* بسم الله الرحمن الرحيم
ربنا اشرح لي صدري ويسر لي أمري واحلل عقدة من لساني
بسم الله الفتاح ♡

Experiment 5

Sterilization Methods and Principles

Introduction

- **Sterilization**: can be defined as any process that effectively **kills or eliminates** transmissible agents (such as fungi, bacteria, viruses and prions) from a surface, equipment, foods, medications, or biological culture medium. In practice sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time.
- **Decontamination**: is the process of **cleansing** an object or substance to **remove** contaminants such as micro-organisms or hazardous materials, including chemicals, radioactive substances, and infectious diseases.
- **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.

* بالبداية بدنا نتذكر كم شغلة :

sterilization ⇐ حكينا انو هي process بتتضمن ازالة أو Killing أو elimination لكل ال microorganism

سواء كانت (fungal, bacteria, virus) يعني ما بخلي ولا اشي موجود، فلما احكي هذا السطح steril معناها انو free 100% من ال M.O.

* طيب كيف بنقدر نعمل ال ~~[illegible]~~ sterilization ؟ ← عن طريق اننا نستخدم ال sterilization technique الي بتأثر على كل الوقت عند اخد dose محددة.

وطرق ال sterilization هي 1 physical method 2 chemical method.

* وعرفنا كمان شو هي ال Decontamination وحكينا انو هي عملية cleaning أو removing لجزء من ال M.O أو ال hazardous materials من مواد ضارة لأنو مش كل ال MO راح.

* وتعرفنا كمان على مصطلح اسمه ال survivor cures : وهي علاقة بتربط بين : أديش ضل عندي MO (يعني كمية ال MO الي ضلو عايشين) مع أديش ال dose أو الوقت الي أنا استخدمته خلال ال sterilization technique

ال (x axis يكون عندي ال time مثلا ، y axis يكون عندي عدد ال M.O)

وعادة بيكون عدد ال curve عطول ب Log scale.

➢ Methods of Sterilization are:

A. 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. In these processes 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

يعني عاتباتو بالحرارة

1.1 Moist Heat Sterilization

Moist heat sterilization involves the use of steam in the range of 121-134 °C. Steam under pressure is used to generate high temperature needed for sterilization. Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures. Boiling water is the most common form of application of moist heat but is not capable of killing endospores or killing all viruses

اسم الجهاز

* هلا حلينا نبدأ نحكي عن ال sterilization method :
كده زي ما حكينا هم ه ينقسموا ل physical method و chemical method

sterilization method

- physical method (mainly ع جهاز)
  1. Heat
  2. Filtration
  3. Radiation
- chemical method (mainly متفرقين)
  - use of chemical Agents
  - نوع فلشطات ال انهم رح نحكيهم عن شكل gas or liquid

* هلا بالنسبة لل sterilization by Heat :

sterilization by Heat (Heat فيقسمني نوعين من ال)

1. Dry Heat
   - Direct Heat
     Ex: flaming or Incineration
     زي لما بالمختبر نعقم inculation Loop لل
   - in Direct Heat
     ex: oven
     ال oven داخل ال powders اي انه مثلا لل حرارة وسينا مدة معينة
2. Moist Heat
   Ex: Autoclave
   لل moist Heat :
   mainly نحكي ال عن ال Autoclaving تحت under pressure نستخدم
   و Vapor مع وجود Humidity (رطوبة)

* يلا خلونا نحكي ل details أكثر :

[1] * طبعاً بدنا نبدأ بال physical method رح نحكي عن ال Thermal (Heat) sterilization وهي من أكثر الطرق المستخدمة وأكثر الطرق الفعالة عشان تعمل sterilization ونتيجة وجود ال temperature وال Heat بيصير فيها ال distruction لل enzyme وال protein ومكونات الخلية فبيصير Killing لل Microorganism .

وزي ما حكينا ال Heat ممكن تكون Dry or Moist Heat .

ومعلومة إنه in general ال moist Heat لوجود ال humidity أو ال Hydration بتسهل أو بتساعد كل ال penetration للسطح أو ال object اللي أنا بدي أعمله sterilization .

* وطبعاً ما بنقدر نستخدم هاي ال method للأشياء بتتأثر بالحرارة *

[2] ال Moist heat sterilization :

⇐ باستخدام هاي الطريقة بترتفع درجة الحرارة ل Range بين ال 121 - 134°C : لأنه عندي Water ويتحول ل steam (بخار) وهاد ال steam بيكون under pressure ، والجهاز اللي بيوفر هاي ال Condition هو ال Autoclave . فوجود الحرارة المرتفعة مع ال steam وال pressure بيعمل destroy لل M.O .

وهاي ال method هي أكثر method مشان أعمل decontamination لل Laboratory waste وفي ال Glass ware وال media بس نتخلص منها ، وهون لازم انتبه ! إنه هاي الطريقة ما بتزبط لكل ال glassware أو كل ال items ، فمثلاً ازا في أشياء بتتأثر بالحرارة زي البلاستيك وال rubber ما فيني أستخدمها معاها ال method . وآخر شغلة ! إنه هي من أكثر الطرق ال effective اللي بتقتل ال M.O

ال M.O أو M.O بس في أجزاء من هاي ال method ما بتقتلها زي ال endospores أو بعض أنواع الفيروسات ممكن تكون resistant لهاي ال method وبتقدر تضل عايشة حتى لو درجة الحرارة المرتفعة وال pressure العالي .

Autoclave

An autoclave is a high-pressure device used to allow the application of moist heat above the normal-atmosphere boiling point of water Exposure to 121°C for 15+ minutes is typically sufficient to sterilize

Again, the material must be 121°C before the clock starts

Large items, large volumes, and items that are poorly penetrated by steam may take much longer than 15 minutes to sterilize

Pasteurization

Pasteurization is the application of moist heat of less-than boiling temperatures to foods to prevent the growth of food-spoiling organisms as well as various heat-labile pathogens

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2 Dry Heat Sterilization

It employs higher temperatures in the range of 160-180 °C and requires exposures time up to 2 hours, depending upon the temperature employed. It is used for sterilizing non-aqueous thermostable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Incineration is another common method of dry heat sterilization, e.g., such as the flame incineration of an inoculating loop

* أكتر جهاز مستخدم عشان أعمل moist sterilization هو الـ Autoclave وهو جهاز بيعطيني High pressure وبيطلع حرارة عالية بتوصل لـ 121°C وعادةً 15 دقيقة دي هي الـ condition قادرة إنها تعمل killing للـ M.O وفي بعض أنواع الـ M.O بيحتاج وقت أكتر. وكمان بتعتمد على الـ items اللي أنا بدي أعمل لها sterilization إذا الحاجة مو كثير permeable للـ steam بتاخذ وقت للـ killing فبيحتاج لوقت أكتر.

* في عندي كمان مثال على الـ moist sterilization بس أقل قوة الـ pasteurization ودي اللي بعمل application لـ moist heat لكن بدرجة حرارة أقل من الـ boiling point وعادةً تستخدم الـ food industry عشان أمنع الـ growth للـ M.O والـ pathogens داخل الـ food

* بيدخل تحت الـ Dry Heat steri. في application على درجات حرارة مرتفعة أكتر من الـ moist steam تتراوح بين 160-180°C وكمان بيحتاج لوقت أطول ممكن توصل لـ ساعتين ويتم استخدامها إذا بدي أعمل sterilization لـ (thermostable powders and thermostable liquids non-aqueous).
والـ dry heat ممكن إنها تكون قادرة تعمل killing لـ أو destroys للـ endotoxins or pyrogens وبالتالي ممكن أستخدمها عشان أعقم الـ glassware (زي لما نعقم الـ inoculation loop بالمختبر)

هلا خلونا نقارنه بين الـ moist والـ dry heat

Comparison between moist and dry heat sterilization method

1. Moist heat is more effective than dry heat at a given temperature or length of exposure
2. Moist heat is also more penetrating than dry heat
3. However, to achieve sterilization employing moist heat requires rather elaborate equipment, i.e., the employment of an autoclave
4. Just as with dry heat, temperature and length of exposure are inversely related
5. penetration is absolutely critical with moist heat, penetration is even more of a problem since penetration of heat without associated penetration of moisture is simply dry heat and dry heat is less effective as an antimicrobial than moist heat; consequently, things that block moisture penetration can inhibit sterilizing efficacy.

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** or **accelerated electrons** has proven to be a useful method for the industrial sterilization of heat sensitive products. But some undesirable changes occur in irradiated products, an example is aqueous solution where radiolysis of water occurs. **Radiation sterilization** is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastic syringes and dry pharmaceutical products.

Ultraviolet (UV) light is electromagnetic radiation with a wavelength between 10 and 400 nm; which bridges the gap between the visible light and X-rays. The germicidal effect of UV light is associated with a specific region of its spectrum known as the UV-C band which practically spans from 200 to 280 nm, with an optimum germicidal effect at 265 nm. Around this wavelength, UV light can destroy microbial cells by either damaging their DNA, mainly through thymine dimer formation, or by directly damaging their proteins. For decontamination purposes, UV light is usually produced by mercury-vapor lamp which gives a maximum emission at near-optimum wavelength of 253.7 nm. Such UV light can be used for water, air and surface sterilization, but it is practically unsuitable for sterilization of pharmaceutical products since it has poor penetrating power through many materials including those commonly used in packaging of pharmaceuticals. On the other hand, the antimicrobial effect UV light is directly related to its dose which is a function of both; light intensity and exposure time.

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.

* وهلأ رح نحكي عن الـ Radiation وهي physical method المناسبة من الـ method :

في عنا عدة أنواع من الـ Radiation مثل الـ electromagnetic ومثال عليها (gamma rays + UV Light) أو particulate radiation ومثال عليها الـ (accelerated electrons).

هلأ غالباً الـ mechanism of radiation إنها تعمل distruction الـ Microbial DNA.

* الـ gamma rays or accelerated electrons منستخدمها عنا بالـ industrial sterilization والها application كتير في الـ heat sensitive product اللي منعبر عنها بـ radiation.

وميزة الـ radiation sterilization إنه in generally بيصير الها application في الـ object اللي dry state زي الـ glassware والـ plastic syringes

* خلونا نحكي عن الـ UV Light :

هلأ احنا منعرف إنه الـ electromagnetic radiation عندي الـ wavelengths من 10 - 400 nm ولو بدي أحدد فيها الـ range الـ UV رح تكون بين الـ Xrays و Visible Light بالنهاية range الـ UV. هلأ في هاد الـ [redacted] range الـ UV في عندي منطقة بنسميها UV-C band وهي أكثر منطقة effective إنها تقتل الـ MO ونسبتها بين الـ 200 to 280nm والأكثر effective فيها optimum نسبتها هي الـ 265nm.

* الـ UV Light قادر إنه يحقق الـ damaging لـ MO إما عن طريق إنه يعمل damage للـ DNA من خلال إنه يعمل thymine dimer formation أو من خلال إنه يعمل distruction للـ protein.

* الـ UV Light ممكن استخدامها نعمل decontamination لـ water أو الـ air أو surface ومناسب عنا نعمل sterilization لـ pharmaceutical product لأنه عنده poor penetration للـ packaging الـ pharmaceuticals product

* ولازم ننتبه لشغلة إنه الـ effect للـ UV Light هو highly dependant على الـ Light intensity والـ exposure time.

3. Filtration Sterilization method

Filtration Sterilization Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. Membrane filters are composed of different types of polymers e.g cellulose, teflon, nylon and others. They have different pore sizes, 0.22um, 0.45um, 0.5um, 1um. Some filters are sterile others are not. Membrane filters have different diameters (2.5 cm, 3cm, 5cm etc). The main mechanism by which these filters work is sieving, where particles (microbes) above the pore size are retained on the filter. Membrane filters with pore size 0.2-0.22 um are used for sterilizing liquid solutions by filtration. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermenters, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing.

* هلأ بدنا نحكي عن آخر method من الـ physical method وهي الـ Filtration sterilization method :

* في هاي الـ method احنا ما بنعمل killing للـ M.O في بس عملية removing للـ M.O وممكن استخدمها عشان نعمل clarification او sterilization لـ liquid أو gas

مبدأها انه بيكون في عنا شي membrane (filter) وهاد الـ filter بيجي عنا منه أنواع مختلفة من الـ polymer زي الـ cellulose أو الـ teflon أو الـ nylon وبرضو بيجي منه different pore size يعني حجم الفتحات الموجودة في هاد الـ filter بتتراوح بين 0.22um لـ 1 um • وبيجي برضو منها لـ diameter مختلفة من (2.2 - 5 cm). خلاصتها انه الـ liquid أو الـ gas لما يمر من خلال هاد الـ filter الـ M.O بتعلق على surface الفلتر (بنحكي عنها seiving) وهيك الـ liquid أو الـ gas بطلع free من الـ M.O وعشان نعمل sterilization غالبا الـ size المستخدم (0.2-0.22) لأنها دائما بتعطي الـ retention للـ M.O على السطح تاعه.

* هاي الـ method من الـ sterilization مفيدة جدا اذا بدي أعمل sterilization لـ heat sensetive material أو pharmaceutical product زي الـ injection, eyedropes biological product وكمان الـ air و other gases.

B. Chemical Method

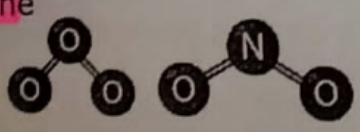
Chemical Sterilization is the process of removal of microorganisms by the use of chemical bactericidal agents. Even if physical methods of sterilization are more appropriate for effective sterilization, it is not always appropriate to use for heat-sensitive materials like plastics, fiber optics, and biological specimens. Under such conditions, chemical either in liquid or gaseous state can be used for sterilization. However, it is crucial to ensure that the materials undergoing sterilization are compatible with the chemical being used. Besides, it is important to adopt safety rules in the workplace safety during the use of chemical agents.

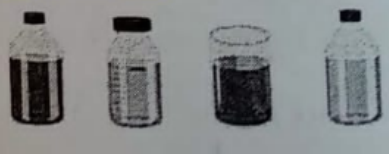
Chemical sterilization is typically used for devices that would be sensitive to the high heat used in steam sterilization, and for devices that may be damaged by irradiation (rubbers and plastics can become more brittle after irradiation.)
Often chemical sterilizers function by using low temperature, highly reactive gases that come into direct contact with the test article (often through a semi-porous membrane or package.) Liquids – for example, bleach – are also used for sterilization.

The chemical method of sterilization can be categorized as liquid and gaseous sterilization.

Chemical Sterilization

Gaseous Sterilization	Liquid Sterilization
- Ethylene oxide	- Hydrogen peroxide
- Formaldehyde	- Glutaraldehyde
- Nitrogen dioxide (NO_2)	- Hypochlorite
- Ozone	





* هلا بدنا نحكي عن الطريقة التانية من طرق الـ sterilization وهي الـ chemical method.

في الطريقة هاي بنستخدم chemical bactericidal agents مشان نعمل removing للـ M.O.

* بالرغم من انه الـ physical method هي effective و appropriate، الا انه في بعض الحالات ما بنقدر نستخدم الا الـ chemical method زي مثلاً إذا كنت بتتعامل مع heat sensitive material أو material بتتأثر بالـ (radiation

فهون بنضطر نستخدم الـ chemical method.

والـ chemical agent يا اما تكون liquid أو gaseous لتقدر تستخدمها في الـ sterilization.

ولازم انتبه لعدة شغلات لما بدي استعمل أو اتعامل مع الـ chemical: انه لازم اتأكد انه الـ material اللي بدي استخدمها compatible مع الـ tools أو الـ items اللي بدي اعملها sterilization، ⓒ لازم اتأكد من الـ safety للـ workplace اللي انا بتعامل أو بشتغل فيه لانه انا بتعامل مع chemical يعني لازم ما يكون toxic.

* واحدة من مميزات الـ chemical method انها بتكون at low temperature مناسبة للـ sensitive material.

زي ما حكينا الـ chemical sterilization بتكون إما liquid أو gaseous زي ما هو مبين بالصورة.

1. Gaseous method

Gaseous Sterilization the chemically reactive gases such as formaldehyde (CH2O) and ethylene oxide (CH2)2O possess biocidal activity. The mechanism of antimicrobial action of this gas is assumed to be through the alkylation of sulphydryl, amino, hydroxyl, and carboxyl groups on proteins and imino groups of nucleic acids. Both of these gases being alkylating agents are potentially mutagenic and carcinogenic.

The efficacy of the gas depends on the concentration of gas available for each article which is greatly assisted by the good penetrating nature of the gas, which diffuses readily into many packaging materials including rubber, plastics, fabric, and paper.

نبدأ بال Gaseous method :

من أهم الـ method يلي بستخدم gas مثل ال formaldehyde أو (CH_2O) وال ethylene oxide $(CH_2)_2O$ وهدولا بيعملوا biocidal activity على ال MOs ، أما ال mechanism اللي بيشتغلوا فيها إنه هو بعمل alkylation لـ sulphydryl / amino / hydroxyl / carboxyl الموجودين بالبروتين وال imino groups بال nucleic acid

وضروري نحكي انه هاي ال material فيها risk انه هو عبارة عن carcinogenic, mutagenic material على اللي رح يستخدم عشان هيك لازم نكون careful واحنا بنستخدمهم.

* ال efficacy عشان نعلي ال MOs killing بتعتمد على أديش تركيز الغاز وال time اللي بترك فيه ال item أو ال object معرضة فيه لل gas .

* وميزة ال gaseous عن ال liquid اللي رح نحكي عنها انه بتوفر ال good penetration لل gas إله على ال items تاعتي فبحكي إني أقدر أستعملها عشان أعمل sterilization لل packaging material اللي بالـ paper, fabric, plastics, rubber .

وآخر اشي

2. Liquid method

Liquid sterilization is the process of sterilization which involves the submerging of equipment in the liquid sterilant for a prescribed period of time at a controlled temperature and concentration to kill all viable microorganisms and their spores. Although liquid sterilization is not as effective as gaseous sterilization, it is appropriate in conditions where a low level of contamination is present. Different liquid chemicals used for liquid sterilization such as hydrogen peroxide.

Hydrogen peroxide is a liquid chemical sterilizing agent which is a strong oxidant and can destroy a wide range of microorganisms. It is useful in the sterilization of heat or temperature-sensitive equipment like endoscopes. In medical applications, a higher concentration (35-90%) is used. H_2O_2 has a short sterilization cycle time as these cycles are as short as 28 minutes where ethylene oxide has cycles that as long as 10-12 hours. However, hydrogen peroxide has drawbacks like low material compatibility, lower capacity of penetration, and associated health risks.