterilization when handled properly.

Application:
EtO is used to sterilize items that are heat or moisture sensitive.

Disadvantages of EtO gas are that it can leave toxic residues on sterilized items and it possesses several physical and health hazards to personnel that merit special attention.

Since EtO poses several health hazards, the alternative technologies that is currently available: a plasma phase hydrogen peroxide-based sterilizing agent .

B. Low Temperature Hydrogen Peroxide Plasma
Low temperature plasma sterilization was introduced to fill the gap between autoclave: high temperature steam sterilization (safest, fastest and least expensive) and EtO gas sterilization, which leaves toxic residuals. It is a low temperature, non-toxic, but fairly expensive sterilization method.

In this process, hydrogen peroxide is activated to create a reactive plasma or vapor. Gaseous plasma is a new physical agent applied recently to sterilisation. High frequency energy initiates generation of the plasma from hydrogen peroxide vapours in a high vacuum and creates reactive species particles from the vapours that collide and kill microorganisms.

Note: Plasma is ionized gas made up of ions and electrons and is distinguishable from solid, liquid, or gas. Plasma is often referred to as the fourth state of matter. The Hydrogen Peroxide Gas Plasma Sterilization system with an operating temperature range of 45-50□C. Operating cycle times range from 45-70 minutes, depending on size of system.

This sterilization system uses a combination of hydrogen peroxide and low temperature as plasma to quickly sterilize most medical instruments and materials without leaving any toxic

residues. Hydrogen peroxide is a known antimicrobial agent that is capable of inactivating resistant bacterial spores. Sterilization by this method occurs in a low moisture environment.

The Hydrogen Peroxide Plasma Process:

The process consists of *two* consecutive and equal sterilization phases.

Vacuum / Preplasma Stage:

When a low pressure is achieved in the vacuum stage, low temperature air plasma is generated. This helps in removing residual moisture from the chamber. The system is then vented to atmospheric pressure at the end of this stage.

Sterilization Stage:

Pressure in chamber is reduced and an aqueous solution of **hydrogen peroxide** is injected and **vaporized** into chamber.

The hydrogen peroxide diffuses throughout the chamber, surrounds the items to be sterilized, and starts the inactivation of the microorganisms.

After the pressure is reduced, applying **radio frequency (RF) energy** creates an electric field and thus **forms low temperature plasma**.

Free radicals are generated in the plasma by breaking apart the hydrogen peroxide vapor.

Once the activated components react with the organisms and kill them, they lose their high energy and re-combine to form oxygen, water vapor, and nontoxic by-products.

This is half of the total sterilization process. The other half of the cycle is completed by repeating the above sterilization steps.

At the completion of the second half cycle, the source of RF energy is turned off, vacuum is released, and chamber is returned back to atmospheric pressure by introduction of filtered air.

Application:

This system is best suited to sterilize heat sensitive medical equipment .

C. Chlorine Dioxide

Chlorine Dioxide is a chemical liquid sterilization process. The best operating temperature range for this process is 25-30□C, while using low concentrations of ClO2. The process requires 6 hours of contact time to achieve sterilization. The presence of organic matter reduces activity. A processor converts a compound of dilute chlorine gas with sodium chlorite to form ClO2 gas and this gas is then exposed to the equipment in a sterilizing chamber.

Note: This alternative may corrode some materials and must be generated onsite. Prehumidification of the ClO2 is also required.

D. Ozone

Ozone sterilizes by oxidation, a process that destroys organic and inorganic matter. It penetrates membrane of cells causing them to explode.

In this process, a generator is used to convert oxygen to ozone, as a 6 to 12 percent concentration of ozone continuously flows through the chamber. Ozone penetration is controlled by vacuum pressure or by adding humidity. After the process is complete, oxygen is allowed to flow through the chamber to purge the ozone. The cycle time may be up to 60 minutes depending on the size of the chamber or load of items to be sterilized.

Ozone is formed by applying electrical energy to the oxygen molecule, which splits some portion of those oxygen molecules in half, into singlets of O. Therefore ozone molecules contain three atoms of oxygen and are unstable. Due to ozone gas being corrosive, and it being able to damage moisture sensitive equipment, there has not been much use of it in the medical industry.

Radiation Sterilization

Non ionising radiations–

Infra Red radiation (rapid mass sterilization of syringes, etc)

Ultra Violet radiation (enclosed areas)

Ultraviolet rays with wavelengths shorter than 300 nm are extremely effective in killing microorganisms. The most effective sterilizing range for UV is within the C bandwidth (UVC). This range is called the germicidal bandwidth. UVC has been used in hospitals for decades to sterilize surgical instruments, water, and the air in operating rooms.

How UV Light Works

Germicidal ultraviolet (UVC) light kills cells by damaging their DNA. The light initiates a reaction between two molecules of thymine, one of the bases that make up DNA. The resulting thymine dimer is very stable, but repair of this kind of DNA damage–usually by excising or removing the two bases and filling in the gaps with new nucleotides–is fairly efficient. Even so, it breaks down when the damage is extensive.

The longer the exposure to UVC light, the more thymine dimers are formed in the DNA and the greater the risk of an incorrect repair or a "missed" dimer. If cellular processes are disrupted because of an incorrect repair or remaining damage, the cell cannot carry out its normal functions. If the damage is extensive and widespread, the cell will die.

Ionising – Gamma, X ray, cathode ray (plastics, syringes, oil, metal foils)

Gamma, Beta Sterilization

Mode of Action

Both, X rays and Gamma rays have wavelength shorter than the wavelength of ultraviolet light. X rays, which have wavelength of 0.1 to 40 nm, and gamma rays, which have even shorter wavelength, are forms of ionizing radiation, so named because it can dislodge electrons from atoms, creating ions. (Longer wavelengths comprise nonionizing radiation.) These forms of radiation also kill microorganisms and viruses and ionizing radiation damages DNA and produces peroxides, which act as powerful oxidizing agents in cells. This radiation can also kill or cause mutations in human cells if it reaches them.

Irradiation is an effective sterilization method, but it is limited to commercial use only. The product to be sterilized is exposed to radiation for 10 to 20 hours, depending on the strength of the source. The highest temperatures reached in gamma sterilization are usually 30-40□C.

Gamma radiation is popular for sterilizing before shipment and it can be done through the packaging. A dose of 2.5 megarad is generally selected for many items. Ionizing radiation produces ions by knocking electrons out of atoms. These electrons are knocked out violently, and strike an adjacent atom and either attach themselves to it, or dislodge an electron from the second atom. The result is ionic energy that becomes converted to thermal and chemical energy.

This energy kills microorganisms by disruption of the DNA molecule, therefore preventing cellular division and propagation of biologic life.

The principal sources of ionizing radiation are **beta** particles and **gamma** rays.

Beta particles, free electrons, are transmitted through a high-voltage electron beam from a linear accelerator. These high-energy free electrons will penetrate into matter before being stopped by collisions with other atoms. This means their usefulness in sterilizing an object is limited by the density, thickness of the object and by the energy of the electrons. These free electrons produce their effect by ionizing the atoms they hit, producing secondary electrons that kill microorganisms.

Cobalt 60 is a radioactive isotope capable of breaking down to produce gamma rays. **Gamma rays** are electromagnetic waves that have the ability to **penetrate a much greater distance than beta rays** before losing their energy from collision. Because they travel with the speed of light, they must pass through a thickness measuring several feet before making sufficient collisions to lose all of their energy. Cobalt 60 is the most commonly used source for irradiation sterilization.

Gamma radiation and electron beams are used to effect ionization of the molecules in organisms. Mutations are thus formed in the DNA and these reactions alter replication. These processes are very dangerous and only well-trained and experienced staff should decide upon the desirability of their use and should ensure monitoring of the processes.

Application:
The radiation can change the properties of some materials like plastics and have adverse affects on glues or adhesives.

Sterilization controls:

Radiation doses should be monitored with specific dosimeters during the entire process. Dosimeters should be calibrated against a standard source on receipt from the supplier and at appropriate intervals thereafter. The radiation system should be reviewed and validated whenever the source material is changed and, in any case, at least once a year.

The bioindicator strains proposed for validation of this sterilization process are: spores of *Bacillus pumilus* (e.g. ATCC 27142) with 25 kGy (2.5 Mrad) for which the D-value is about 3 kGy (0.3 Mrad) using 10^7-10^8 spores per indicator; for higher doses, spores of *Bacillus cereus* (e.g. SSI C 1/1) or *Bacillus sphaericus* (e.g. SSl C_1A), *M. radiodurans* are used

E-Beam Radiation

In this process, the E-beam generator delivers a high dose of electrons in a narrow beam at the items to be sterilized. The electrons from the E-beam generator have limited penetrating power, less than gamma radiation. For example, a 10MeV Ebeam will penetrate about 5 cm of a unit-density material.

X-Ray Sterilization

This is a new developing process that is based on obtaining X-rays through conversion of electron beams. The X-rays produced have the same penetrating properties as the rays produced by Cobalt-60. But with this, treatment is faster, more flexible, and more environmentally friendly.
X-rays offer excellent product penetration in sterilization, thoroughly treating the surface and interior of a product.

Disinfection

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction of number of contaminating organisms to a level that cannot cause infection, i.e. pathogens must be killed. Some organisms and bacterial spores may survive.

Disinfectants are chemicals that are used for disinfection. Disinfectants should be used only on inanimate objects.**Antiseptics** are mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

The common disinfectants used in the medical & veterinary laboratories and hospitals are as follows:

A. Glutaraldehyde

Glutaraldehyde, which has been a known *disinfectant* in the medical industry.

Glutaraldehyde is an organic compound with the formula $CH_2(CH_2CHO)_2$.
A pungent colorless oily liquid, glutaraldehyde is used to disinfect medical and dental equipment. It is also used for industrial water treatment and as a preservative. It is mainly available as an aqueous solution, and in these solutions the aldehyde groups are hydrated.

No carcinogenic properties.

3.4% alkaline glutaraldehyde solution, has tuberculocidal and highlevel disinfection capabilities. It achieves high-level disinfection in 20 minutes at 25 □C and has up 28-day reuse life.

2.4% alkaline glutaraldehyde solution, which has tuberculocidal and high-level disinfection capabilities. It achieves high-level disinfection in 45 minutes at 25 □C and has up to a 14-day reuse life.

It is used to disinfect medical instruments and endoscopes. This solution can also be used in an automated reprocessor. (An automated reprocessor is the machine used to disinfect endoscopic and medical devices with a high level disinfectant solution.)

Both the concentrations have been used as a cold liquid high-level disinfectant for heat sensitive equipment.

*Note: G*lutaraldehyde products are being withdrawn from the European market due to concerns that it is toxic and harmful to health care staff in hospitals. Also, the U.S. market is requiring glutaraldehyde-free chemical solutions, which led to the formulation of the Cidex OPA solution. Cidex OPA solution is now known as the alternative to glutaraldehyde.

B. Ethanol

The effectivity of ethanol as e.g. desinfectant or antiseptic agent depends on the concentration of ethanol-water-mixture: An ethanol percentage of 50-80% destroys the cell wall/membrane of bacteria by denaturing their proteins and dissolving their lipids (effective against most bacteria, fungi and some viruses; ineffective against bacterial spores). Therefore, the ethanol has to pass the bacterial membrane/wall to get into the bacteria - if you use 100% ethanol instead, the bacteria get 'sealed' and they will survive... An other mechanism is the high osmotic pressure of ethanol/water-mixtures; and the 70% has the highest one.

C.Formaldehyde

Formaldehyde is used as a disinfectant and sterilant in both its liquid and gaseous states.

Formaldehyde is sold and used principally as a water-based solution called formalin, which is 37% formaldehyde by weight. The aqueous solution is a bactericide, tuberculocide, fungicide, virucide and sporicide.

It is indicated that formaldehyde should be handled in the workplace as a potential carcinogen and set an employee exposure standard for formaldehyde that limits an 8-hour time-weighted average exposure concentration of 0.75 ppm . The standard includes a second permissible exposure limit in the form of a short-term exposure limit (STEL) of 2 ppm that is the maximum exposure allowed during a 15-minute period .

Ingestion of formaldehyde can be fatal, and long-term exposure to low levels in the air or on the skin can cause asthma-like respiratory problems and skin irritation, such as dermatitis and itching. For these reasons, employees should have limited direct contact with formaldehyde, and these considerations limit its role in sterilization and disinfection processes.

Mode of Action. Formaldehyde inactivates microorganisms by alkylating the amino and sulfhydral groups of proteins and ring nitrogen atoms of purine bases .

Microbicidal Activity. Varying concentrations of aqueous formaldehyde solutions destroy a wide range of microorganisms.
Inactivation of poliovirus in 10 minutes required an 8% concentration of formalin, but all other viruses tested were inactivated with 2% formalin 72.
Four percent formaldehyde is a tuberculocidal agent, inactivating 104 M. tuberculosis in 2 minutes 82, and
2.5% formaldehyde inactivated about 107 Salmonella Typhi in 10 minutes in the presence of organic matter .

The sporicidal action of formaldehyde was slower than that of glutaraldehyde in comparative tests with 4% aqueous formaldehyde and 2% glutaraldehyde against the spores of B. anthracis. The formaldehyde solution required 2 hours of contact to achieve an inactivation factor of 104, whereas glutaraldehyde required only 15 minutes.

Uses. Although formaldehyde-alcohol is a chemical sterilant and formaldehyde is a high-level disinfectant, the health-care uses of formaldehyde are limited by its irritating fumes and its pungent odor even at very low levels (<1 ppm). For these reasons and others—such as its role as a suspected human carcinogen linked to nasal cancer and lung cancer . When it is used, direct exposure to employees generally is limited; however, excessive exposures to formaldehyde have been documented for employees of renal transplant units and students in a gross anatomy laboratory .
Formaldehyde is used in the health-care setting to prepare viral vaccines (e.g., poliovirus and influenza); as an embalming agent; and to preserve anatomic specimens; and historically has been used to sterilize surgical instruments, especially

when mixed with ethanol. A 1997 survey found that formaldehyde was used for reprocessing hemodialyzers by hemodialysis centers.

If used at room temperature, a concentration of 4% with a minimum exposure of 24 hours is required to disinfect disposable hemodialyzers reused on the same patient .

Paraformaldehyde, a solid polymer of formaldehyde, can be vaporized by heat for the gaseous decontamination of laminar flow biologic safety cabinets when maintenance work or filter changes require access to the sealed portion of the cabinet.

Exercise:

1. Draw well labelled diagrams of: Autoclave, Hot Air Oven, Seitz Filter, Sintered Glass Filter, Membrane Filter Assembly, & Syringe Filter.
2. What is rectified spirit? Write its role in disinfection.
3. Write the grades of Membrane filter used for filtration to remove the viruses.

References

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