STERILIZATION AND ASEPSIS

Mrs. Srushti S. Patil. Assistant Professor. Department of Biotechnology (Entire)

• General principals of asepsis are laid down by Hungarian.

• These principles were accepted after **Joseph Lister (Father of antiseptic surgery**) studied prevention of wound infection(1865-1891).

DEFINATIONS

• **CLEANING** - It is a process which removes **visible contamination** but does not necessarily destroy micro organisms. It is necessary prerequisite for effective disinfection or sterilization.

• ASEPSIS -Term used to describe methods which prevent contamination of wounds and other sites, by ensuring that only sterile object and fluids come into contact with them. ANTISEPSIS - It is the procedure or application of an antiseptic solution or an agent which inhibits the growth of microorganisms, while remaining in the contact with them.

DISINFECTION - it is a process which reduces the number of viable microorganisms to an acceptable level but may not inactive some viruses and bacterial spores.

STERLIZATION - it is the process of destruction or removal of all microorganisms from article, surface or medium, including spores. Sanitizing - process that reduces microbial population on object to a safe level.

Decontamination - process that removes pathogenic microorganisms from an object to make it safe to handle.

DISINFECTION

- Process that eliminates defined pathogens Not all microbial forms
- Main difference with sterilization = the lack of

sporocidal activity

- Categorized into 3 levels:
 - High,
 - Intermediate
 - Low

Resistance of Microorganisms



Dental instruments are classified into three categories –

critical, semi critical, or non critical depending on their risk of transmitting infection and the need to sterilize them between uses.

SPAULDING CLASSIFICATION

	Item	comes in contact with	Туре
	recommended		
0	Critical	Tissue, vascular space	Sterilization
0	Semicritical	Mucous membrane	High level disinfection
		Non intact skin	High level disinfection
0	Noncritical Intermediate or	Intact skin only	
		not mucous membranes	low level disinfection

Critical

Semicritical

Noncritical







To achieve sterilization of any instrument three definite stages are to be completed-

- Pre sterilization cleaning
- Sterilization process
- > Aseptic storage

PRESTERILIZATION CLEANING

• <u>Objective</u>-

Removal of the organic matters, blood and saliva which provide protective barrier for microorganisms and prevents its destruction.

- There are three methods for cleaning
 - -Manual
 - -Ultrasonic
 - -Mechanical washing

MANUAL CLEANING

- Simplest and the cheapest method, but time consuming and difficult to achieve.
- heavy duty gloves and glasses must be worn to protect needle stick injury and to protect eye.
- Material used for manual cleaning
 - -Soaps
 - -Detergents

ULTRASONIC CLEANING



Principle- conversion of electrical energy into vibratory sound waves which pass through a soap solution containing the instrument.

Used mainly for burs, bone files, bone cutter, artery forceps, saw etc.

MECHANICAL WASHING



<u>**Principle</u>**- High-pressure jets of water with or without a detergent which removes debris from instrument.</u>

Small instrument like burs, blade are not suitable for this type of cleaning.

<u>Classification of the method of</u> <u>sterilization/Disinfection</u>

A. PHYSICAL

- 1- Sun Light
- 2- Drying
- 3- Heat i- Dry
 - ii-Moist
- 4- Filtration
- 5- Gas
- 6- Irradiation
- 7- Ultra sonic cleaning

B. CHEMICAL

- 1. Phenol Derivatives : Phenol, Cresol, resorcinol, chloroxylenol
- Oxidizing agents :Pot.Permanganate, Hydrogen Peroxide,Benzoyol Peroxide
- 3. Halogens : Iodine, chlorine
- 4. Biguanide : Chlorhexidine
- 5. Quarternary Ammonium (Cationic) : Cetrimide, Zephiran
- 6. Alcohols : Ethanol, Isopropanol.
- 7. Aldehydes : Formaldehyde, Glutaraldehyde
- 8. Acids : Boric acid, acetic acid
- 9. Metallic salts; Silver Nitrate, Zince Sulfate, Zinc Oxide, calamine,
- 10. Dyes : Gentian violet, proflamine, Acriflamine
- 11. Furan derivatives : Nitro flurazone



Most common and one of the most effective methods of sterilization. Factors influencing sterilization by heat are : -

- i. Nature of heat
 - a. Dry
 - b. Moist
- ii.Temperature & time
- iii. No. of organism present
- iv.Whether organism has sporing capacity
- v. Type of material from which organism is to be eradicated



Killing is due to :

- Dehydration and oxidation of organisms
- Protein denaturation
- Toxic effects of elevated levels of electrolytes
- **1.** <u>Red Heat</u> : It is used to sterilize metallic objects by holding them in flame till they are red hot. Example : inoculating wires, needles, forceps etc.
- Flaming : The article is passed over flame without allowing it to become red hot. Example : Glass plates, Cotton wool plays and glass slides.

3. Hot air oven :

It is used to sterilize items, which do not get damaged by high temp. such as laboratory glass, flasks, instruments with sharp cutting edges, B.P. handles, Powders, Dapen dishes, mouth mirrors.



<u>Temp. & Time</u>: The sterilization is complete if these two factors are achieved throughout the load.

Temperature	Time(Min)
140°C	180
150°C	150
160°C	60
170°C	45
180°C	18
190°C	7.5

Sterilization Control of Hot Air Oven

• The spores of non-toxigenic strain of Bacillus subtilis and Clostridium tetani are used as a microbiological test of dry heat.



Browne's test strip available that contain a chemical indicator.



4. <u>GLASS BEADS STERILIZER</u> :

- •The media used are glass beads, molten metal and salt.
- •The temperature achieved is of 220°C.
- •The method employs submersion of small instruments such as Endodontic files, artery forceps, scissors and burs, into the beads; and are sterilized in 10 seconds provided they are clean.
- •A warm-up time of at least 20 minutes to ensure uniform temperatures in these sterilizers.



B. <u>Moist heat</u>

Causes denaturation and coagulation of proteins.

1. Pasteurization :

The temperature employed is either 63° C for 30mins (**Holder method**) or 72° C for 15-20 seconds (**Flash method**) followed by cooling quickly to 13° C.

Method is used for heat sensitive liquid and pharmaceutical products.

2. <u>Tyndallisation</u> :

Named after John Tyndall.

Exposure of 100°C for 20 min for 3 successive day.

Principle: 1st exposure kills all vegetative bacteria & **spores**, since they are in a favorable medium, will germinate and be **killed on subsequent** occasions.

3. <u>AUTOCLAVE</u> :

Steam is the effective means of sterilization, because of its

- **1.** High penetrating capacity.
- **2.** It gives of large amount of heat to surface with which it comes in contact.





Autoclaves, or steam sterilizers essentially consist of following:

i) A cylindrical or rectangular chamber, with capacities ranging from 400 to 800 liters.

ii) Water heating system or steam generating system

iii) Steam outlet and inlet valves

iv) Single or double doors with locking mechanism.

v) Thermometer or temperature gauge

vi) Pressure gauges

• To achieve sterility, a holding time of at least 15 minutes at 121 °C (250 °F) or 3 minutes at 134 °C (273 °F) at 15 psi (100 kPa) above atmospheric pressure is required.

• To Avoid corrosion *Crawford and Oldenburg* recommended addition of ammonia to the autoclave

Sterilization control of the moist heat

Physical Indicator- an alloy designed to melt only after being subjected to relevant holding time.

Chemical indicator- Strips or tapes that change color once the correct conditions have been met.

Biological indicator- Spores of Geobacillus stearothermophilus are used as the test organisms as it is toughest organism for an autoclave to destroy.

Its spores require an exposure of 15 mins at 121°c to be destroyed.

FILTRATION

- Help to remove bacteria from heat labile liquids.
- As viruses pass through ordinary filters, it can be used to obtain bacteria free filtrates of virus isolation.
- TYPES:
 - Candle filter
 - Asbestos filter
 - Sintered glass filter
 - Membrane filter

IRRADIATION

Radiation used for sterilization is of two types

- 1. Ionizing radiation, e.g., X-rays, gamma rays, and high speed electrons .
- 2. Non-ionizing radiation, e.g. ultraviolet light, and infrared light.

These forms of radiation can be used to kill or inactivate microorganisms.

1. Ionizing Radiation

X-rays, gamma rays and cosmic rays are highly lethal to DNA and other vital constituents.

They have high penetration power.

There is no appreciable increase in temperature, thus referred to as **cold sterilization**.

Commercial plants use gamma radiation for sterilizing plastics, syringes, swabs, catheters etc.

2. Non-ionizing radiation

Two types of non-ionizing radiations are used for sterilization:-

• A. Ultraviolet -

Short range UV(UVC) is considered **"germicidal UV".** At a wavelength of **2537 Angstroms** UV will destroy micro-organismal DNA.

Used mainly for air purification and water purification in hospitals.

o B. Infrared –

It is most commonly used to purify air, such as in the operating room. Infrared is effective, however, it has no penetrating ability.

ETHYLENE OXIDE STERILIZATION (ETO)

- Used almost exclusively to sterilize medical products that cannot be steam sterilized or sensitive to radiation.
- *Mechanism of action:* It destroys micro-organisms by alkylation and cause denaturation of nucleic acids of micro-organisms.
- At 30 °C 60°C with relative humidity above 30 % and gas conc. between 200 and 800 mg/l for at least 3 hours.



- Ethylene oxide is a colorless liquid with a boiling point of 10.7 °C.
- Highly penetrating gas with sweet ethereal smell.
- Highly inflammable & in conc. greater than 3%, highly explosive.
- By mixing with inert gases such as CFC or CO2, explosive tendency is eliminated.
- Plastics, rubber & photographic equipments can be sterilized by this method.
- Also used for mass sterilization of disposable items, plastic
 - syringes, needles, catheters, blades etc.

• Disadvantages

- Lengthy cycle time
- $-\operatorname{Cost}$
- Potential hazards to patients & staff

• Advantage:

Can sterilize heat or moisture sensitive medical equipments.

Aseptic storage

The maintenance of sterility during transportation and storage is of utmost importance.

- Instruments are kept wrapped until ready for use .
- To reduce the risk of contamination, sterile packs must be handled as little as possible.
- Sterilized packs should be allowed to cool before storage; otherwise condensation will occur inside the packs.
- To prevent contamination from rodents, ants, and cockroaches, the store must be subjected to adequate pest control.
- Materials should be stored at least 8" off the floor and 18" from the ceiling
- Sterile packs must be stored and issued in correct date order. The packs, preferably, are stored in drums which can be locked. Preset trays and cassettes, are useful as, the instruments can be organized as per the procedure

FUMIGATION OF OPERATION THEATRE

- Fumigation of the operation theatre is achieved by fumigator and potassium permanganate reaction technique.
- The chemical used is 40% formaline.



Factors influencing the fumigation of the theatre :

1. Relative humidity

Relative humidity plays a major role in fumigation. A minimum of 70% is essential. Water used in fumigator with fumigant helps to achieve and maintain humidity.

2. Temperature

temperature for effective fumigation is 30°-40°C.

3. Formaldehyde levels in the Air in the operation theatre

The dose of formaline is usually decided by the size of the room. As a rule, 180 ml is used for a room of the size 1000 cubic feet.

CHEMICAL METHODS

No available chemical solution will sterilize instruments immersed in it.

Secondly, there is a risk of producing tissue damage if residual solution is carried over into the wound while it is being used.





Mechanism of action of chemical disinfectants :

The mechanism of action of most of the chemicals are nonspecific and complex but most of them effect microorganisms by one of the following mechanisms.

- 1. Cell membrane injury.
- 2. Coagulation and Denaturation.
- 3. Interactions with functional groups of proteins.

1. ALDEHYDE COMPOUNDS

a. <u>Formaldehyde</u>:

A broad-spectrum antimicrobial agent, used for disinfection, has limited sporicidal activity.

Hazardous substance, inflammable and irritant to the eye, skin and respiratory tract.

- a. <u>Glutaraldehyde</u>:
- It is a high level disinfectant

A solution of 2% glutaraldehyde (Cidex), requires immersion of 20 minutes for disinfection; and 6 to 10 hours of immersion for sterilization.

2. ALCOHOLS

Act by **denaturing bacterial proteins**.

Solutions of **70% ethanol** are more effective than higher concentrations, as the presence of water speeds up the process of protein denaturation as reported by Lawrence and Block (1968).

Frequently used for skin antisepsis prior to needle puncture.

Isopropyl alcohol is preferred as it is a better fat solvent, more bactericidal and less volatile. Used for disinfection of clinical thermometer.

3. IODOPHOR COMPOUNDS

Many studies have shown, that, iodophor compounds are the most effective antiseptics,. Iodine is complexed with organic surface-active agents, such as, polyvinylpyrrolidone (Betadine, Isodine). Their activity is dependent on the release of iodine from the complex.

These compounds are effective against most bacteria, spores, viruses, and fungi. These are the most commonly used surface disinfectants along with hypochlorite.

4. <u>Biguanide</u> :

Most commonly used biguanide compound is chlorhexidine.

It is a powerful non-irritating antiseptic that disrupts bacterial cell membrane.

It persists on skin for longer period of time and that is why it is extensively used for surgical scrubbing, neonatal bath, mouth wash and a general skin antiseptic.

5.Hydrogen peroxide

• Strong oxidant.

• Oxidizing properties allow it to destroy wide range of pathogens.

• Biggest advantage is short cycle time.

• Used in 35% to 90% concentration.

Operation theatre procedure :

Antiseptic environment :

The principle is to minimize bacterial contamination, especially, in the vicinity of operating table; the concept of zones is useful, and must be employed.

•Outer and general access zone- patient reception area and general office.

•Clean or limited access zone- the area between reception & general office and corridors & staff room.

•**Restricted access zone**-f or those properly clothed personnel engaged in operating theatre activities, an esthetic room.

Aseptic or operating zone- the operation theatre.

HAND WASHING

• Three types of hand washing-

- Social hand washing
- Clinical hand washing
- Surgical hand washing

SOCIAL HAND WASHING

- Recommended following social-type contact with clients, after going to the toilet and after covering a cough or sneeze.
- A plain liquid soap is often used.

CLINICAL HAND WASHING

- A clinical hand wash is used before clinical procedures on clients, when a client is being managed in isolation, or in outbreak situations.
- An anti-microbial soap, containing an antiseptic agent, is used.

SURGICAL HAND WASHING

- A surgical hand wash is required before any invasive or surgical procedure requiring the use of sterile gloves.
- An antimicrobial skin cleanser, usually containing chlorhexidine or detergent-based povidone-iodine, is used.

Surgical Scrubbing :

The purpose is to reduce resident and transient skin flora (bacteria) to a minimum.

Proper hand scrubbing and the wearing of sterile gloves and a sterile gown provide the patient with the best possible barrier against pathogenic bacteria in the environment and against bacteria from the surgical team.

The following steps comprise the generally accepted method for the surgical hand scrub-



1. Palm to palm



 Palm to palm fingers interlaced



5. Rotational rubbing of right thumb clasped in left palm and vice versa



2. Right palm over left dorsum and left palm over right dorsum



4. Backs of fingers to opposing palms with fingers interlocked



6. Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa



1. DRY HANDS.



2. PICK UP GOWN.



3. LET GOWN UNFOLD.

 OPEN TO LOCATE SLEEVE / ARMHOLES.



 SLIPARMS INTO SLEEVES.



 HOLD ARMS OUT AND SLIGHTLY UP.



 CIRCULATOR PULLS GOWN ON.

HM3f0204



THANKYOU



1. Disinfection is :

a. removal of visible contamination but does not necessarily destroy micro organisms
b. reduction of number of viable microorganisms but may not inactive some viruses and bacterial spores.
c. destruction or removal of all microorganisms including spores.

d. destruction or removal of all microorganisms excluding spores.

- 2. Sterility assurance level (SAL) acceptable for critical item is :
 - a. 4
 - b. 5
 - c. 6
 - d. 7

- 3. Which of the following is semicritical?
 - a. mucous membrane
 - b. intact skin
 - c. tissue space
 - d. vascular space
- 4. Minimum Relative humidity essential in fumigation is :
 - a. 50%
 - b. 70%
 - c. 90%
 - d. 100 %
- 5. Cidex consist of
 - a. 2% glutaraldehyde
 - b. 4% glutaraldehyde
 - c. 2% chlorhexidine
 - d. 4% chlorhexidine

6. Tyndallisation is :

a. Exposure of 100 C for 20 min for 3 successive day.

b. Exposure of 100 C for 10 min for 3 successive day

- c. Exposure of 100 C for 30 min for 2 successive day
- d. Exposure of 100 C for 10 min for 2 successive day

7. Which of these is an appropriate sterilizing cycle?

- a. 121 degree centigrade for 15 min
- b. 134 degree centigrade for 3 min
- c. 109 degree centigrade for 10 min
- d. A or B

e. all of these

- 8. Which of these is used for gas sterilization ?
 - a. Glutaraldehyde
 - b. Liquid Nitrogen
 - c. Ethylene oxide
 - d. All of these

9. Which of these is used for liquid sterilization ?

- a. Ethylene oxide
- b. Liquid Nitrogen
- c. Glutaraldehyde
- d. All of these

10. What would you use to clean a flexible endoscope after use ?

- a. Sterilization
- b. Paracetic acid
- c. Alcohol
- d. Autoclave