STERILIZATION

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CONTENTS

- Definition
- Various agents used in sterilization
- Physical agents:

  Sunlight

  Drying

  Dry heat :Flaming,Inceneration,Hot air.

  Moist heat: Pasteurisation,boiling,steam under normal pressure, steam under high pressure.

  Filteration: candels asbestos pads,membranes

Cont....

- **Chemical agents:**
  - Alcohols : Ethyle, Isopropyl, Trichlorobutanol.
  - Aldehydes :Formaldehyde, Glutaraldehyde.
  - Dyes
  - Halogens
  - Phenols
  - Surface active agents
  - Metallic salts
  - Gases: ethylene, oxide,formaldehyde, beta propiolactone.
- Generally used sterilization methods in laboratory.
- Asepsis in the operating room
- Summery and conclusion

Definition of sterilization:

- Sterilization is making a substance free from all micro organisms both in vegetative and sporing states.

#Spore is a reproductive structure that is adapted for dispersal and surviving for extended periods of time in unfavourable conditions.

Spores form part of the lifecycles of many bacteria, plants, algae , fungi and some protozoa.

Terms used in sterilization:

- **Disinfection:**
  - The destruction or removal of all pathogenic organisms capable of giving rise to infection.

Disinfection does not affect spore state organisms.

- **Antisepsis:**
  - The term is used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues.
  - This is done by the antiseptics
  - Chemicals or disinfectants which can be safely applied on skin or mucous membrane to prevent infection by inhibiting the growth of bacteria.
- **Bactericidal agents / germicides:**
  - Those which able to kill bacteria.

Bacteriostatic agents:

- Only prevent multiplication of bacteria, but they remain alive.

Cleaning:

- Important preparatory step before sterilization or disinfection, by removing soil and other dirt.

Decontamination:

- The process of rendering an article or area free of contaminants, including microbial, chemical, radioactive and other hazards.

METHODS/AGENTS OF STERILIZATION

Classification of Sterilization:

Physical agents:

- Sunlight
- Drying
- Heat

  Dry heat: flaming, incineration, hot air

  Moist heat: pasteurization, boiling, steam under pressure.
- Filtration: candles, asbestos pads, membranes
- Radiation
- Ultrasonic and sonic vibrations.

Chemicals agents:

- Alcohol
- Ethyl, isopropyl, trichlorobutanol
- Aldehydes
- Formaldehyde, glutaraldehyde
- Dyes
- Halogens
- Phenols
- Surface active agents
- Metallic salts
- Gases: Ethylene oxide, formaldehyde, beta propiolactone

Physical methods of Sterilization

Sunlight:

- Action primarily due to UV rays however, effects vary due to places

Eg: In tropical country, the germicidal effect is better than 4 seasoned countries.

Bacteria in water are readily destroyed by sunlight.

Drying:

- Moisture is essential for growth of bacteria.
- Drying in air has deleterious effect on many bacteria.
- However, spores are unaffected.

Therefore, it is not really unreliable.

Heat:

- Most reliable method of sterilization and should be the method of choice.

Dry Heat & Moist Heat.

The factors influencing sterilization by heat:

- Nature of heat-dry or moist
- Temperature and time
- Number of microorganisms present
- Characteristics of organisms –species, strain, sporing capacity
- Type of material from which organism have to be eliminated.
- Killing effect is due to protein denaturation, oxidative damage and toxic effect of elevated level of electrolytes.
- Killing effect of moist heat due to denaturation and coagulation of proteins.

Thermal Death Time:TDT

"Minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.
Thermal death time is inversely proportional to temperature.
TDT is increased in presence of organic substance, proteins, nucleic acid, starch, gelatin , sugar , fats, oils."

Dry heat:

Flaming:

- Heating over fire, till they become red hot.
- Instruments like: Point of Forceps, Spatulas, Inoculating loops and Wires.

(Inoculating loop is better dipped in disinfectant first before flaming to prevent spattering)

Incineration:

- It is a process that involves the combustion of organic substances contained in waste materials.
- Items: contaminated cloth, animal carcasses and pathological material. PVC, polythene can be dealt.

(However, polystyrene will emit black smoke. Hence should be autoclaved in appropriate container.)

Hot air oven:

- Hot air ovens are electrical devices used in sterilization.
- The oven uses dry heat to sterilize articles.
- Generally, they can be operated from 50 to 300 C (122 to 572 F) .
- There is a thermostat controlling the temperature.
- This is the most widely used method of sterilization by dry heat.

Items: glassware, forceps, scissors, scalpels, all-glass syringes, swabs, liquid paraffin, dusting powder, fats, grease.

(Materials should be properly arranged to allow free circulation of air)


HOT AIR CIRCULATING OVEN

Precautions:

- Glass wares should be dry.
- Oven should not be over loaded.
- Articles are to be arranged in a manner to allow free circular of air.
- Door of the Oven should be opened after it cools down (2Hours).

Temerature (c)	Holding time(in minutes
160	45
170	18
180	7.5
190	1.5

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Advantages & Disadvantages:

- They do not require water and there is not much pressure build up within the oven, unlike an autoclave, making them safer to work with.
- Suitable to be use in a laboratory environment.
- They are much smaller than autoclaves but can still be as effective.
- As they use dry heat instead of moist heat, some organisms like prions, may not be killed by them every time.

Moist heat:

Moist heat can be categorized into 3 groups:

- **Temperature below 100 C**
- **Temperature at 100 C**
- **Temperature above 100 C**

Pasteurisation of milk

- **Holding period: 63 C, 30 minutes (holder method) ; or 72 C, 15-20 minutes followed by cooling quickly to 13^0c or lower.**
- **Target: all nonsporing pathogens**

Eg: mycobacteria, brucellae, salmonella.Coxiellaburnetti, relatively heat resistant, may survive the holder method.

Temperature at 100 C:

Boiling

Not recommended for sterilising but used for disinfection. sterilization may be promoted by addition of 2% sodium bicarbonate to the water.

Holding period: 10-30 minutes.

Steam at atmospheric pressure (100 C)

Used to sterilize culture media.

This is an Inexpensive method

Holding period: 100 C, 20 minutes on three succesive days (intermittent sterilization).

Principle: first exposure kills vegetative bacteria and then the next exposure will kill vegetative bacteria that matures from the spore.

Steam under pressure

- Autoclave/steam sterilizer--: autoclave is a device that uses steam to sterilize equipment and other objects.
- This means that all bacteria, viruses, fungi, and spores are inactivated.
- However, prions may not be destroyed by autoclaving at the typical 134 C for 3 minutes or 121 C for 15 minutes.

Principle:

Water boils when its vapour pressure equals the surrounding atmosphere. Thus, when pressure inside closed vessels increases, the temperature at which water boils increases too.

Holding period: varies.

Temperature: between 108 C and 147 C.

Items: dressings, instruments, laboratory ware, media and pharmaceutical products.

Several types of steam sterilizer:

- Laboratory autoclaves
- Hospital dressings sterilizers
- Bowl and instrument sterilizers
- Rapid cooling sterilizers
- Even a domestic pressure cooker can be used as a steriliser.

Recommended temperature and duration:

Temperature(c)	Duration(min)
121	15
126	10
134	3

Disadvantges of autoclave

- Some plastic ware melts in the high heat, and sharp instruments often become dull.
- Moreover, many chemicals breakdown during the sterilization process and oily substances cannot be treated because they do not mix with water.

Filtration:

- Helps to remove bacteria from heat labile liquids

Items: sera and solutions of sugars or antibiotics.

Principle: as viruses pass through the ordinary filters, filtration can be used to obtain bacteria-free filtrates of clinical samples for virus isolation.

Types of filters:

- Candle filters
- Asbestos filters
- Sintered glass filters
- Membrane filters.

Candel filters:

- Used for purification of water for industrial and drinking purposes.
- These are manufactured under different grades of porosity.

There are 2 types of candel filters

1)Unglazed ceramic filters

2)Diatomaceous filters.

Asbestos filters:

- Disposable, single-used disc
- Tend to alkalinise filtered liquids.

Usage is discouraged because of its carcinogenic property.

Eg: Seitz and Sterimat filters

Sintered glass filters:

- Has low absorptive properties
- Brittle and expensive.

Membrane filters:

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

Radiation:

2 types of radiation: Ionising radiation &Non-ionising radiation

Non-ionising radiation

- Infrared-Used for rapid mass sterilization of prepacked items such as Syringe,Cathaters
- UV
- Used for disinfecting enclosed area such as entryways, operation theatres and labs.

Ionising radiation

Gamma rays:

X-rays:

Used for sterilising plastics, syringes, swabs, catheters, animal feeds, cardboard, oils, greases, fabric and metal foils.

Testing - Efficacy of Radiation

Radiation control indicator disc:

Special paper sticker which is yellow in colour, is sticked on the articles. After proper sterilization disc colour changes to red.

Chemical methods of Sterilization

Chemical agents:

Action of chemical agents:

- Protein coagulation
- Disruption of cell membrane resulting in exposure, damage/loss of contents
- Removal of sulfhydryl group essential for normal functioning of enzyme
- Substate competition.

Commonly used chemical

Alcohol

- Frequently used are Ethyl alcohol ,Isopropyl alcohol
- These must be used at concentration 60-90%.
- Isopropyl alcohol used in disinfection of clinical thermometer.
- Methyl alcohol is effective against fungal spores, treating cabinets and incubators.
- Methyl alcohol is also toxic and inflammable.

Aldehyde

Formaldehyde:

- Having Bactericidal, sporicidal and has lethal effect on viruses.
- Used to preserve anatomical specimens, destroying anthrax spores on hair and wool.

Glutaldehyde:

- Effective against tubercle bacilli, fungi, viruses.
- Less toxic and irritant to eyes, skin
- Used to treat anaesthetic rubber, face masks, plastic endotracheal tubes, metal instruments and polythene tubing.

Dyes:

2 groups of dyes:

1. Aniline dye
2. Acridine dye

- ✓ Both are bacteriostatic in high dilution but are of low bactericidal activity.
- ✓ Aniline dye is more active against gram +ve than gram-ve organisms.

Some important dyes:

Proflavine
Acriflavine
Euflavine
Aminacrine

- ✓ These Impair the DNA complexes of the organisms and thus kill or destroy the reproductive capacity of the cell.

Halogens

Iodine

- ✓ Used as Skin disinfectant
- ✓ Having Active bactericidal activity &moderate action on spores.

Chlorine

- ✓ Used to disinfect Water supplies, swimming pools and food and dairy industries.
- ✓ Along with hypochlorides are bactericidal. Also act on viruses.

Phenols

- ✓ These are obtained from distillation of coal tar between 170-270 C.

Lethal effects are:

- ✓ Capacity to cause cell membrane damage, releasing cell contents and causing lysis.
- ✓ Low concentration will precipitate proteins.

Gases:

Types of gases used for sterilization:

Ethylene oxide
Formaldehyde gas
Beta propiolactone (BPL).

Ethylene oxide

- ✓ Action is due to its alkylating the amino, carboxyl, hydroxyl and sulphydryl groups in protein molecules.
- ✓ Also on DNA and RNA.

Items: heart-lung machines, respirators, sutures, dental equipment, books, clothing.

Formaldehyde gas:

- This is widely employed for fumigation of OT and other rooms.
- Formaldehyde is produced by adding 150g of KMnO4 to 280ml of formalin for every 1000cu.ft of roomvolume, after closing the windows and other outlets.
- After fumigation, the doors should be sealed and left unopened for 48 hours.

Betapropiolactone:

- ✓ Product of ketane and formaldehyde with a boiling point of 163 C.
- ✓ Having rapid bactericidal activity but carcinogenic.
- ✓ Capable of killing all microorganisms and is very active against viruses.

Surface-active agents:

Substance that alter the energy relationship at interfaces, producing a reduction of surface or interfacial tension is called surface active agents.

These are widely used as wetting agents, detergents and emulsifiers.

There are 4 main groups:

- ✓ **Anionic**
- ✓ **Cationic**
- ✓ **Nonionic**
- ✓ **Amphoteric**

Metallic salts:

- ✓ Though all salts have a certain amount of germicidal action depending on their concentration,salts of heavy metals have a greater action.

Eg: salts of silver, copper and mercury

These are Protein coagulants and have capacity to combine with free sulfhydryl group of cell enzymes.

Monitoring the Effectiveness of Sterilization

- To ensure that sterilization has been successful the process of sterilization (and not the end product) is tested.
- Indicators have been developed to monitor the effectiveness of sterilization by measuring various aspects of the process through different indicators.

Mechanical indicators

- These indicators, which are part of the autoclave or dry-heat oven itself, record and allow you to observe time, temperature, and/or pressure readings during the sterilization cycle.

Chemical indicators

- Tape with lines that change color when the intended temperature has been reached.
- Pellets in glass tubes that melt, indicating that the intended temperature and time have been reached.
- Indicator strips that show that the intended combination of temperature, time, and pressure has been achieved.
- Indicator strips that show that the chemicals and/or gas are still effective.
- Chemical indicators are available for testing ethylene oxide, dry heat, and steam processes. These indicators are used internally, placed where steam or temperature take longest to reach, or put on the outside of the wrapped packs to distinguish processed from nonprocessed packages.

Biological indicators

- These indicators use heat-resistant bacterial endospores to demonstrate whether or not sterilization has been achieved.
- If the bacterial endospores have been killed after sterilization, you can assume that all microorganisms have been killed as well.
- After the sterilization process the strips are placed in a broth that supports aerobic growth and incubated for 7 days.
- The advantage of this method is that it directly measures the effectiveness of sterilization.
- The disadvantage is that this indicator is not immediate, as are mechanical and chemical indicators.
- Bacterial culture results are needed before sterilization effectiveness can be determined.

Ideal antiseptic/disinfectant should be:

- Effective against all microorganisms
- Be active in presence of organic matter
- Effective in acid and alkaline media
- Have speedy action
- Have high penetrating power
- Stable
- Compatible with other antispetics and disinfectant
- Should not corrode metals
- Not cause local irritation or sensitisation
- Not interfere with healing
- Not toxic if absorbed into circulation
- Inexpensive and easily available.

Factors determine the potency of disinfectants:

- Concentration of the substance
- Time of action
- pH of the medium
- Temperature
- Nature of the organisms
- Presence of extraneous material.

Testing of disinfectants

- ✓ There is no single reliable test available to determine the efficiency of a disinfectants due to the number of parameters which influence disinfectant activity.
- ✓ Traditionally in such tests phenol is taken as standard.

Generally used sterilization methods in laboratory

Dry Heat

- Glassware and plastic ware (empty vessels), and instruments may be sterilized by dry heat in an oven at 160-180 C for 3 hr.
- But most people prefer to autoclave.
- More recently, glass bead sterilizers (300 C) are being employed for the sterilization of forceps, scalpels, etc.; these devices use dry heat.

Flame Sterilization

- Instruments like forceps, scalpels, needles, etc. are ordinarily flame sterilized by dipping them in 95% alcohol followed by flaming.
- These instruments are repeatedly sterilized during the operation to avoid contamination.
- It is customary to flame the mouths of culture vessels prior to inoculation/subculture.

Autoclaving

- Culture vessels, etc. (both empty and containing media) are generally sterilized by heating in an autoclave or a pressure cooker to 121 C at 15 (1.06 kg/cm2) for 15 (20-50 ml medium) to 40 (21 medium) minutes.
- Sterilization during autoclaving depends mainly on temperature.
- Certain types of plasticware and some instrument, e.g., micropipettes, etc., are also autoclavable.
- Care should be taken to properly stopper all the vessels and to open the autoclave only when its pressure gauge indicates zero pressure.

Ayurvedic concept of Sterilization

- Nirjantukarana is equivalent term for sterilization.
- No direct reference is available.
- Scattered references gives the idea of aseptic approach to yantra and shastra karma.
- Prior to surgical procedure preparation of patient and surgeon by removing hairs, cutting of nails and wearing of clean and washed cloths have been advocated.

References of Nirjantukarana

ALRÉJÉÉ AIÉMIÉÉ
VÉÉXGÉ NÀÛSLÉÀ MÉÉMÜ PÉRÉQÉÇ
XRÉÉIÉÇ II
(XÉÑ, ÍCÉ, 2/46) QÛSWÛHÉ

Dhupana vidhi:

Exposing the part or the article by Dhuma of krimighna drugs. Vruna dhupana prevents affliction of krimis and rakshasas.

Dhupana dravyas:

- **Guggulu, Agaru, Sarshapa, Vacha, Nimba patra, Sarjarasa, Lavana, Ghruta etc.**
- **Dhupana has to be given two times in a day.**
- **Dhupana vidhi has been mentioned for bndha dravyas, patients dresses, beddings and also for Shastragara and Vrunitagara.**

Summary:

- Sterilization is a process or killing all microorganisms (including spores) on or in a material or object.
- The factors that determine the type of sterilization or disinfecting process to be used include time, temperature ,stage of growth of the organism ,nature of the medium in which the organism is suspended (air, gas ,liquid) and the number of organism present.
- Sterilization and disinfection can be achieved by using heat, filtration, chemical or radiation etc.
- Overall, heat is the best means of sterilization, but other methods are used for heat labile objects.
- Dry heat requires more time than wet heat to kill organisms ,boiling kills most vegetative cells but not bacterial spores & pressure cookers &autoclaves achieve sterilization.

Conclusion:

- Sterilization is the most important procedure.
- Sterilization has major share in Success of surgical management.
- No elaborate description is available in Ayurvedic texts.
- Hints are given to perform surgeries under aseptic precautions.
- Dhupana dravyas are to be studied for their krimighna properties.
- An attempt to evolve Ayurvedic sterilization procedures is absolute necessity.

Thank you

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