

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.

الهدف هو الصفاء على بيئة معقمة

Decontamination involves removing or neutralizing contaminants from objects or environments.

Survivor curves are used to study the relationship between the duration or intensity of exposure and the fraction of surviving microorganisms.

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.

وهي علاقة اللوغاريتم النسبة المتبقية مقابل وقت التعرض للتعقيم

> Methods of Sterilization are:

كل ما زاد وقت التعقيم قل عدده الميكروبات الحية

• Physical Method

طرق التعقيم الفيزيائية

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.

بال Autoclave البخار يضرب المواد بسهولة بسبب ضغط البروتينات (Protein denaturation) داخل البكتيريا

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 mins or 72°C for 15 secs) to kill pathogens while preserving food quality.

المستخدمة لتعقيم المواد الغذائية من الطهي هذه العملية تقتل البكتيريا الضارة فقط مع الحفاظ على جودة الطعام

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.

الحرارة الرطبة أسرع وأكثر فعالية

لأنه يعمل Protein denaturation بسرعة

التعقيم هو عملية القضاء الكامل على جميع أشكال الحياة الميكروبية

إزالة التلوث Survivor curves هي رسوم بيانية توضح نسبة الميكروبات التي تبقى ماثقة كما ما زاد وقت أو شدة التعقيم

تدمير الأنزيمات واضطراب المكونات الخلوية الأساسية

تعقيم على البخار

البلدان يكون عند درجة حرارة 100°C لكن ما يقتل جميع ال spores هناك ما ينبغي عنه تعقيم كامل

تعقيم على الهواء الساخن بدون بخار

الحرق أي حرق ال inoculation loop على اللهب هي طريقة تعقيم فعالة

هذه الطريقة يتكون المثل فعالية إذا كانت الصلابة فيها ماء

الحرارة الرطبة

المستخدمة لتعقيم المواد الغذائية من الطهي

هذه العملية تقتل البكتيريا الضارة فقط مع الحفاظ على جودة الطعام

الحرارة الجافة

160-180°C

up to 2 hours

Incineration

Moist vs. Dry Heat Comparison

الحرارة الرطبة أسرع وأكثر فعالية

لأنه يعمل Protein denaturation بسرعة

تستخدم للواد التي تتحمل درجات حرارة عالية جدًا

ميزة الحرارة الجافة إنها يدمر ال endotoxins عنان هيئ بنمطه مع الإبرة والمصاق

- 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.

الحرارة الجافة مناسبة للمواد التي يمكن تصريفها أو تصدأ مع الرطوبة في الزيوت والزيجاج المعادن

2. Radiation Sterilization method

بعض هذه لطعام الاشعاع للتدمير الجائحات الدقيقة

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. →

الحمض الثيميبي للأشعاع هو DNA الضام بالميكروبات لا يستطيع الضلية الانقسام أو التكاثر

- Radiation sterilization with high energy (Gamma rays and electron beams) are used for industrial sterilization of heat-sensitive items.

تستخدم في الصناعة الدوائية لتعقيم مواد حساسة للحرارة

- 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.

تستخدم لتعقيم الهواء / الماء / الأسطح لكن مشكلتها قدرة اختراق ضعيفة بحيث تعقيم الأسطح فقط

يتمتع بقدرة عالية على تكوين Thymine dimers داخل DNA وهذا يمنع تضاعف أو DNA داخل الخلايا الميكروبية

- 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

التعقيم بالترشيح

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.

① ② ③ ④ ⑤
 ① غربلة ② لاصقة الميكروبية ③ لاصقة المادة ④ لاصقة الفلتر ⑤ حبس الجزيئات

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

هنا الطريقة لا تقتل الميكروبات بل تزيلها من السائل أو الغاز تستخدم عادة لا تكون المادة حساسة للحرارة تزيل اللقاحات والمضاد الحيوي

Chemical Method

كلما ما اقدم لمضغ حرارة أو شمعي بمضغ هذه الطريقة الكيميائية

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.

تعمل Alkylation على ① Proteins ② DNA بالتالي بؤة الميكروب

2. Liquid Sterilization

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

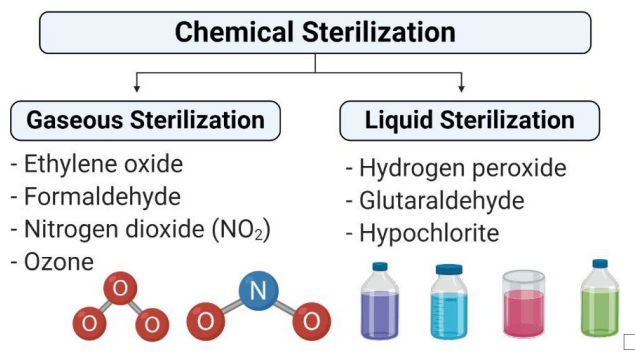
- Effective for low levels of contamination.
- Hydrogen peroxide (35–90%) is a strong oxidizer used in medical fields.
- Shorter sterilization cycles (~28 min) than gaseous methods.
- Limited penetration and material compatibility.

التعقيم بالسوائل تعتمد على عوامل مؤكسدة قوية كيميائية تسمى Hydrogen peroxide strong oxidizing agent

عند قدرة اختراق عالية ليس مشكلتها انه سام وممكن يسبب سرطان

تعقيم سريع

عيبه ① فترة اختراق محددة ② محتما ما يصاب بعضها المواد



- Moist Heat
- Dry Heat
- Radiation
- Filtration
- Gaseous
- Liquid

أفضل طريقة تعقيم (Autoclave) للزجاج والزيوت والمساحيق للأدوات الحساسة للحرارة للمحاليل مثل اللقاحات للبلاستيك والأجهزة عند عدم إمكانية استخدام الطرق الأخرى

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 their lids, and incubate them at 37 °C for 24 hours.

ما ر 2 /
يكون في
توبجيري
لأنه الأوتوكلاف
لأنتين
المختبريا واليدرات
عشان هيلك كيناعه
هوا نضل طريقة لتعقيم

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

1. Place the two bacterial suspension test tubes provided to you in a water bath at 100 °C.
2. After 5 min, take one test tube, wait to cool and prepare a bacterial lawn by dipping a sterile cotton swab in a test tube and then spread the bacterial inoculum across the entire surface of a nutrient agar plate.
3. Cover the plate with their lids, and incubate them at 37 °C for 24 hours.
4. After 15 min, take the second test tube, wait to cool and prepare a bacterial lawn as we did in the previous step.
5. Cover the plate with their lids, and incubate them at 37 °C for 24 hours

ليه ال UV غير فعال للتعقيم الكامل ؟
لأنه ← poor penetration في شرفه
عنه ال surface

11. Sterilization by moist heat usually uses an instrument called Autoclave.
12. UV light forms Thymine dimers in DNA.
13. Dry heat sterilization usually requires temperatures around 160 - 180 °C.
14. Ethylene oxide is used in Gaseous sterilization.
15. UV light is effective mainly on the Surface of materials.