MICROBIOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 7

Sterilization & Disinfection (Pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

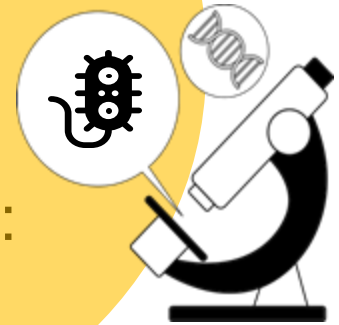
اللهم استعملنا ولا تستبدلنا

Written by:

- Leen Mamoon
- Heba sleman

Reviewed by:

- Raneem AH



Quiz for sterilization& disinfection pt.1

say bismillah يلّلا

اللّهم علّمنا ما ينفعنا وانفعنا بما علّمتنا وزدنا علمًا

Lecture 8 – part 2

Sterilization&

Disinfection

Objectives

Physical methods for sterilization

Moist heat (Autoclave)

Dry heat

Ionizing radiation

Filtration

ربما على حاء الحيره نقطة لا تراها "

Physical methods for sterilization

I) Moist heat above 100°C (Autoclave)

***Recall that there are three levels of Moist Heat Treatment used in microbial control:**

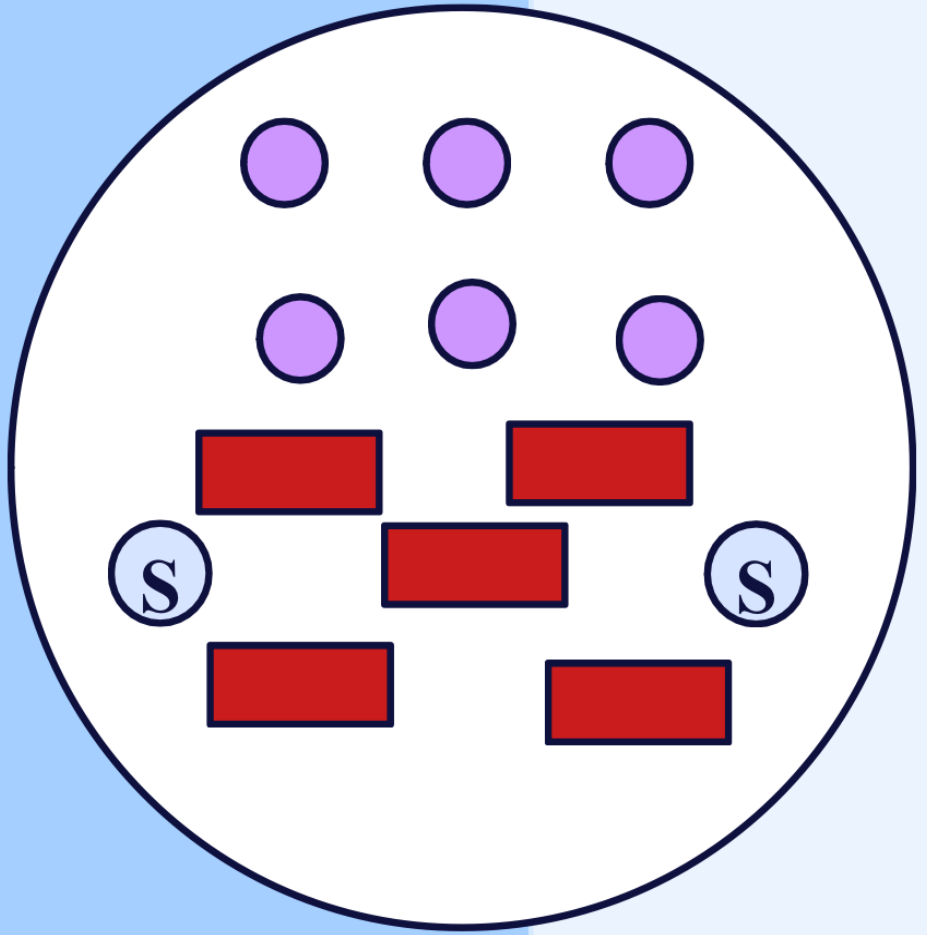
1. **Below 100°C: This level is primarily used for disinfection,** effectively killing most bacteria but not all resistant spores.
2. **At 100°C: Boiling at this temperature also serves for disinfection,** killing many pathogens but not all spores.
3. **Above 100°C : This level is used for sterilization.**

Sterilization

- **What do we mean by sterilization?**

Killing all microbes including bacterial spore.

- **sterilization is an absolute term,** it means “complete eradication of all microorganisms,” while disinfection is less absolute, as it varies in effectiveness based on the microorganisms targeted.



Moist heat above 100°C (Autoclave)

Steam

⇧

Pressure

⇧

Temp.

Time.

- An autoclave operates on the same principle as a pressure cooker.
- pressure cooker has a container that is a sealed pot that holds water, When heated, the water boils and turns into steam. Because the steam can't escape when it is closed, the pressure inside the cooker increases, which raises the boiling point of the water above 100°C. So, at certain time it will achieve sterilization process.

- An autoclave is a device that uses high-pressure steam to sterilize equipment and supplies. The key components include:

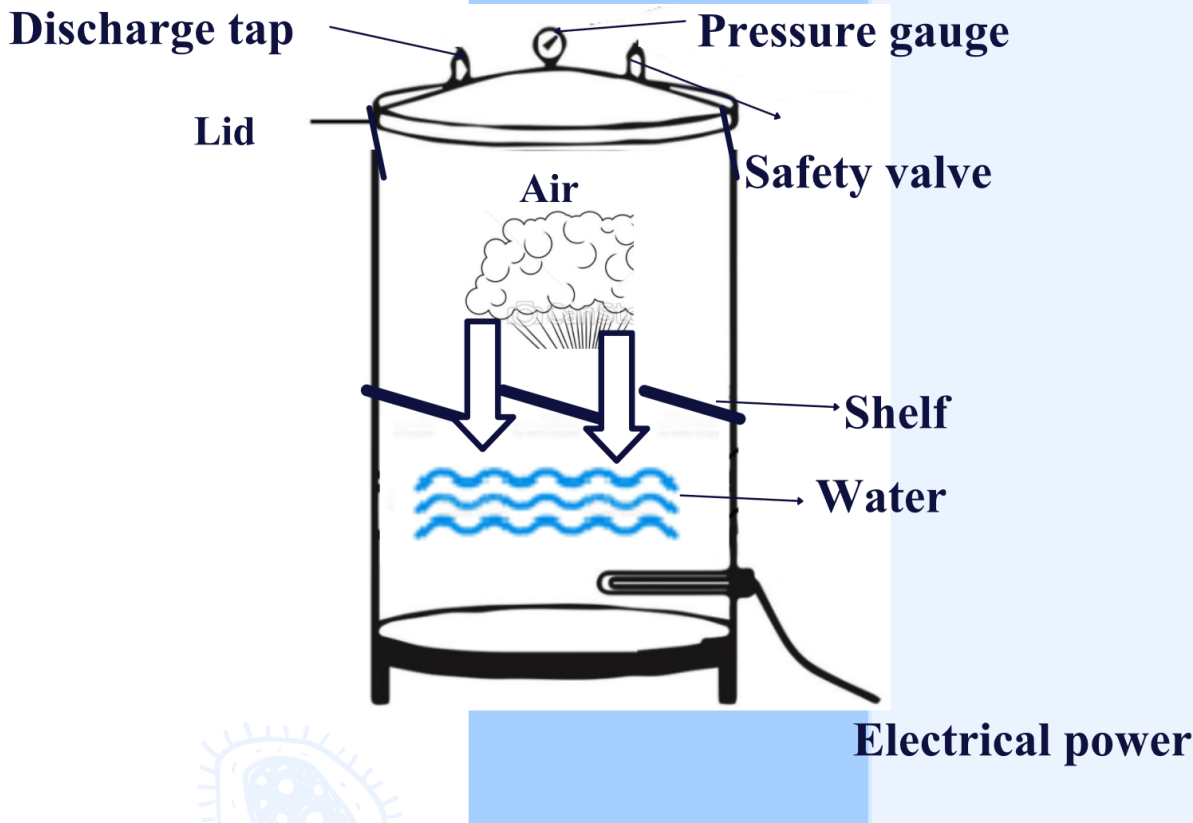
Electrical Power The autoclave is powered by electricity, which heats the **water** in the chamber to generate steam. Inside the autoclave, there is **shelf** where items to be sterilized are placed. There's **safety valve** is included to release excess pressure if it exceeds safe limits.
The autoclave is equipped with a **pressure gauge** to measure pressure and tight-fitting lid that seals the chamber, After the sterilization cycle is complete, the **discharge tip** allows for the safe release of steam and pressure from the chamber.

Moist heat above 100°C (Autoclave)

Pressure: 2 | 3

Heat: 121 °C | 134°C

Time: 20 min. | 6 min.



Autoclave (conventional) Process

The autoclave operates by using electrical power to heat the water within its chamber, initiating the boiling process and generating steam. As steam is produced, it fills the container.

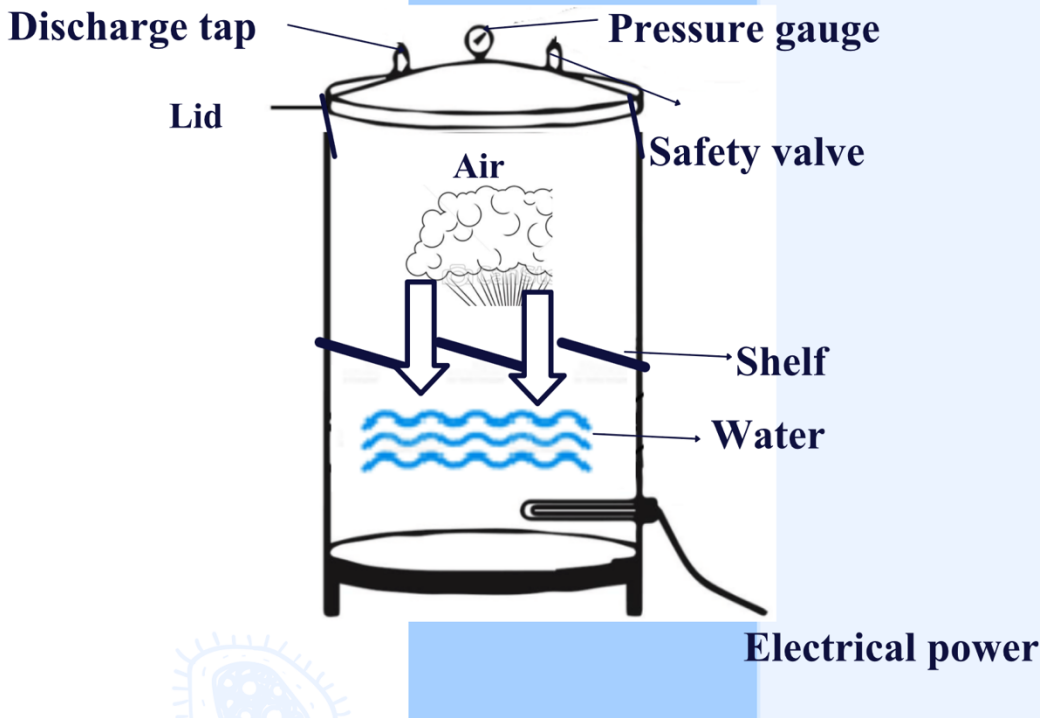
Initially, the chamber contains some air, which is displaced by the rising steam. The discharge tip allows for the release of some of this initial steam and air mixture, facilitating the expulsion of air from the chamber.

As the process progresses, the air is completely expelled, and the chamber becomes entirely filled with steam, particularly after sealing the discharge tip. This steam generates substantial pressure on the water inside the container, significantly elevating the temperature beyond 121°,The combination of high pressure and elevated temperature creates the ideal conditions for effective sterilization, ensuring that any microorganisms present are eradicated.

Moist heat above 100°C (Autoclave)

- When we have these conditions, this achieves sterilization.
- If we increase the pressure to 3 atm, the temperature will rise to 134°C, and the sterilization time will be 6 minutes. However, this will be very dangerous.

Pressure: 2 atm	3
Heat: 121 °C	134°C
Time: 20 min.	6 min.



Moist heat above 100°C (Autoclave)

Prevacuum autoclave

Gravity displacement autoclave

- **The principle of an advanced autoclave system:**

  In this advanced type of autoclave, steam is introduced from an external source through a tube, facilitating the rapid replacement of air within the chamber. The density of steam is significantly lower than that of air, which allows the lighter steam to displace the denser air more quickly and efficiently. By efficiently removing the denser air, the external steam creates a more uniform and effective sterilization environment.

Moist heat above 100°C (Autoclave)

- **The principle of how autoclaves work involves two key processes:**

Denaturation

- Denaturation is the process where proteins lose their natural structure due to the application of heat, steam, or pressure. This loss of structure disrupts the functions of essential proteins and enzymes, rendering the microorganisms inactive and unable to reproduce.

Coagulation

- Coagulation is the process by which denatured proteins clump together, leading to irreversible changes in their structure. In microorganisms, coagulation further disrupts cellular functions and integrity, ultimately resulting in cell death.

Moist heat above 100°C (Autoclave)

- Autoclaves are used for a variety of purposes:

Surgical instruments

- In surgical instruments, we actually prefer another method than autoclaving because, during the process of autoclaving, a little bit of water may remain in the container. This residual moisture can lead to corrosion (rust) unless we use another technique that ensures better drying.

Bed linen

Surgical dressings

Gauze

Cotton

Moist heat above 100°C (Autoclave)

Advantages

High penetration

Latent heat

Non-toxic

Rapid (20 min)

Moist heat above 100°C (Autoclave)

Disadvantages

- we are talking about 121°C, not all objects are suitable for this method.

Not suitable for heat-sensitive objects

Sterilized objects -

moist

making rust

Physical methods for sterilization

I) Moist heat above 100°C (Autoclave)

Monitoring of steam sterilizer

How??

Let's see how

Monitoring of steam sterilizer

1

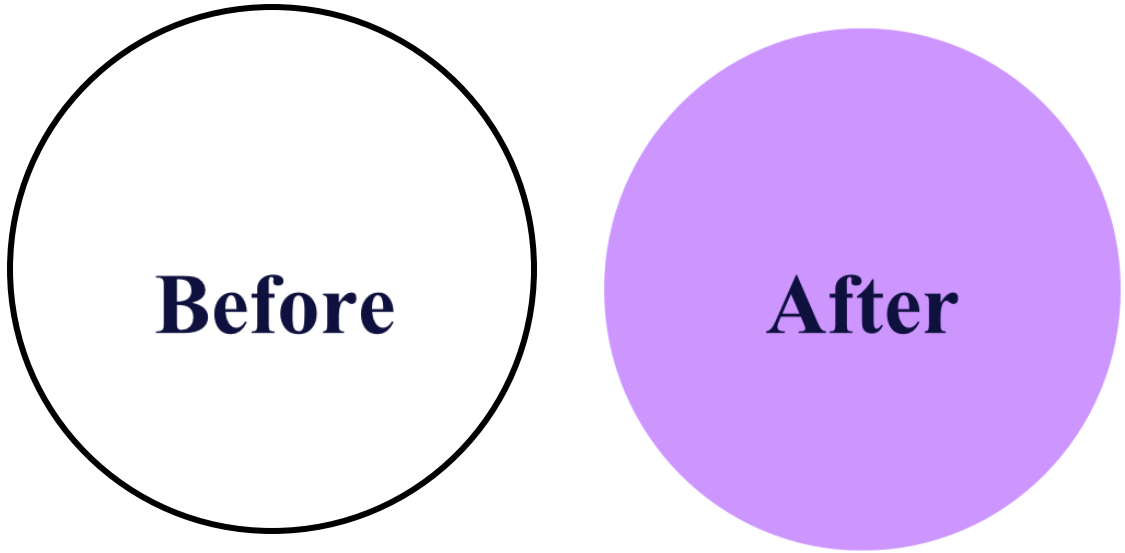
Mechanical indicators

- It is a machine that indicates the pressure, temperature, and duration of the sterilization process.

Monitoring of steam sterilizer

2

Chemical indicators



- In chemical indicators, we have filter paper that contains specific chemicals, which are colorless at first. If it changes color , that means sterilization is complete.

Monitoring of steam sterilizer

3

Biological indicators

Geobacillus Stearothermophilus

G. Stearothermophilus

See next slid :)

- **About biological indiators:**

it involves a specific type of spore forming bacterium (Geobacillus stearothermophills), which ls used to test the efficacy of sterilization processes, primarily autoclaving, with the main aim of killing spores, We place this bacterium in the items being sterilized, and after the autoclave cycle is complete, we take out the test tube and make a subculture, if growth occurs in the culture then this indicates failer of sterilization; if there is no growth, it means that the sterilization was successful.

Physical methods for sterilization

Dry heat (Three ways)

Dry heat

1) Incineration

2) Direct flame

3) Hot air oven

1) Incineration

Burning of Contamination Materials

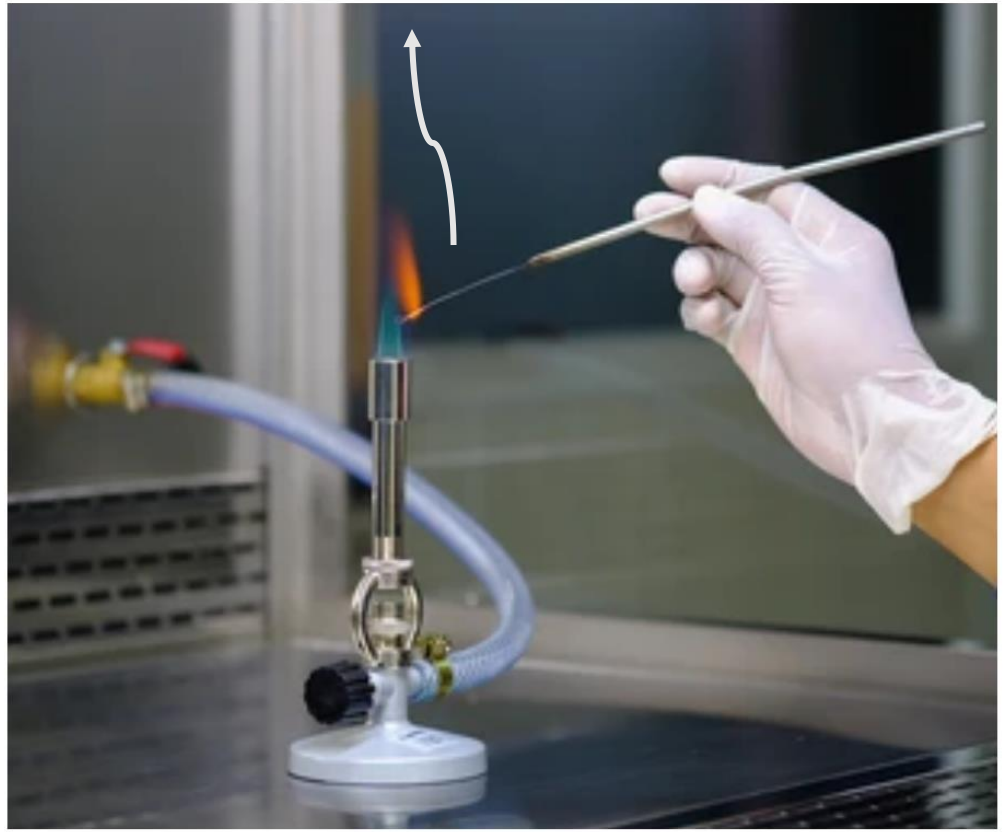
2) Direct Flame

- Must reach the red flame (red flame=sterilization).

Loop

Points of

forceps --> Mouth of test tube



- We use loops in cultures preparation .
- The loop should be sterilized to prevent false results.

3) Hot air oven

An electrically powered device

Heat 160°C -2 hr.

Heat 170°C – 1hr.

Both effectively achieve sterilization.

3) Hot air oven

- **Some uses:**

Sterilization of

Glass-ware

Powders → **This is done to avoid altering the properties of the powder,** so it does not dissolve or form a solution.

Oils

Surgical instruments

Hot air ovens are preferable for sterilization of surgical instruments compared to autoclaves.

3) Hot air oven

Advantages

Non-toxic

Inexpensive

Not corrosive

3) Hot air oven

Disadvantages

Slow heat penetration

Time consuming

Not suitable for heat-sensitive objects

Physical methods for sterilization

Radiation

Radiation

Emitted from

Radioactive

Cobalt 60

(Gamma rays)

- **Cobalt-60 emits gamma rays that can damage DNA.**
  Leading to the death of bacteria .

Radiation

Breaks DAN

Radiation

used in:-

Gloves

Catheters

Surgical sutures

Physical methods for sterilization

Filtration

سبحان الله وبحمده سبحان الله العظيم.
خلصتوا أكتر من نصف الملف :)

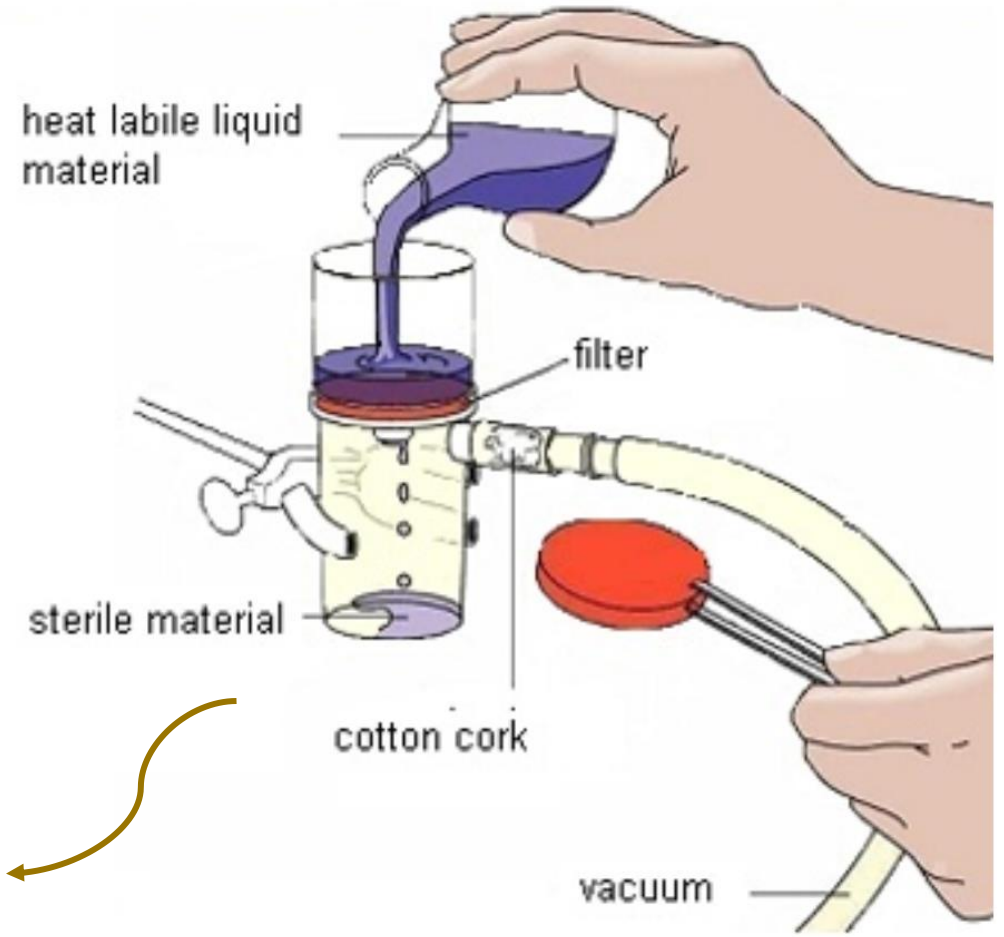
Filtration

Remove microorganisms from biological fluids

Boiling destroys their characteristic.

e.g. Serum, Plasma, Hormones & Vitamins

- Pass the fluid through a tube containing a filter. The filter has pores that allow the liquid to pass through while trapping microorganisms.
- This membrane is usually made from **cellulose nitrate** or **polyester**.



Filtration

Very small pores prevent the passage of viruses.

Membranes made from:-

Cellulose nitrate

Polyester

Filtration

HEPA filters

(High Efficiency Particle Arresters)

- **Used in:**

Operation room

Drug filling cubicles

Chemical methods for sterilization

- Gaseous
- Ethylene oxide
- Peracetic acid

We will focus on:

Gaseous

Chemical methods for sterilization

1) Gaseous

Plasma gas sterilizers

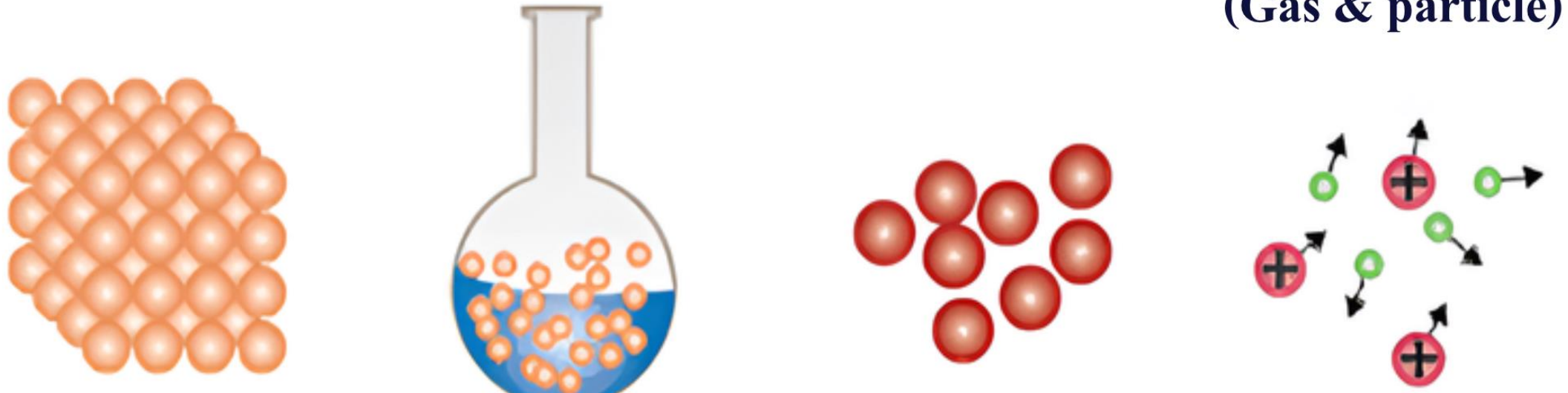
قال ابن تيمية رحمه الله " وليكن هجيراه لا حول ولا قوة إلا بالله، فإنها بها تحمل الأثقال،
وتُكابد الأهوال، ويُنال رفيع الأحوال ".
وهي كنزٌ من كنوز الجنّة.

Plasma gas sterilizers

PHASES OF MATTER

An ionized gas (cloud of gases with electrons and ions) **is produced as a result of radiation exposure.**

(Gas & particle)



Solid

Liquid

Gas

4th state of matter
plasma

Low

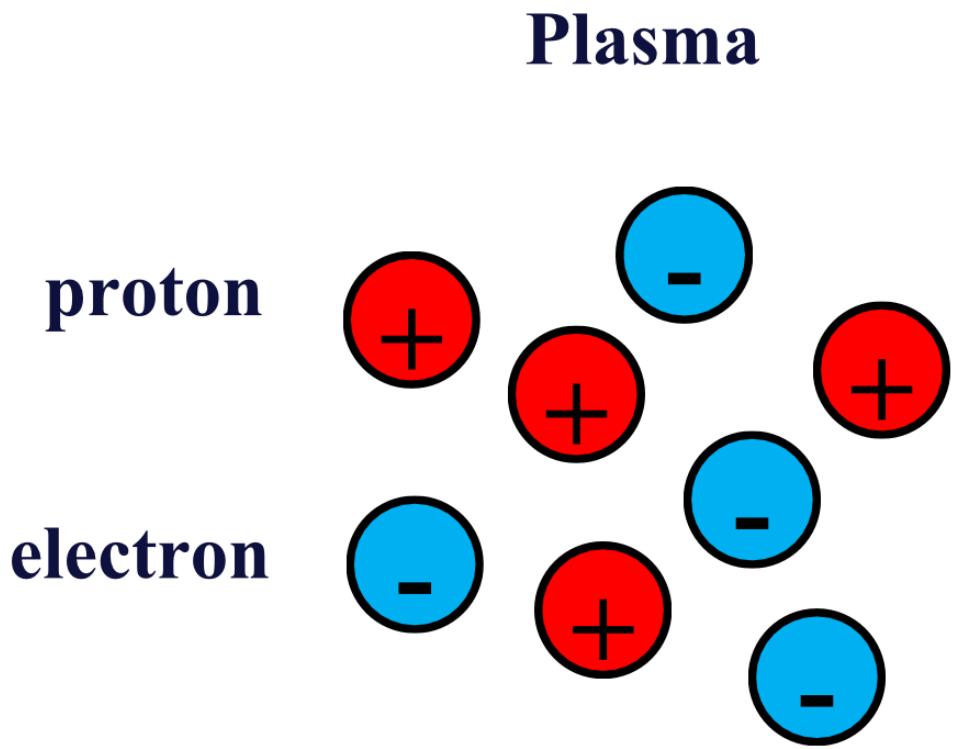
Temperature of Energy

High

Plasma gas sterilizers

Plasma = any gas that

contains electrons, ions



Plasma gas sterilizers

- **Examples:**

1) Hydrogen peroxide gas plasma

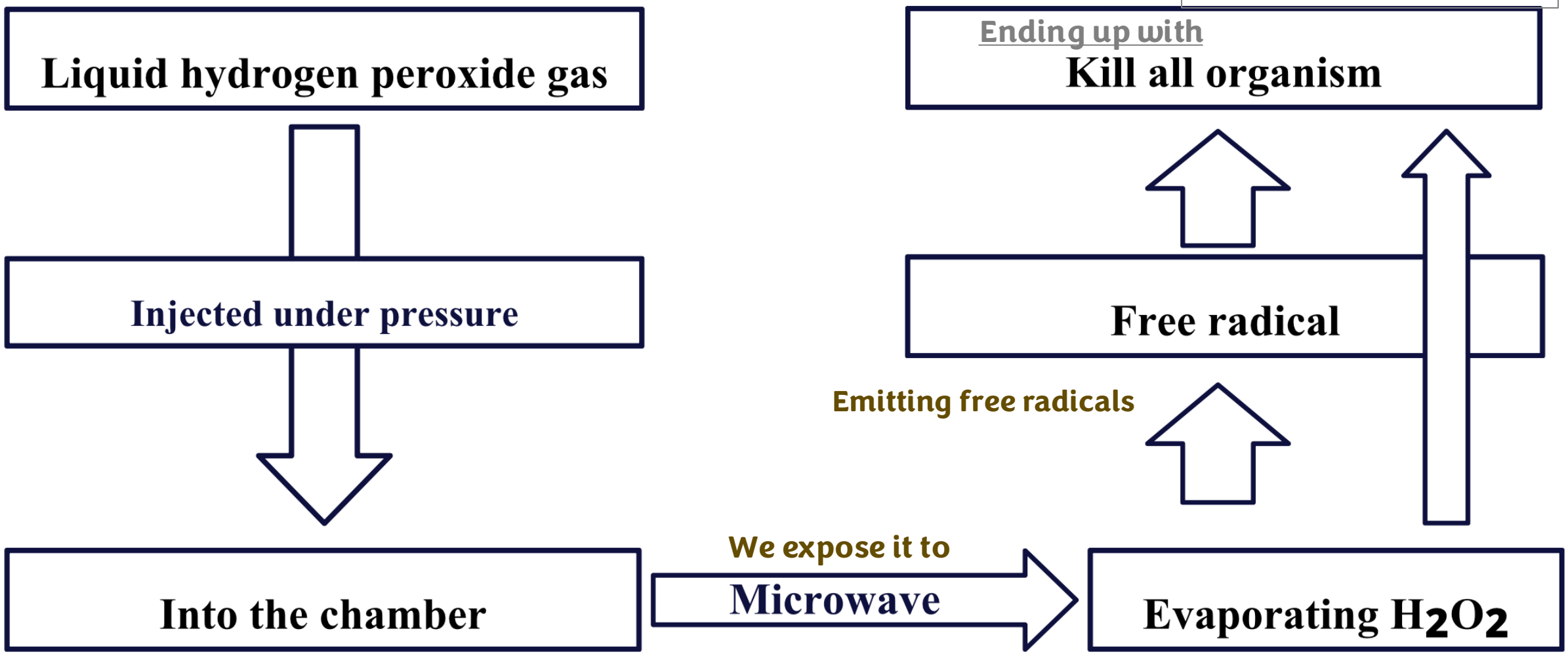
2) Ethelene oxide gas sterilization

3) Peracetic acid gas sterilization

- They all share the same mechanism...

1) Hydrogen peroxide gas plasma

Both H_2O_2 and the resulting **free radicals** contribute to bacterial killing.



1) Plasma gas sterilizers

Used for:-

- **Heat sensitive devices e.g.**
- **Plastic** Because it cannot withstand heat.
- **Laparoscopes** مناظير البطن
- **Arthroscopes** مناظير المفاصل

1) Plasma gas sterilizers

Advantages

- Non toxic
- Suitable for Heat sensitive

1) Plasma gas sterilizers

Disadvantages

- Total time of sterilization cycle is about 50 minutes

Chemical methods for sterilization

I) Gaseous

2) Ethylene oxide (EO)

Same mechanism

2) Ethylene oxide (EO)

EO gas is a highly lethal alkylating agent

Kill all organism including spores

2) Ethylene oxide (EO)

- Items exposed to EO at 55°C for 3-6 hr.

- Then aerated for 8-12 hr. to remove any trace of the gas

Chemical methods for sterilization

I) Gaseous

3) Peracetic acid

3) Peracetic acid

Acetic acid and hydrogen peroxide

- **It exists in liquid or gas form.**

3) Peracetic acid

- How does it work as a sterilant?

Denaturation

Oxidation (enzymes)

Disrupt cell wall

The best sterilant

Chemical solution

Glutaraldehyde 2%

Peracetic acid

Chemical solution

Glutaraldehyde 2%

High level disinfectant (for 20 min.)

Sterilization (10hrs)

Sterilization methods

Physical methods	About the method	Uses	Advantages	Disadvantages
Autoclave	• It is a moist heat above 100° C. • Influencing factors: Pressure & Heat Duration: 20min 2 ~> 121 C Duration: 6min 3 ~> 134 C • Prevacum (Gravity displacement) autoclave: external steam pumping. • Mechanism of work: Denaturation & Coagulation. • Monitoring: 1) Mechanical indicators. 2) Chemical indicators. 3) Biological indicators.	Sterilization of: • Surgical instruments. • Bed linen. • Surgical dressing. • Gauze. • Cotton.	• High penetration. • Latent heat. • Not-toxic. • Rapid.	• Not suitable for heat-sensitive objects. • Sterilized objects are moist.
Dry heat	1) Incineration: burning. 2) Direct flame. 3) Hot air oven: (160 C-2 h) or (170 C-1 h). We will discuss only the hot air oven method in the adjacent columns.	Sterilization of: • Glass-ware. • Powders. • Oils. • Surgical instruments.	• Non-toxic. • Inexpensive. • Not corrosive.	• Slow heat penetration. • Time consuming. • Not suitable for heat-sensitive objects.
Radiation	• Example: Cobalt-60. • Emits γ rays. • Breaks **DNA**.	Sterilization of: • Gloves. • Clatheters. • Surgical sutures.	—	—
Filtration	• Sterilize solutions. • Filter membrane made from: Cellulose nitrate & polyester. • HEPA filters: Operations & Drug filling cubicles.	• HEPA applications.	—	—

Sterilization methods

Chemical methods	About the method	Uses	Advantages	Disadvantages
Gases	• Plasma gas sterilizers: 1) hydrogen peroxide gas plasma. 2) Ethelene oxide. 3) Peracetic acid "BEST STERILANT". • They all have same mechanism that involves Microwave radiation. • EO is lethal alkylating agent. (Exposure duration: 3-6 h @55 C) then (Aerate duration: 8-12 h).	Sterilization of: • Heat sensitive devices. • Plastic. • Laparoscopes & Arthroscopes.	• Not-toxic(the plasma gas sterilization) • Suitable for Heat sensitive instruments .	• EO gas is highly lethal alkylating agent • Long duration (50 minutes).
Solutions	• Glutaraldehyde 2%. • Acts as both: high level disinfectant ~>(20 minutes). Sterilant (10 h).	—	—	—

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	23(last note in the slide) 54(gases advantages &disadvantages)	-The culture should be sterilized -Not toxic	-The loop should be sterilized -Not toxic(the plasma gas sterilization) -EO gas is highly leathal alkylating agent **(added to disadvantages)**
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

(وَقُل رَّبِّ اغْفِرْ وَارْحَمْ وَأَنتَ خَيْرُ الرَّاحِمِينَ)

اللهم انصر أهل غزة وثبّتهم واربط على قلوبهم وارحم
شهداءهم وتقبّلهم في علّيين
اللهم ارحم ضعفنا وقلّة حيلتنا واستعملنا ولا تستبدلنا يا
رب العالمين