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Chapter 3

Sterilization

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Abstract: Microbial cultivation is a challenging task as it always ends up with contamination by other microorganisms. Sterilization is one of the principle techniques used in microbiology to reduce and to avoid the contamination with microorganisms of not required. Wet heat sterilisation like Autoclaving can be used to sterilize materials which are sensitive to be sterilized by Dry heat method.

Keywords: Autoclaving, Contamination, Dry Heat sterilisation, Wet Heat sterilisation.

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5. Sterilization:

Sterilisation is defined as destruction of complete life (i.e., both vegetative and spore forms), carried out with means of various physical and chemical methods. Technically, there is reduction of around ≥106 log colony forming units (CFU) and most of the resistant spores usually done at half of the regular cycle.

5.1. Dry heat sterilization:

Dry heat sterilization is one of the most preferable methods of sterilization used to sterilize industrial ovens with hot air blown inside to the chamber.Dry heat sterilization is carried out at very often due to the predictable results and can be modified according to the sterilization procedures required.

5.1.1. Dry-Heat Sterilization Working principle:

Dry air is blown directly on the object with force (force air) and the energy is transferred to the object through conduction which destroys living organisms associated with object.

oven has circulating coils instead of static air but blowing air is preferred as it touches the object with homogeneity. Typically, sterilization using hot air oven can be performed at 160 °C (320 °F) for two hours, 170 °C (340 °F) for one hour, and up to 190°C (375°F) for 6 to 12 minutes. The commonly used time scales for dry heat sterilization will vary depending up on the requirement of time scale and can be varied accordingly. Depending on the oven and blower unit the times of cycle required to achieve sterilization may vary. In case of oven with large blower unit with powerful fans that deliver large amount of hot air on to the object the time cycle can be reduced to half.

The heat that is passed on the object cause denaturation of proteins in all biological agents like bacteria, fungi, Prions and spores. In viruses they cause denaturation of capsid and permanently damage the nucleic acids either DNA or RNA through denaturation. Removal of moisture in the air blown inside the chamber is imperative as it affects the degree of denaturation there by finally affecting the efficacy of sterilization.

There are different types of dry heat sterilization which are explained below:

a) Red Heat :
Red heat sterilization is the process of instant sterilization by holding the instrument directly in the Bunsen flame until it becomes red hot. Usually inoculation loops, wires and tips of forceps are sterilized using this method.
b) Flaming:
Flaming is a type of dry sterilization that involves flaming of metallic objects till the dust and microorganisms are removed or killed.Objects sterilized by using flaming should be dipped in ethanol before exposed to flame.
c) Incineration:
Incineration is the process of sterilization with reduction in amount of waste generated after the process. Normally hospital waste meant for final disposal is usually subjected to ash that can be disposed later.Incineration is generally carried out using a chamber called Incinerator.
d) Infrared radiation:
Infrared radiation (IR) is a method of thermal sterilization where radiation is transformed to thermal energy and the objects exposed will retain temperature of about 180^0c for 17 minutes. IR sterilization is generally used for bulk sterilization of objects like catheters and syringe disposals.
e) Hot air oven:
It uses the principle of conduction where thermal energy is conducted through object inner layer from the outer surface after exposure to hot air in the chamber. Generally the temperatures used in Hot air oven for sterilizing the samples include 160 °C (320 °F) for

two hours, 170 °C (340 °F) for one hour, and up to 190°C (375°F) for 6 to 12 minutes. Hot air oven sterilization is used to sterilize petri plates, glass tubes and even powder samples.

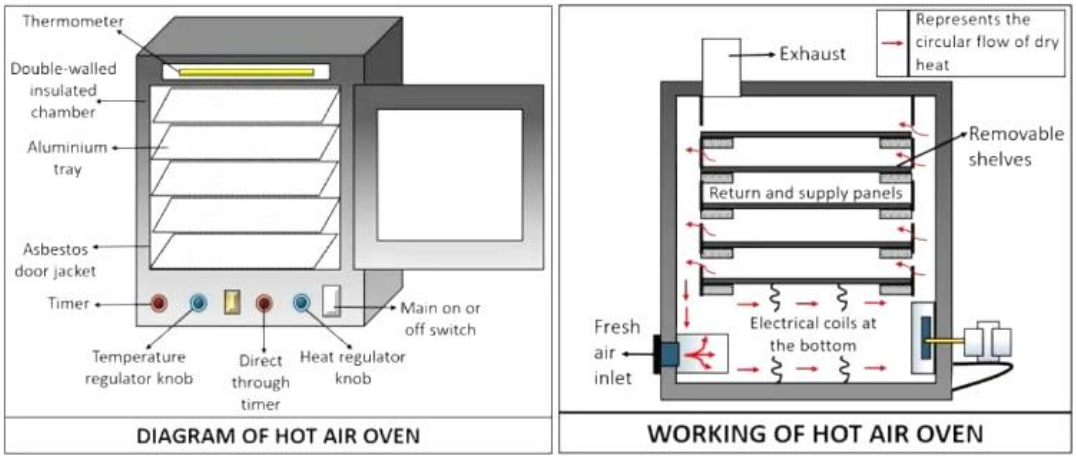

Figure: 3 Outline of Hot air oven and working principle of the oven. [Taken from Alkadhim, Saif Aldeen Saad, Hot Air Oven for Sterilization: Definition & Working Principle (December 14, 2018). Available at SSRN: https://ssrn.com/abstract=3340325 or http://dx.doi.org/10.2139/ssrn.3340325].

5.1.2. Advantages of Dry-Heat Sterilization:

- Dry heat ovens are really cheap at cost
- The maintenance cost and cycles required for sterilization is generally low.
- The heat can penetrate deeply and can achieve sterilization of objects packed inside.
- Metallic objects can be sterilized quickly at high temperatures as they can hold higher temperatures.
- Dry heat is non corrosive due to presence of less moisture
- The process involves no toxic agents and so hazardous substances are not delivered in to environment.
- Requires no human presence or intervention during operation.
- The object can be used immediately after sterilization as cooling occurs rapidly.

5.1.3. Disadvantages Compared to Other Processes

- The dry heat require more time compared to steam, radiation, flaming and Incineration.
- The heat can cause warping of thin sheets or temperature sensitive things.
- The high temperatures can irreversibly damage plastics, rubber, so these items cannot be sterilized using dry heat.
- Overexposure to heat can cause irreversible damage to the chemical structure of the materials carried out for sterilization.

5.1.4. Common Applications of Dry Heat Sterilization

Wide range of materials and objects can be sterilized using hot air oven and the examples include metals of all kinds, petri dishes, glassware, chemicals not altered due to moisture and medical instruments such as syringes or surgical equipment and surgical tools.

5.2 Moist heat sterilization:

It is one of the affordable method of sterilization and done with the help of an autoclave. An autoclave works on the principle of steam under pressure. Hence moist heat sterilization is also known as steam sterilization.The water used in autoclave is boiled at 121^0c - 134^0c at a pressure of 15psi. This may cause coagulation of proteins so the microbes are effectively killed.

5.2.1. Autoclave:

Lethality of microorganisms depends on:

- Degree of exposure to heat
- Duration of exposure to heat and
- Moisture content

Heat based sterilization kills the microbes by denaturation of proteins with in the cells. In moist heat sterilization moist heat hit the cooling surface of the object and there by undergo condensation from gas to water. During condensation process the steam releases thousands of calories there by killing the microorganisms that undergo penetration by steam. If moisture cannot reach the object that has to sterilized like oil that cannot be

sterilized effectively using moist steam.The basic steam sterilization cycle involves three major steps:

- Premaintainance of the chamber by removing the air and filling with saturated steam and load in to the chamber
- The sterilization cycle chosen
- Steam removal and release of pressure

In order to create the steam the water should reach the temperature of 100^0c to 121^0c by applying 15 pounds per square inch of pressure above the atmospheric pressure.The steam sterilization cycle begins by heating the walls of the container so that water inside the chamber will boil. The materials to be sterilized should contain certain amount of moisture level with in them otherwise the steam may not be penetrated inside and the sterilization may not be carried out properly. For steam sterilized solutions, glass containers should be used. Plastic syringes or containers usage may lead to bursting at high pressure.

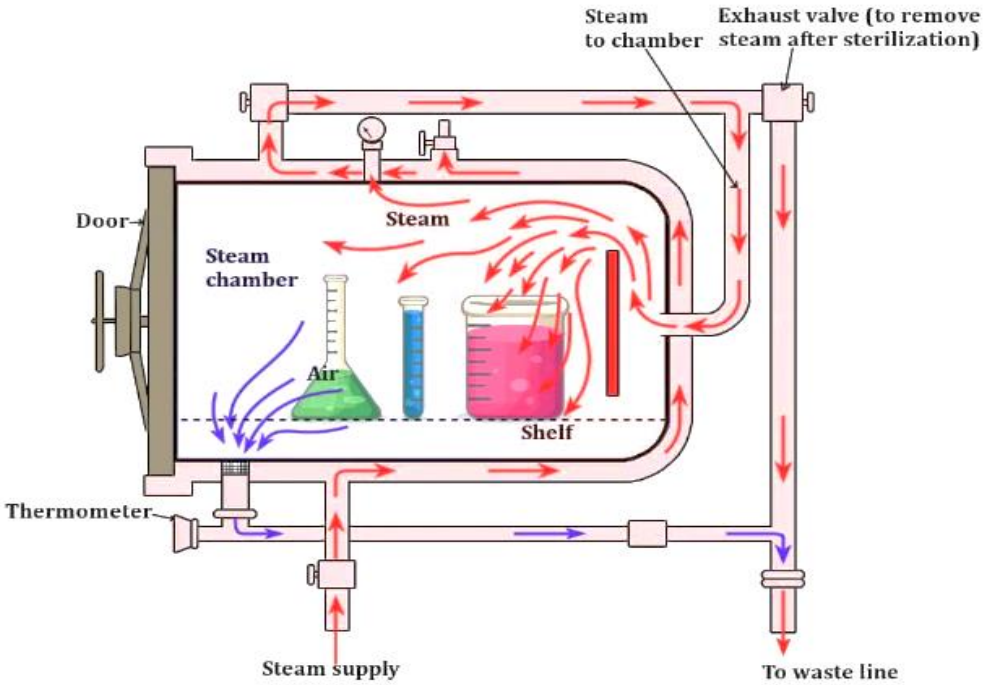

Figure:4 Working principle of Autoclave [Taken from Cappucino JG and Sherman N (1996). Microbiology, A Laboratory Manual 4th edition. Benjamin Cumings Inc. California

The greatest problem with the steam sterilization is mainly due to change in integrity of the compound subjected to sterilization. Oils or dry packed items may not be be sterilized effectively using moist heat due to improper penetration of steam.

5.3 Tyndallization:

Tyndallization is a method of sterilization used for sterilization of media containing sugar and gelatin at 100^0c for 30 min on three successive days with out decomposition of sugar at high temperatures. Moist heat at 100°C is applicable for contaminated bedding, plastics and dishes not soiled and for objects that are not temperature sensitive.

5.4 Filtration:

The process of filtration involves removal of dirt, living and non living organisms with out destroying them. Further the method can be used for both sterilization and clarification of liquids and gases. The primary mechanism involves sieving, adsorption and trapping the living particles inside the matrix. Filtration uses membranous filters that can allow small particles to travel stopping the large particles such as bacteria passing through the filter.

High-efficiency particulate air (HEPA) filters can remove up to 99.997% of particles >0.3mm in diameter and their microbial removing efficiency will be very high as most of the microorganisms found associated along with the dust. Other applications of the filter include displacement of air in tissues or microbial culture, Sterilization of venting, treatment of exhausting air from microbial bio safety cabinets, Decontamination of air from ventilators and sterilization of liquids and surgical gases.

5.5 Radiation:

Irradiation is the process of exposure to different radiations and majorly electromagnetic radiation is generally preferred for sterilization purpose. The main target of radiation is microbial DNA prone to permanent damage caused through free radicals produced by ionization (gamma-rays and electrons) or excitation (UV light). Non ionizing light rays like UV radiation can result formation of pyrimidine dimer which leads to mutation and finally death.Ionizing Radiation X-ray and gamma rays are the commonly used ionizing radiations of sterilization. Exposure to the radiation results in formation of toxic O2 metabolites like hydroxyl radical, super oxide ion, and H2O2 by ionization. These are highly oxidative and can kill microbes by ionization targeting their cellular components.

5.6 Chemical Sterilization:

Chemical sterilization involves process of killing the microbes using chemical bactericidal agents.

1) Gaseous sterilization: Gaseous sterilization involves exposure of objects to different gases in the preheated chamber. It is a effective method of sterilization as the gas covers larger area and usually gas is passed through a small orifice. Materials needs to be sterilized is placed in a chamber and heated before exposure to gas as heating may increase the efficacy of sterilization by gas. Different gases will work with different principles.

B] Ethylene oxide (EO) gas is commonly used gas for sterilization due to its major compatibility with most of the objects. Ethylene oxide gas can kill all the bacteria and spores. Formaldehyde, Ozone and Nitrogen dioxide are examples of other gaseous sterilants.

5.7 Liquid sterilization:

Liquid sterilization is a process where the material to be sterilized is dipped in the chemical sterilant and this method is not considered to be effective compared to gas but it is often used when there is less contaminants associated with the objects. Hydrogen peroxide is a liquid sterilant which can be used to kill majority of the microorganisms due to its strong oxidizing properties. Glutaraldehyde is an effective chemical sterilant but requires long immersion time and it requires about 22 hrs of immersion to kill all the spores. Hypochlorite solution, also known as household bleach is an example of liquid sterilant used widely for disinfection.

5.8 Disinfection: Disinfection involves the process of destroying all the microbes except spores on the inanimate objects and surfaces. Sterilization involves killing of almost all living microorganisms including spores. Disinfection utilizes various physical methods like radiation and chemical substances for sterilization. So, disinfection can be used to reduce the microbial load on the surface. Many factors can effect the effectiveness of the disinfection like depending on the microbial load, organic and inorganic compounds associated with the object, physical and chemical nature of the object exposed and duration of time exposure, characteristics of the sterilant used. Other factors include temperture, humidity, pH, water hardness etc., Physical method of disinfection using temperature depends on certain processes to target diseased microbes.

5.9 Sanitation:

Sanitation reduces microorganisms but not targets the microbial load. Hence sanitizers are not sterilizing agents. Antimicrobial liquids or disinfectants are classified based on how effectively they reduce the microbial population on the environment surfaces. FDA approves sanitizing agent as the liquid solution used to reduce the number of microorganisms on the inanimate objects of the environmental surfaces.

5.9.1 Antisepsis sterilants: A sterilant is a chemical used to kill the microbes like bacteria on non living objects including spores.. Ethylene oxide, glutaraldehyde, hydrogen peroxide gas, and per acetic acid are some of the examples of sterilants. An antiseptic is described as agent that kill microorganisms on the living tissues or surfaces. Iodophors, chlorhexidine, and the alcohols (ethanol and isopropanol) are commonly used antiseptics.

5.9.2 Fumigation: Fumigation is a one of the procedure used in older days to sterilise operation theatres and cinema halls. One of the first uses of formaldehyde gas is to fumigate rooms and as the fumes are very pungent and powerful so, before fumigation the rooms should be sealed and closed properly. There are, however, automatic, low temperature steam formaldehyde sterilizers that are effective in sterilizing plastic items and heat sensitive agents. As mentioned formaldehyde in gas form is highly irritant to skin, eyes and respiratory tract hence its usage in gas form should be limited.

Determination of Phenol coefficient of a disinfectant:

Phenol Coefficient:

The effectiveness of a chemical can be determined in a number of ways from the previous times. The effectiveness of a chemical is usually compared with phenol the first antiseptic used by Joseph Lister. In 1903, British chemists Samuel Rideal (1863–1929) and J. T. Ainslie Walker (1868–1930) established a protocol for testing the effectiveness of various chemicals with phenol using test organisms *Staphylococcus aureus* (a gram-positive bacterium) and *Salmonella enterica* serovar typhi (a gram-negative bacterium). They allowed the test organisms to expose to various chemicals diluted with water for 7.5 minutes. A chemical with phenol coefficient equal to 1 indicates that it is equally efficient as that of phenol. If the phenol coefficient is less than 1 it indicates that the chemical is less efficient than phenol. For example formalin show phenol coefficient of 0.3 with test organism (*S. aureus*) and 0.7 (*S. enterica* serovar Typhi). A chemical agent with phenol coefficient greater than 1 indicates that it is more effective than phenol for example chemical chloramine with phenol coefficients 133 and 100 respectively. Although Phenol

coefficient is used very often to describe the effectiveness of the disinfectant it is used no more because the conditions and the required organism is chosen arbitrarily in now a days.

Determination of phenol coefficient: Phenol coefficient of a disinfectant is calculated by dividing the dilution of the test compound by dilution of phenol with calculations under predetermined conditions.

Rideal Walker method: Phenol dilution is carried up to 1:400 to 1:800 and the test disinfectant is diluted from 1:95 to 1:115. Their effectiveness is tested against the test cultures S. typhii organism. Sub cultures are prepared using both from the phenol tested cultures and test compound at intervals of 2.5, 5, 7.5 and 10 minutes. Plates are incubated for 48-72 hrs at 37^0c. Phenol coefficient of test compound can be obtained by dividing the dilution of test compound by dilution of the phenol used. For example, after 7.5 minutes, S.typhii killed with the test compound at dilution 1:600 and at the same time the test organism killed with phenol at a dilution rate of 1:200. So, phenol coefficient is calculated as

Phenol coefficient = 600/200 = 3

Chick Martin test: This method or test is also used to test the phenol coefficient of the test compound but instead of carrying dilution of test compound in water the disinfectant is allowed to act in the presence of yeast suspension or 3% dried human faeces to stimulate in the presence of organic matter. Here the test organisms used are S.typhi and S.aureus. This test gives lower phenol coefficient compared to Rideal Walker method.

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