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Different Methods of Sterilization

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ABSTRACT

Sterilization is a critical process in healthcare facilities, ensuring the complete eradication of microorganisms from surfaces, objects, and fluids to prevent the spread of infections. This comprehensive review outlines various methods of sterilization, including physical, chemical, radiation, and mechanical approaches. We delve into the principles, instruments, applications, advantages, and disadvantages of each method. From the heat-based techniques of dry and moist heat sterilization to chemical methods like hydrogen peroxide, alcohols, and aldehydes, and ionizing radiation, this article provides a comprehensive overview of how healthcare professionals safeguard patient and worker safety through sterilization processes. Mechanical sterilization, achieved through filtration, offers an effective way to obtain sterile fluids by excluding particles and microorganisms based on size. Understanding these methods is crucial for maintaining the highest level of microbial elimination in medical and healthcare settings

KEYWORDS: Sterilization, Healthcare facilities, Microorganisms, Infections, Physical methods, Chemical methods, Radiation methods, Mechanical methods, Dry Heat Sterilization, Moist Heat Sterilization, Hydrogen peroxide, Alcohols, Aldehydes, Ionizing radiation, Non-ionizing radiation, Filtration, Sterility Assurance Level (SAL), Disinfection, Sanitization, Pasteurization, Heat-resistant items, Steam sterilization, Ethylene oxide, Hydrogen peroxide gas plasma, Peracetic acid, Critical items, Surgical instruments, Biopsy forceps, Implants, Heat-sensitive materials, HEPA filter, High Efficiency Particulate Air filter, Indoor air quality, conclusion .

I. INTRODUCTION

Sterilization is a meticulous and critical process employed in healthcare facilities to completely eradicate all forms of life, particularly microorganisms like bacteria, fungi, spores, and even viruses, from various surfaces, objects, or liquids. This comprehensive procedure is achieved through a range of methods, including the application of heat, chemicals, irradiation, high pressure, or filtration. It is essential to distinguish sterilization from other related terms like disinfection, sanitization, and pasteurization. Unlike these methods, which aim to reduce but not entirely eliminate harmful microorganisms, sterilization ensures that once an item has undergone this process, it is rendered entirely free from any potentially hazardous biological agents, making it sterile or aseptic.

Sterilization is not to be confused with disinfection, a process that involves the removal or killing of organisms that are capable of causing infections, although it may not necessarily result in complete sterilization. Common disinfectants include substances like phenol, formaldehyde, chlorine, and iodine. Sterilization, on the other hand, goes the extra mile by achieving the total removal of all types of microorganisms, including both vegetative and spore forms. This is accomplished through a combination of physical and chemical techniques, resulting in the elimination of approximately 106 log colony-forming units.

The primary objective of sterilization is to prevent the growth of microorganisms that might proliferate on the surface of an object if left untreated. It stands apart from disinfection and sanitization, where only a reduction in microorganisms occurs rather than their complete elimination. After undergoing the sterilization process, an object becomes sterile or aseptic, making it safe for use in medical and healthcare settings.

To quantify the effectiveness of sterilization, a concept known as the Sterility Assurance Level (SAL) is utilized. The SAL represents an estimate of the lethality of the entire sterilization process and is calculated conservatively. In the United States, dual SALs are employed, with different levels for various medical items – for example, a 10^-3 SAL may be suitable for blood culture tubes and drainage bags, while a more stringent 10^-6 SAL is applied for items like scalpels and implants. This choice of SAL levels is arbitrary and has not been linked to adverse outcomes, such as patient infections.

In the realm of medical devices, those that come into contact with sterile body tissues or fluids are categorized as critical items. These items must be sterile when used, as any microbial contamination could potentially lead to the transmission of diseases. Such critical items encompass surgical instruments, biopsy forceps, and implanted medical devices. For heat-resistant items, steam sterilization is the recommended method due to its reliability, consistency, and proven lethality. However, items that are sensitive to heat and moisture require low-temperature sterilization technologies, such as ethylene oxide, hydrogen peroxide gas plasma, or peracetic acid.

Sterilization and disinfection play integral roles in hospital infection control activities. Hospitals and healthcare facilities routinely perform various surgical and invasive procedures. Medical devices and surgical instruments that come into contact with sterile tissues or mucous membranes during these procedures carry an increased risk of introducing pathogens into a patient's body. Furthermore, there is a potential for infection transmission from one patient to another, from patients to healthcare personnel, and vice versa. There is also a risk of infection transmission from the environment to the patient through improperly sterilized or disinfected devices. Thus, it is imperative for medical personnel, laboratory staff, and healthcare providers to have a thorough understanding of these techniques to effectively prevent the spread of pathogens.

In essence, sterilization is the rigorous process of entirely eradicating all forms of life, especially microorganisms, from surfaces, objects, or fluids, ensuring their safety for use in critical medical and healthcare settings. It is a cornerstone of infection control and patient safety, aiming for the highest level of microbial elimination to protect patients and healthcare workers.

II. Method of sterilization

1) physical method :

2) chemical method :

3) Radiation method :

4) mechanical method :

1) physical method

Physical methods of microbial control aim to eliminate or neutralize microorganisms through the use of extreme temperatures, removal of water, radiation, sound waves, or filtration. These techniques exploit the vulnerability of microorganisms to temperature variations, desiccation, genetic mutations from radiation, cell breakdown through sonication, and filtration to block their passage. Sterilization is the complete eradication of all forms of microorganisms, while disinfection reduces them but may not achieve complete sterility. Medical devices that contact sterile body tissues or fluids must be sterilized to prevent disease transmission, with steam sterilization being the preferred method for heat-resistant items. Understanding these techniques is crucial in preventing the spread of pathogens in healthcare settings. Sterilization removes or deactivates all life forms, while disinfection and sanitization reduce them, and pasteurization only partially eliminates microorganisms.

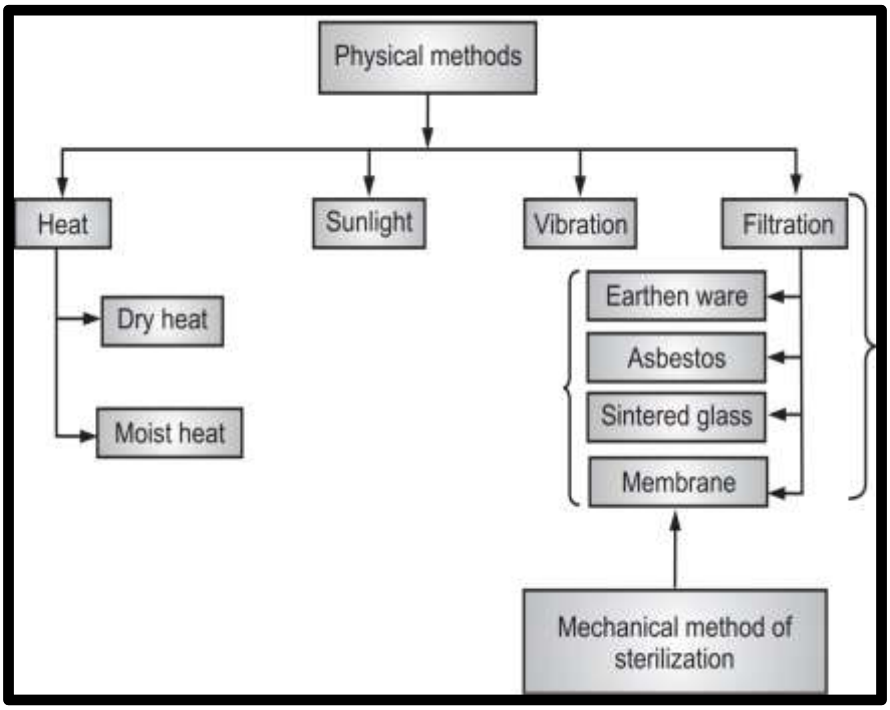

Fig 1 : Physical method of sterilization

• There are two methods involve in physical method of sterilization.

1) Dry Heat Sterilization :

2) Moist Heat Sterilization :

1) Dry Heat Sterilization -

Dry heat sterilization in microbiology is a method that relies on elevated temperatures, typically exceeding 356°F (180°C), and does not involve moisture. This high temperature effectively eliminates microorganisms through a process known as destructive oxidation, which targets and breaks down essential cell components, particularly proteins. This results in the death of the microorganisms. Maintaining this temperature for an extended period, often around an hour, ensures the destruction of even the most resilient bacterial spores.

In microbiological applications, dry heat sterilization is valuable for decontaminating various materials such as glassware, metal instruments, paper-wrapped items, and syringes. These items are suitable for dry heat sterilization due to their heat-resistant or heat-stable nature. Additionally, substances like powders, anhydrous oils, and fats, which are impermeable to moisture, can also be effectively sterilized using this method. Dry heat sterilization plays a crucial role in ensuring the microbiological safety of laboratory equipment and materials.

• Dry Heat Sterilization Involve following methods :

•Sunlight:

Sunlight can provide dry heat, which may help sterilize objects to some extent.

•Red Hot:

Heating an object until it becomes red hot can effectively sterilize it through dry heat.

•Flaming:

Using an open flame, such as a Bunsen burner, is a common laboratory technique for dry heat sterilization.

•Incineration:

This extreme form of dry heat sterilization involves completely burning an object to ashes to ensure sterilization.

• Instrument Involve in Dry Heat Sterilization :

HOT AIR OVEN

•Principle

Hot air ovens operate on the principle of sterilization using dry heat through a combination of convection, conduction, and radiation. They employ heating elements to raise the temperature of the air inside the chamber, which is then evenly circulated by fans. This hot, dry air contacts the surfaces of items to be sterilized, leading to the transfer of heat from the exterior to the center. The elevated temperature causes microorganisms to lose water, leading to damage in their cellular components, protein denaturation, electrolyte imbalance, and ultimately, their demise.

• Construction :

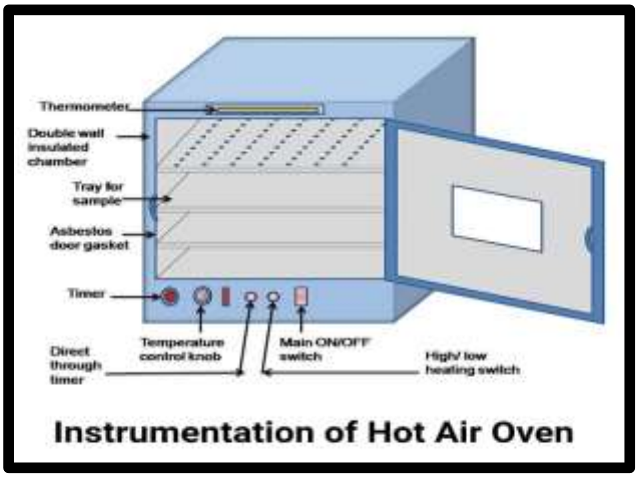

Fig 2 : Hot Air Oven

• It is consist of a alluminium and other parts

• chamber (Heating materials placed)

•Dubble walled door (insulated)

•fan

•on/off nobe

• Temperature regulator hobe

•Thermometer.

• vents (for releasing hot at end)

•tryst

• Working of Hot Air Oven :

1) Loading Materials :

•Open the front door of the hot air oven.

•Place trays with the items to be sterilized on the slots inside the chamber.

2) Sealing and Switching On :

•Close and lock the door securely.

•Turn on the switch to activate the oven.

3) Heating Begins :

•When switched on, the air heaters start generating heat.

•The heat is distributed inside the chamber, causing the temperature to rise.

4) Air Circulation :

•As the temperature increases, a fan starts to circulate the hot air.

•This airflow ensures even heat distribution throughout the chamber.

•In some cases, the presence of a fan depends on the material and customer requirements.

5) Insulation :

•The oven is equipped with insulation on the outside and the door, which traps heat inside the chamber.

•This gradual increase in temperature is reflected on the control panel's indicator.

6) Setting Time and Temperature :

•Set the desired time and temperature for the sterilization process using the control panel.

7) Heating Process :

•The hot air gradually increases the temperature of the materials.

•Heat first affects the outer layers and then penetrates deeper layer by layer.

8) Sterilization :

•The heating process continues until the materials reach the desired temperature for sterilization, effectively destroying bacteria.

9) Monitoring :

- The oven is kept running for a specific time to ensure the sterilization process is completed.

10) Alarm and Shutdown :

•When the set temperature limit is reached, an alarm sounds to signal completion.

•The laboratory oven is then turned off.

11) Safety Precautions :

•Materials are removed from the hot air oven using safety gloves.

•Allow the materials to cool down before using them for the next application.

• Application :

1) used to Sterilize glass wares such as Spatula , scissors, Scalpels , wire loop .

2) To sterilize chemical like liquid paraffin fats , zinc oxide , kaolin, glycerine etc .

• Advantages :

1) Non toxic and not harmful to environment

2) Simple and easy to use

3) Not required water and vaccum

4) Safe

• Disadvantages :

1) Not suitable for heat sensitive materials like - Rubber , pipette , plastic .

2) Slow Speed .

2) Moist Heat Sterilization

Moist heat sterilization, using high-pressure steam in devices like autoclaves, is a cost-effective and fast method to eliminate harmful microorganisms on objects without toxic chemicals. This widely used technique is crucial in healthcare for sterilizing equipment, such as surgical tools, and relies on pure steam and efficient air removal. Monitoring temperature and using indicators like Bowie-Dick tape ensure its effectiveness. It's the go-to method for medical device sterilization, known as steam sterilization.

•Moist heat Sterilization Involve following methods :

•Below 100°c :

In this method of sterilization heat sensitive materials can be sterile .

•At 100°c :

Boiling at 100°c for 20-30 minutes kills all microorganisms and save vegetative spores. Also called tyndallization process .

•Above 100°c :

In this method temperature is maintain above 100°c by providing saturated steam .

•Instrument Involve in moist Heat Sterilization :

AUTOCLAVE

Principle :

An autoclave functions based on the principle of using high-pressure steam to eliminate microorganisms, such as bacteria, viruses, and fungi, through a process known as steam sterilization. This involves subjecting items to direct steam contact at a specific temperature, pressure, and duration. The elevated pressure within the autoclave chamber increases the boiling point of water, facilitating the deep penetration of heat into equipment. The moisture in the steam causes microbial proteins to coagulate, leading to irreversible loss of their functions and, ultimately, their destruction. To effectively sterilize materials, autoclaves typically operate at 121°C and 15 pounds per square inch (psi) of pressure for around 20 minutes, as most bacteria cannot survive under these conditions.

• Construction :

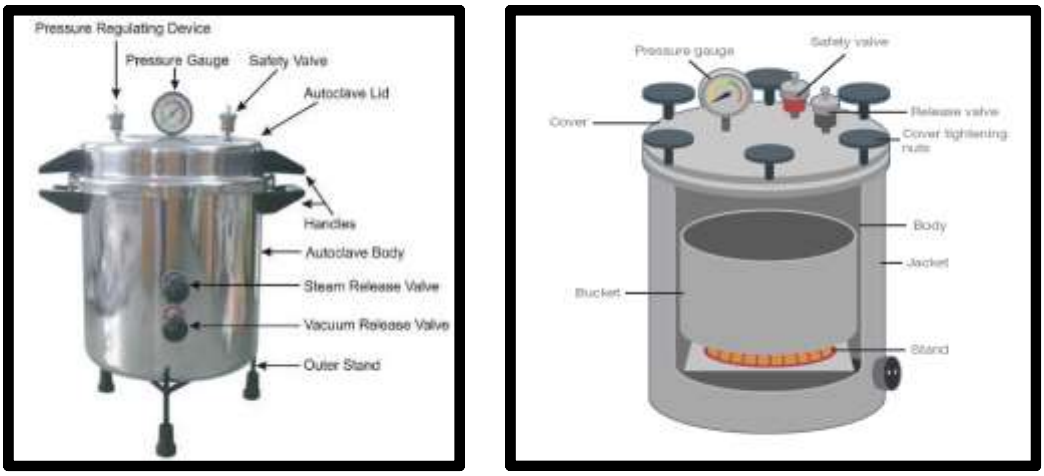

Fig 3 : Autoclave

•Auto clave is made up of stainless steel and covered by jacket It consist :

• Heating element.

•Temperature Controller.

•pressure Sensor. (pressure gauge)

•water level sensor.

•Steam generator.

•vaccum pump.

•chamber.

•steam release valve.

•lid

• Working :

1) Initialization Phase :

•Prior to operation, ensure the autoclave is clear of any remnants from the previous cycle.

•Fill the chamber with an appropriate amount of water.

2) Loading Phase :

•Place the items to be sterilized into the chamber.

•Seal the chamber tightly by closing the lid and securing it.

3) Heating Phase :

•Activate the electric heater to initiate the sterilization process.

•Adjust the safety valves to maintain the required pressure within the chamber.

4) Air Removal Phase :

•As the water inside the chamber heats up, it generates steam, displacing the air-water mixture.

•Release this air-water mixture through the discharge tube until no more air bubbles are visible.

5) Pressure Buildup Phase :

•Close the drainage pipe to create a pressurized environment inside the chamber.

•Once the desired pressure (typically around 15 lbs) is reached, a whistle may sound to release excess pressure.

6) Sterilization Phase :

•Continue the autoclave operation for a specified holding period, usually around 15 minutes, at the set temperature (commonly 121°C).

7) Cooling Phase :

•Switch off the electric heater and allow the autoclave to cool naturally.

•Monitor the pressure gauge until it indicates a return to atmospheric pressure.

8) Air Entry Phase :

•Open the discharge pipe to introduce external air into the autoclave, equalizing pressure.

9) Finalization Phase :

•Finally, open the lid and remove the sterilized materials from the chamber.

• Application :

1) To sterile biological media , saline solution.

2) To sterile rubber products , dressing instruments , and thermostable liquids .

•Advantages :

1) Very fast

2) more effective than hot air oven

3) Bacteria spores also killed

4) East to use

•Disadvantages :

1) Not suitable for anhydrous materials.

ex : powders and oils .

2) Can't use for very heat sensitive materials that decomposed above 100°c temperature .

2) CHEMICAL METHOD OF STERILIZATION

Chemical sterilization is a vital method used across various industries, including healthcare, food production, and agriculture, to eliminate harmful microorganisms such as bacteria, fungi, and viruses. This technique is particularly advantageous when other sterilization methods like heat, high pressure, irradiation, or filtration are unsuitable for the materials involved. Chemical sterilization offers several benefits, including affordability, speed, efficiency in eradicating target microorganisms, and minimal reliance on complex equipment.

Chemical sterilization methods can be categorized as liquid or gaseous. The choice between them depends on factors like material type and heat sensitivity. The Food and Drug Administration (FDA) regulates the chemicals employed in sterilization processes and tends to recommend gaseous techniques for enhanced sterility assurance.

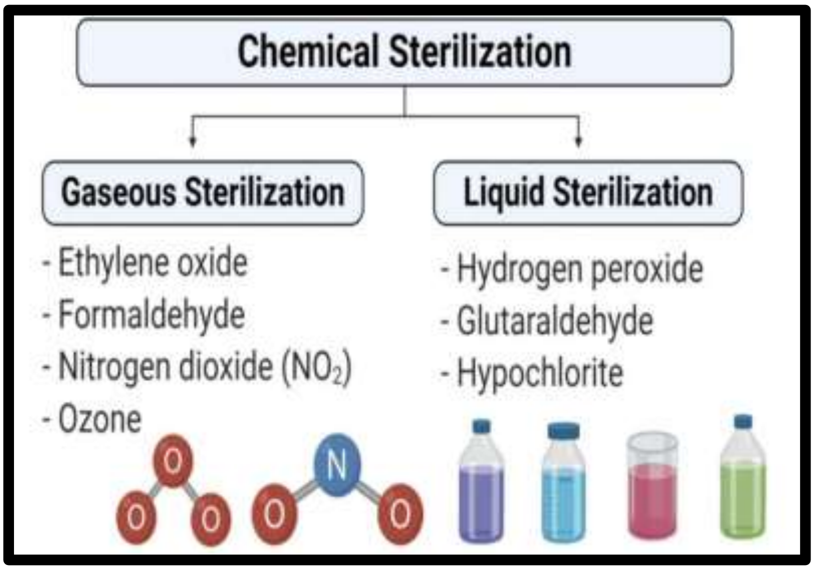

Fig 4 : Chemical method of sterilization

•Common chemical sterilizing agents include:

1. Hydrogen peroxide: Widely used for decontaminating food packaging materials and contact surfaces, it's subsequently dried with hot sterile air.

2. Alcohols: Typically, 70% alcohol solutions, such as methyl, isopropyl, and ethyl alcohol, are used for bacterial eradication.

3. Aldehydes: Solutions like formaldehyde and glutaraldehyde serve as effective surface disinfectants.

4. Halogens: Chlorine compounds, iodine compounds, and chlorine bleach act as antiseptics and direct bacterial impact.

5. Heavy Metals: Copper sulfate, mercuric salts, and silver nitrate, along with dyes like aminacrine and acridine dyes, can be utilized for sterilization.

6. Gaseous sterilization: Gases like formaldehyde and ethylene oxide are effective in eliminating bacterial spores.

• Applications :

•In operation theaters.

• To prepare aseptic area.

• In microbiology laboratories.

•for sterilization of Heat sensitive materials.

•For sterilization of Food, Heaty equipment, books.

• Advantages:

•Best Penetrating ability to gas.

• Best for heat sensitive materials.

•Effective.

• Disadvantages:

1) Gas hand carcinogenic activity.

2) Toxicity - Acute irritation

- Nasal irritation

- Eye irritation

3) RADIATION METHOD OF STERILIZATION

Radiation is a non-thermal sterilization technique that eradicates microorganisms in various products using gamma radiation, electron beams (beta particles), or ultraviolet light. Unlike many sterilization methods, it doesn't rely on high temperatures. This method has gained popularity since the late 1950s and is especially valuable in medicine and healthcare, as it can effectively sterilize products like tissue allografts, pharmaceutical packaging, and medical devices.

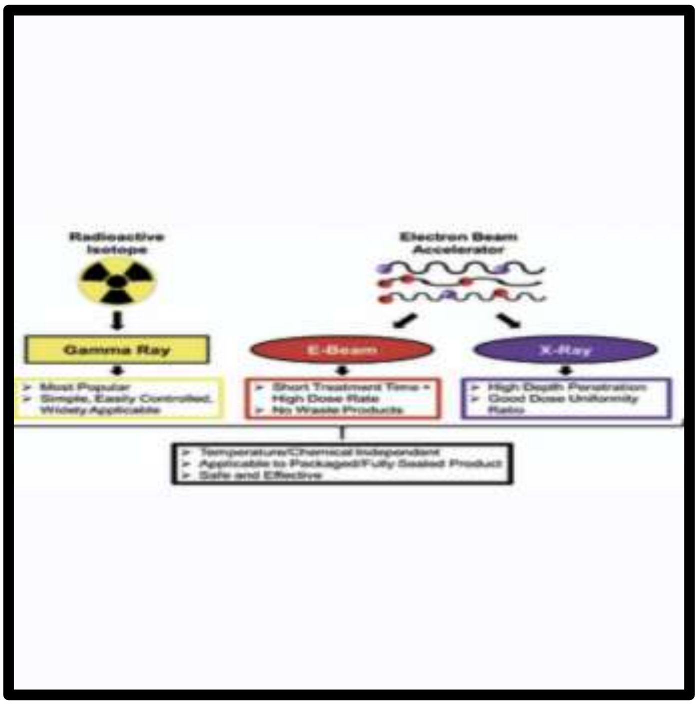

Fig 5 : Radiation methods of Sterilization

•There are two main types of radiation for sterilization:

1) Ionizing radiation :

2) Non-ionizing radiation :

1) Ionizing radiation, such as gamma or X-rays, uses short-wavelength, high-intensity radiation to damage microorganisms' DNA. Non-ionizing radiation, like ultraviolet light, has longer wavelengths and lower energy, making it suitable for surface sterilization.

2) Non-ionizing radiation can also break down ozone in water to kill bacteria, though it requires subsequent treatment to make the water safe for use.

In medical device sterilization, ionizing radiation is often used, with gamma rays from a cobalt 60 isotope source or machine-generated accelerated electrons. Gamma irradiation is the preferred choice when materials are sensitive to high temperatures but compatible with ionizing radiation. It exposes packages to a 60Co source for a specified time, typically at a dose of 25 kGy.

The bactericidal effect of gamma irradiation relies on oxidizing biological tissue, making it a quick and effective sterilization method, although it does come with high capital costs. While most metal-based medical devices can be sterilized this way, gamma irradiation can cause physical changes in biomedical polymers, including embrittlement, discoloration, odor generation, and changes in properties like stiffness, softness, and molecular weight.

• Application :

1) In hospital operation room

2) laboratory wards

3) In packeged food - Sterilization

4) In Sterilization of syringe , plastic etc .

• Advantages :

1) Best for Heat sensitive products.

2) Better Penetrating power.

3) very effective.

4) clean Proccess.

• Disadvantages :

1) Also affect to humans.

2) Require very qualified person.

3) Expensive.

4) MECHANICAL METHOD OF STERILIZATION

The mechanical method of sterilization, also known as the filtration method in microbiology, involves the use of membranous filters with small pores to effectively sterilize liquids or gases by excluding particulate matter and microorganisms based on their size. This process consists of three essential steps: sieving, adsorption, and trapping.

1. Sieving: Filtration works like a sieve, where a porous membrane filter with specific pore sizes is used. These pores act as a physical barrier, preventing particles and microorganisms larger than the filter's pore size from passing through.

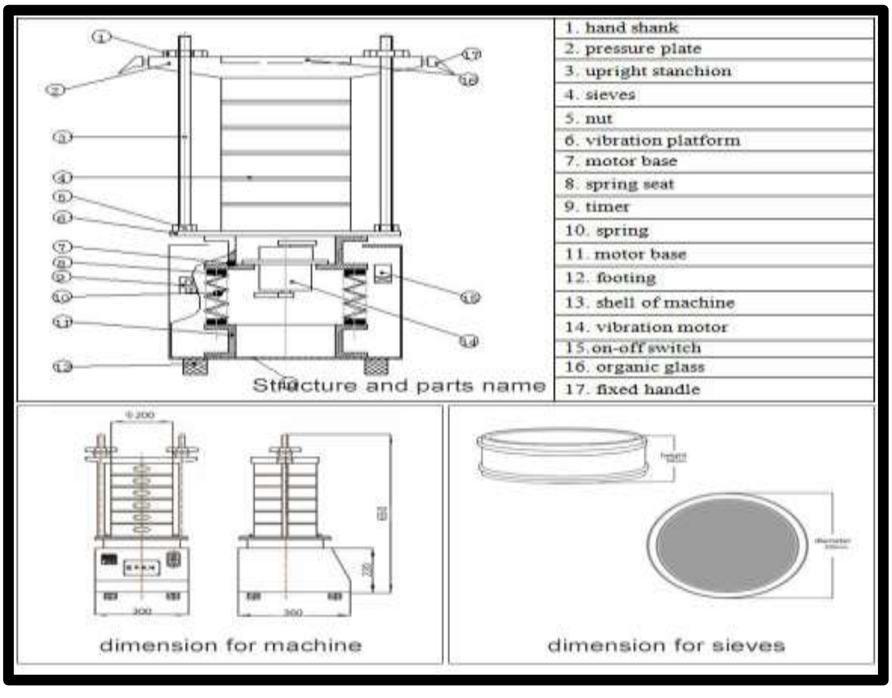

Fig 6 : Sieve shaker

2. Adsorption: Contaminants, such as microorganisms and particles, are not just physically blocked by the filter; they can also be adsorbed or attracted to the filter material's surface. This helps to capture and immobilize the contaminants.

3. Trapping: The matrix of the filter material itself plays a role in trapping contaminants. Microorganisms and particles that are too large to pass through the pores or have been adsorbed are effectively trapped within the filter material, ensuring they do not pass through.

Filtration is a versatile method for sterilization and clarification of various liquids and gases, making it suitable for applications like sterilizing antibiotic solutions, sera, carbohydrate solutions, and more. It doesn't destroy microorganisms but removes them, leaving the filtered solution or gas sterile.

The choice of filter with specific pore size is crucial, as it determines the size range of microorganisms that can be effectively excluded. For example, filters with 0.01 µm pores can trap even the smallest viruses, while those with 0.45 µm pores are effective at trapping larger bacteria. Filters with 1.2 µm pores can capture protozoa and the smallest unicellular algae.

the mechanical method of sterilization, or filtration, is a highly effective way to obtain sterile filtrates by utilizing porous membrane filters that operate through sieving, adsorption, and trapping mechanisms, ensuring the exclusion of both viable and non-viable particles and microorganisms based on their size.

• Instruments use for mechanical Sterilization/ filtration.

• HEPA FILTER :

A HEPA filter, which stands for High Efficiency Particulate Air filter, is a type of air filter that is designed to trap and remove very fine particles from the air. HEPA filters are highly efficient at capturing particles like dust, pollen, pet dander, and even microscopic allergens. They are commonly used in air purifiers, vacuum cleaners, and HVAC systems to improve indoor air quality by removing contaminants. HEPA filters are known for their ability to remove particles as small as 0.3 microns with a high efficiency rate, making them essential for environments where clean air is crucial, such as hospitals and laboratories.

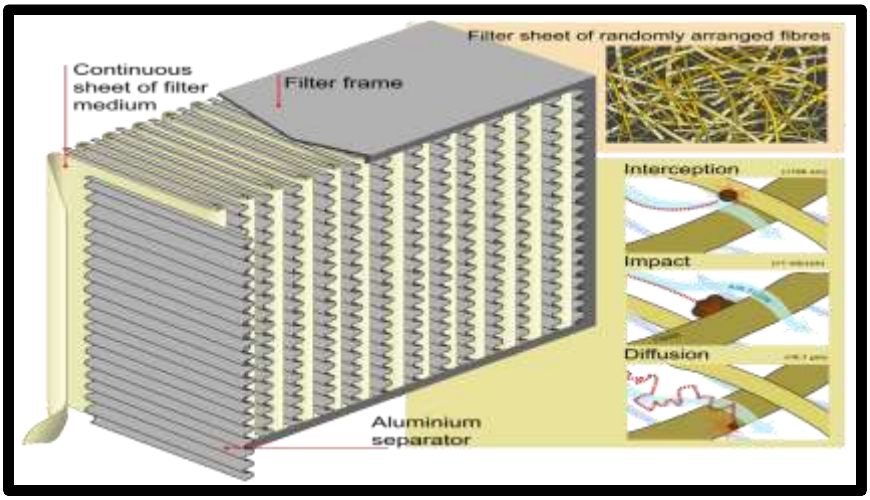

Fig 7 : Hepa filter

• Application :

1) gases for supply to aseptic area.

2) In Sterilization of heat sensitive injections.

3) separation of opthalmic solution , biological products.

• Advantages :

1) Best for heat sensitive materials.

2) Also useful for clarification.

3) All microorganisms are separated

• Disadvantages :

1) It does not differentiate between viable (living) and non- viable (non- living) particles.

2) Not 100% reliable , it requires sterility test .

III. CONCLUSION

In conclusion, sterilization is a crucial process in healthcare and various other industries to completely eliminate all forms of microorganisms from surfaces, objects, or fluids, ensuring their safety for use in critical settings. Sterilization methods can be categorized into physical, chemical, radiation, and mechanical methods, each with its own advantages and disadvantages.

1. Physical methods of sterilization utilize extreme temperatures, removal of water, radiation, sound waves, or filtration to eliminate microorganisms. Dry heat sterilization and moist heat sterilization are key examples, with the former using high temperatures without moisture and the latter relying on high-pressure steam to achieve sterilization.

2. Chemical methods of sterilization involve the use of chemicals like hydrogen peroxide, alcohols, aldehydes, halogens, heavy metals, and gaseous sterilization agents to eliminate microorganisms. Chemical sterilization is effective for heat-sensitive materials but requires careful handling due to potential toxicity.

3. Radiation methods of sterilization use ionizing radiation (such as gamma rays or X-rays) or non-ionizing radiation (like ultraviolet light) to damage microorganisms' DNA, ensuring their destruction. This method is suitable for various applications, including medical devices and food sterilization.

4. Mechanical methods of sterilization, specifically filtration, utilize porous membrane filters with specific pore sizes to exclude particulate matter and microorganisms based on size. This method is versatile and commonly used in various industries to obtain sterile liquids or gases.

Each sterilization method has its advantages and limitations, and the choice of method depends on the specific requirements of the materials being sterilized. Sterilization is a cornerstone of infection control, ensuring the highest level of microbial elimination to protect patients and healthcare workers. It is essential in various applications, including hospitals, laboratories, and the sterilization of medical devices, pharmaceuticals, and food products, ultimately contributing to public health and safety.

IV. REFERENCE

[1] https://www.ansell.com/in/en/faq/what-is-sterilization

[2] https://byjus.com/biology/principle-of-sterilization/

[3] https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/index.html

[4] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7158362/

[5] https://en.m.wikipedia.org/wiki/Sterilization_(microbiology)

[6] https://www.jove.com/science-education/13242/methods-of-sterilization-i-physical-methods#:~:text=The%20physical%20method%20includes% 20 steam,quickly%20warms%20and%20penetrates%20fabrics.

[7]https://study.com/learn/lesson/physical-methods-microbial-control-agents-types-effectiveness.html#:~:text=Physical%20methods%20rely%20upon %20techniques,eliminate%20or%20control%20microbial%20growth.

[8] https://princesterilization.com/difference-between-moist-heat-sterilization-dry-heat-sterilization/#:~:text=Dry%20heat%20helps%20kill%20the, difficult%20of%20the%20resistant%20spores.

[9] https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3340325#:~:text=Hot%20air%20ovens%20use%20extremely,the%20center%20of%20the%2 0item.

[10] https://microbenotes.com/hot-air-oven/

[11] https://youtu.be/aPcHJo-VjeU?si=krPIvCCj2JR0PA5Y

[12] https://youtu.be/aDJeoydNLzU?si=5yj0gEISzko0CByz

[13] https://youtu.be/aPcHJo-VjeU?si=vAv3mxJ-_MWQnwpH

[14] https://tempoinstruments.com/blog/hot-air-oven-working/

[15] https://www.testing-instruments.com/blog/what-is-hot-air-oven-and-how-does-it-work/

[16] https://study.com/academy/lesson/what-is-the-moist-heat-sterilizationmethod.html#:~:text=Moist%20heat%20sterilization%20is%20a,bacteria% 2 C%20viruses%2C%20and%20spores

[17] https://ethidelabs.com/sterilization-by-moist-heat/

[18] https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/steam.html#:~:text=The%20basic%20principle%20of%20steam,pressure %2C%20temperature%2C%20and%20time.

[19] https://www.plantcelltechnology.com/blog/autoclave-principle-types-and-precautions/

[20] https://www.fcbios.com.my/blogs/news-insight/autoclave-machine-principle-how-to-use-and-maintenance

[21] https://www.steris.com/healthcare/knowledge-center/sterile-processing/everything-about-autoclaves#:~:text=An%20autoclave%20is%20a%20machine,a%20given%20amount%20of%20time.

[22] https://www.mlsu.ac.in/econtents/2214_Unit%201_Autoclave.pdf

[23] https://consteril.com/how-does-a-laboratory-autoclave-work/

[24] https://study.com/learn/lesson/chemical-sterilization-methods-types.html#:~:text=fungi%2C%20and%20spores.-,Chemical%20sterilization% 20involves%20the%20use%20of%20chemical%20agents%20to%20kill,all%20viable%20microorganisms%20and%20spo res.

[25] https://www.sciencedirect.com/topics/medicine-and-dentistry/chemical-sterilisation

[26] https://www.ssqllp.com/physical-chemical-method-sterilization/

[27] https://ethidelabs.com/sterilization-by-radiation/#:~:text=Radiation%20is%20a%20non%2Dthermal,temperature%20in%20order%20to%20sterilize.

[28] http://large.stanford.edu/courses/2018/ph241/goronzy2/

[29] https://www.rpi.edu/dept/chem-eng/Biotech-Environ/Projects00/sterilize/radiation.html

[30] https://www.sciencedirect.com/topics/medicine-and-dentistry/radiation-sterilization

[31] https://pharmacyscope.com/mechanical-method-of-sterilization/

[32] https://byjus.com/biology/principle-of-sterilization/

[33] https://en.m.wikipedia.org/wiki/HEPA

[34] https://www.iso-aire.com/blog/what-is-a-hepa-filter-and-how-does-it-work

[35] https://smartairfilters.com/en/blog/what-is-hepa-filter-how-hepa-air-filter-work/