تفريغ لاب مايكرو

محاضرة: Exp 5

الصيدلاني\ة: RabaF Zyoud

لجان الدُّفعات

Soul Academy

Experiment 5
Sterilization Methods and Principles

❖ Objectives:

The aim of this experiment is to:

- Understand the different methods of sterilization.
- Evaluate the effectiveness of various sterilization methods under laboratory conditions.

❖ Introduction

- **Sterilization** is the process of **eliminating** all forms of microbial life, including bacteria, viruses, fungi, and spores, from surfaces, instruments, media, and other materials. It is essential for maintaining aseptic conditions in medical, laboratory, and industrial environments.

- **Decontamination** involves **removing** or neutralizing contaminants from objects or environments.

- **Survivor curves:** They are plots of the logarithm of the fraction of survivors (microorganisms which retain viability following a sterilization process) against the exposure time or dose.

➢ Methods of Sterilization are:

- **Physical Method**

1. Thermal (Heat) Sterilization methods

Heat Sterilization: is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. Both dry and moist heat are used for sterilization. The process is more effective in hydrated state. This method of sterilization can be applied only to the thermostable products

I. Moist Heat Sterilization

Moist heat uses steam at temperatures between 121°C to 134°C and is highly effective for sterilizing metal instruments, glassware, and culture media. Autoclaves are standard equipment in labs for moist heat sterilization, operating at 121°C for at least 15 minutes under pressure. It is more efficient than dry heat due to better penetration and protein denaturation.

- Boiling water (~100°C) is a simple method but does not kill all spores.
- Pasteurization is an application of moist heat. It is used for food and beverages, applying lower temperatures (e.g., 63°C for 30 mints or 72°C for 15 secs) to kill pathogens while preserving food quality.

ال Survivor Curve عبارة عن Curve يمثل العلاقة بين ال Survivor

ال exposure time لـ sterilization method معينة ، مثلاً بتطون هاي لا method تنعم لخمس دقائق و بعد ال 5 دقائق

رح أخذ volum محسب من ال MO وهيك رح احدد ال Fraction وبالتالي بقدر احدد عدد ال MO بالعينة الاصلية (لازم أحسب ال conc ال log ليه)

Mosit ← Faster and more effective than dry ← بسبب ال Penetration

Mosit ← Need temp less than dry

II. Dry Heat Sterilization

Dry heat sterilization employs high temperatures (160-180°C) for extended periods (up to 2 hours). It's used for materials that can withstand such heat, like glassware and metal tools.

• Incineration is a common method where items are burned (e.g., flaming an inoculation loop).

• Dry heat destroys bacterial endotoxins and is useful for sterilizing powders and oils.

III. Moist vs. Dry Heat Comparison

• Moist heat is faster and more effective than dry heat.

• Moist heat requires penetration of both heat and moisture.

• Dry heat is suitable for materials that might corrode or degrade under moist conditions.

• Equipment for moist heat (autoclaves) is more complex.

2. Radiation Sterilization method

Radiation Sterilization many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light), particulate radiation (e.g. accelerated electrons). The major target for these radiations is microbial DNA.

- Radiation sterilization with high energy (Gamma rays and electron beams) are used for industrial sterilization of heat-sensitive items.
- UV-C light (200–280 nm, optimal at 265 nm) is used for disinfecting air, water, and surfaces. UV sterilization is limited by its poor penetration but is effective at the surface level. It forms thymine dimers in DNA, preventing replication.
- **Radiation sterilization** is generally applied to articles in the dry state; including surgical instruments, unit dose ointments, plastic syringes and dry pharmaceutical products

3. Filtration Sterilization method

Filtration removes microorganisms from liquids and gases using membrane filters. It is used for heat-sensitive solutions like vaccines and antibiotics, the major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material.

.

- Filters have pore sizes (0.22um, 0.45um, 0.5um, 1um µm) that physically block microbes.
- Materials include cellulose, nylon, or Teflon.
- Used in pharmaceutical industry, sterile ventilation systems, and sterility testing.

- **Chemical Method**

1. Gaseous Sterilization

Uses gases like ethylene oxide (EtO) or formaldehyde. They act by alkylating proteins and DNA.

• Suitable for plastics, electronics, and other heat-sensitive materials.

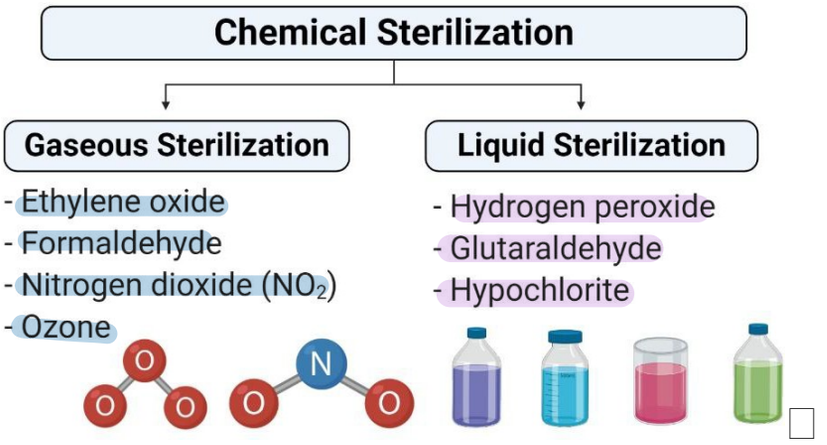
• High penetration power but toxic and potentially carcinogenic.

• Requires proper aeration post-sterilization to remove toxic residues.

2. Liquid Sterilization

Submersion in chemicals like hydrogen peroxide is used when heat or radiation is not suitable.

- Effective for low levels of contamination.
- Hydrogen peroxide (35–90%) is a strong oxidizer used in medical fields.
- Shorter sterilization cycles (~28 min) than gaseous methods.
- Limited penetration and material compatibility.



Practical Part

Test 1: Test for red heat sterilization

1. Divide the nutrient agar plate into four quadrants and label 1 through 4.
2. Quadrant 1 is your negative control. Do not touch it.
3. Gently resuspend the bacterial suspension provided to you.
4. Sterilize the wire loop by Bunsen burner. Then allow it to cool before picking up any microorganisms.
5. Use the sterilized wire loop to inoculate a sample.
6. Streak it on the surface of the second quadrant of petri dish.
7. Sterilize the wire loop by Bunsen burner. Then allow it to cool before picking up any microorganisms.
8. Use the sterilized wire loop to inoculate a sample.
9. Immerse the loop in ethanol 70% in a 50 ml beaker.
10. Wait to dry, and then Streak it on the surface of the third quadrant of petri dish.
11. Sterilize the wire loop by Bunsen burner. Then allow it to cool before picking up any microorganisms.
12. Use the sterilized wire loop to inoculate a sample.
13. Sterilize the wire loop by Bunsen burner. Then allow it to cool before picking up any microorganisms.
14. Streak it on the surface of the fourth quadrant of petri dish.
15. Cover the plates with their lids, and incubate them at 37 °C for 24 hours.

Test 2: Test for Sterilization by Ultraviolet Light

In this practical, we are going to investigate the bactericidal effect of UV light as a function of exposure time in addition to evaluating its penetrating power through different materials.

1. prepare a bacterial lawn by dipping a sterile cotton swab in a diluted overnight culture (of

selected bacteria) and then spread the bacterial inoculum across the entire surface of a nutrient agar plate.
2. covering part of the plate by a tin foil in one time and a piece of paper in the other.
3. Place an inoculated plate under the UV lamp, with the lid removed, for each of the following exposure times: 2 or 5 minutes.
4. Re-cover the plates with their lids, and incubate them at 37 °C for 24 hours.

Test 3: Test for moist heat sterilization at temperature above 100°C (Autoclaving)

1. Under aseptic technique, prepare a bacterial lawn by dipping a sterile cotton swab in a decontaminated (autoclaved) bacterial suspension provided to you, and then spread the bacterial swab across the entire surface of a nutrient agar plate.
2. Cover the plate with it's lid, and incubate them at 37 °C for 24 hours.

Test 4: Test for moist heat sterilization at atmospheric pressure and 100 °C

1. Divide the agar plate in to three sections and label correctly.
2. Take an inoculum using a cotton swap then spread the bacterial inoculum across the first section of a nutrient agar plate (this inoculum will be labeled at time zero and also considered a positive control).
3. Place the bacterial suspension test tube provided to you in a water bath at 100 °C.
4. After 5 min, take one test tube, take an inoculum using a cotton swap then spread the bacterial inoculum across the second section of a nutrient agar plate.
5. After 10 min (Total of 15 min), take an inoculum using a cotton swap then spread the bacterial inoculum across the third section of a nutrient agar plate.
6. Cover the plate with its lid, and incubate it at 37 °C for 24 hours