



تفريغ لاب مايكرو

EXP 2

محاضرة:

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الصيدلانية:



لجان الرفعات



Experiment 2

Preparation of culture media under aseptic conditions.

Quality assurance –microbial monitoring of environment

➤ **Objective:**

The aim of this experiment is to:

1. Prepare culture media under aseptic conditions
2. Conduct quality assurance tests-microbial monitoring of environment

➤ **Introduction:**

Source of microbial contamination: لما بدأنا نشتغل لازم الشغل يكون بـ Aseptic conditions لانه البيئة الى حوالينا وحتى احنا حلينا surface of microorganisms والهواء etc

1. Personal contamination

The population of microorganisms that inhabit the skin, mucous membranes or any other anatomical site of healthy normal persons is called **normal microbial flora**

Normal flora is classified into two types:

1. Resident flora

Consists of relatively fixed types of organisms which are regularly present in a particular area and when disturbed it reestablishes itself like *Esch. coli* is a normal inhabitant of the intestine. The microbes of the normal resident flora are harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. They may produce disease if introduced into foreign locations in large numbers and if predisposing factors are present.

2. Transient flora

Consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks; it is derived from the environment, does not produce disease, and does not establish itself permanently on the surface. Members of the transient flora are generally of little significance as long as the normal resident flora remains intact. However, if the resident flora is disturbed, transient microorganisms may colonize, proliferate, and produce disease.

****During lab work normal flora can cause contamination if not controlled:**

a. contamination from hands

Your fingers are NOT clean. The surface of your skin is home to thousands of bacteria. These bacteria are essential for our health. However, they will easily contaminate media, plates and glassware in the lab. Therefore, you need to be careful not to touch pipette tips, the insides of the plate lids. Or any other surfaces which are sterile.

b. contamination from hair

c. contamination from respiratory system

2. Contamination from air

3. Contamination from water → should be sterile

4. Contamination from surfaces

Preparation of Culture Media under Aseptic Conditions

لحنا بنحضر ال media بشكل تادي ودهيت بنحطها بال sterilization Autoclave وبتعملها

To grow bacteria, we should provide them with suitable environmental conditions and suitable media to allow them to divide by binary fission to increase in cell number (not in cell size).

a. Environmental conditions:

1. **Temperature:** Most microorganisms live within restricted ranges of temperature with a range of tolerance (minimum and maximum tolerated temperature). The **minimum**

مللا ان E.coli لما تنتقل الى urinary tract و تصيب راح نقل infection (مسار Transient Flora)

موجودة في منطقة الجسم

بكونوا harmless بالمكان الي الموجودين فيه وضعه مفيدو بالمكان الموجودين فيه والاكل حماية من نموهم other ليس اذا اقلعوا لمكان تاني غير pathogenic يصير

بسبب اعدادها القليلة و بسبب وجود Normal flora الي بتكون خط حماية

الحسم كله مغطى بال normal flora فانا لما امسك ال sample باليدي نصيب يكون نقلت البكتيريا الموجودة لا الايدي لا surface الي مسكته

لما بدنا نعقم للايدي صح راح نقل ال transient flora بس راح نقل جزء من ال normal flora وعشان هيك مصنوي نعمل ال sample الامت اللوات الخارجية الي تحب non-sterile

المصنوي لانه اهو الي يطلع منه الفم مصنوي يقرب من مكان الشغل

ناخذ مثلا E.coli اذا الشخص اخذ antibiotic لفترات طويلة برتوية ليس بعد فترة راح الالحظ انهم كونوا حالهم مرة لانية reestablish

مصنوي نشتغل غير لما نشتغل انا benign burner اهو حواله يكون sterile يعني صافي microorganisms

مصنوي مصنوي

في ال Range الحرارة حتى البكتيريا تقدر تعيش فيه و تقدر تقا Fission

growth temperature is the standard temperature at which a species will grow, **the optimum growth temperature** is the temperature at which it grows best, and **the maximum growth temperature** is the highest temperature at which growth is possible.

البكتيريا تقدر تعيش تحت ال minimum و فوق ال maximum ليس يمكن ما يغير الهم Fission

بلاب المايكرو بي نشغل درجة 37° اي تكون optimum للبكتيريا

اعلى حرارة البكتيريا تقدر تعمل عليها Fission هون ال time اي بي حاجة ال فission اقل ما يمكن

2. pH: Most bacteria prefer neutral pH (6.5-7.5). Molds and yeast grow in wider pH range while it prefers a pH between 5 and 6.

3. Osmotic Pressure: Cells have about 80 to 90% water of their structure, and normally the salt concentration of microbial cytoplasm is about 1%. **isotonic** معظم البكتيريا تعيش بـ isotonic
What is the effect of presence of microbes in hypertonic or hypotonic solution?

Halophile عيارة عن بكتيريا تعيش High concentration of salts

shrinking

4. Other factors: Like light and time.

كل ما خلتنا ال time اكثر كل ما مار ال growth اكثر

b. Bacterial culture media (nutrient)

The mixture (in which the nutrients are supplied) is referred to as **growth medium or culture medium**.

A growth medium or culture medium is a **liquid** or **gel** designed to support the growth of **microorganisms** or **cells**, or small **plants**

Generally, the growth medium contains:

- 1. Moisture (water)** → water تحتاج ال water كل cell
- 2. An energy source**, for example: glucose, amino acids, nitrite and nitrate
- 3. Nutritionally suitable sources of carbons**, nitrogen, sulfur and oxygen to synthesis amino acid, RNA, DNA → اي يحتاجهم ال Cell Division
- 4. Organic growth factors**, for example: amino acids, minerals

Media must be prepared in such a way that it is **sterile** prior to being inoculated with a bacterial sample, so that when a particular type of bacteria is cultured (cultivated) on that medium, it is the only type of bacteria present.

لما احضر ال media مش لازم يكون sterile ليس لها اعطيت تحضير بحطها بال Autoclave ال بي عمل ال sterilization من صون والحط ال بعد لازم يكون aseptic condition

Autoclave: It is a **sterilization procedure** performed by means of temperature and pressure. It is used only for the sterilization of heat stable media and equipment. An autoclave is basically a **huge steam cooker**. Steam enters into a jacket surrounding a **chamber**. The pressure will go up over **15 pounds per square inch (psi)**; at this point the timer begins to count down usually for **15 minutes**. The high pressure in a closed container allows the temperature to go around **121°C (249 °F)**. Therefore, the parameters for sterilization with an autoclave are 121°C at ≥15 psi for 15 minutes. Fifteen Minutes is the thermal death time for most microorganisms (except some really hardy spore-formers).

بجهد Huge temp and Pressure

درجة حرارة 121°C
 د ضغط 15 psi
 لمدة 15 min
 كفاية لقتل أي organism هيلك صار ال medium sterile

Aseptic techniques applied to prepare sterile culture media

Aseptic technique is a method designed to prevent contamination from microorganisms. It involves applying the strictest rules and utilizing what is known about infection prevention to minimize the risks that you'll experience an infection

Aseptic techniques that should be followed to prepare a sterile culture media:

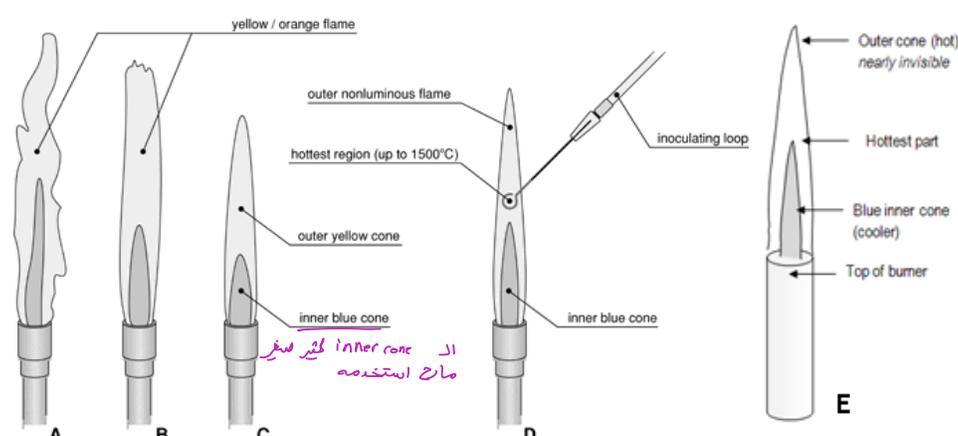
- 1. Doors and windows are kept closed** → عشان ما تدخل الهم

- Hand hygiene "as described previously"
- Decontaminate the surface of your bench at least one time using 70% alcohol
- Turn on the Bunsen burner and make sure that the flame is no more than four inches high and that the blue inner cone more than two inches high. (Figure below D and E)
- Agar plates are held in a manner that minimizes the exposure of the surface to the environment. The Petri dish tops are lifted with the left hand and replaced immediately as the plate is poured
- When removing lids from tubes, lids are held in the hand and not placed on the countertop during the transfer of materials from one tube to another.
- Sterilize all needed glassware in autoclave prior to use.

اد Flam مش
طويل كثير واللون
مش يرتقالي
ليحت ليغير ازرق
ليش هيا
الخطوة 5 الجواب
محتوي على 4

نمسك الخطارة
بالايد الشمال ونحط
صن ال sample باليد اليمين

يس بدنا تصب ال media فيه بسرعة وبسرا
برفقه هون بنصب
بسرعة بسر سرعة



ال area الى رح اشتعل
فيها تقريباً رح تكون
شكل دائريه قطرها
25cm
اد Bunsen Burner

اللون رح يكون اصفر ليرتقالي
ودسويل مش

ال outer cone لونها ازرق
وال inner cone بشكل اعفق
رح استخدمها

Classification of culture media:
A. Classification based on consistency

- Liquid (Broth) medium:** these media contain specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests.
- Solid medium (Agar):** is media containing agar (at a concentration of 1.5-2.0%) or some other, agar is mostly inert solidifying agent. Solid medium has physical structure, and this allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). solid medium is useful for isolating bacteria or for determining the characteristics of colonies.
- Semisolid media:** they are prepared with agar at concentrations of 0.5% or less. They have soft custard-like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

رح تفيل Liquid
سوسا و Room temp
او في درجات
الحرارة العاليه

agar at 121°C → liquid
at lower temp (تقريباً) 45°C او 40°C → solidification
رح يصير له Solidification

بالا بيده ايها agar
ينزل الحرارة و اذا بيده
ايضا له يفرج الحرارة

هون سبب استخدام
ال agar

العوام يشبه الكاستر

لايس فيه هاي الطريقة 1+2

Agar:

The discovery of agar (a polysaccharide derived from red algae) has revolutionized the study of microbiology because of its distinctive properties:

- Agar at a concentration of about 1 – 1.5 % (w/v) will provide a firm gel that cannot be liquefied by the enzymes normally produced during bacterial growth.
- Agar is semi-translucent → من خلال ال agar بقدر اشوف له موجود بالوجه اللامعة
- An agarose medium is porous → المواد الي تفرزها ال microorganism رح تتعلق عن طريق ال agar
- Fluid agar solutions set (solidify) at approximately 40°C, but do not reliquefy on

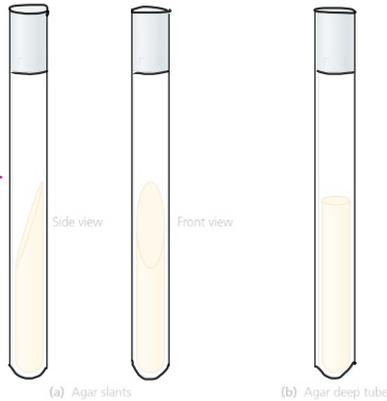
heating until the temperature is more than 90°C.

Thus, agar forms a firm gel at 37°C which is the normal incubation temperature for many pathogenic microorganisms. And when used as a liquid at 45°C is at a sufficiently low temperature to avoid killing microorganisms (this property is important in pour plate counting method).

Forms of solidified agar:

A. Slant (test tube):

- 1/3 full → *عينة ثلاث الـ test-tube وخليته يتشكل ماثل*
- Solidified in a slant position



B. Deep (test tube):

- 1/3 – 1/2 full → *عينة من ثلثه لنهيه وخليته زي ما هو*
- Solidified in an upright position
- For reduced O₂ environment → *للبيكتريا الـ non-aerobic*
- Or for observation of in-media growth

C. Plates (Petri-dish)

□

Practical part *الـ media جاهز وعاطلينه الـ sterilization وحسب موجوده*

Part 1: Preparation of Culture Media under Aseptic Conditions

الـ oven على درجة حرارة 50°C @ 55°C

Preparation and preservation of media

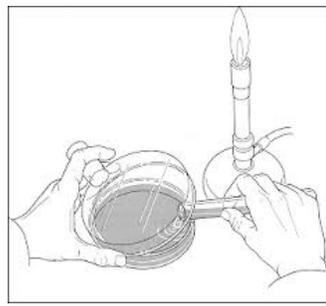
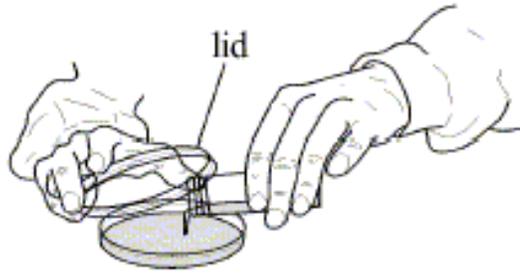
Most culture media are sterilized by autoclaving. Certain media that contain heat labile components like glucose, antibiotics, urea, serum and blood are not autoclaved only they are filtered and may be added separately after the medium is autoclaved.

Prepared media may be held at 4-5 °C in the refrigerator for 1 to 2 weeks. Certain liquid media in screw capped bottles or tubes or cotton plugged can be held at room temperature for weeks.

Pouring Solid media:

Sterilized agar should be allowed to cool to about 50 °C (the agar should be warm and molten, but not too hot to handle in its flask). Pour agar solution into plate.

- Pouring procedure should be performed in an aseptic manner to prevent contamination: speech is prohibited, and Bunsen burner should be ON
- Open the cover of Petri-dish with one hand and while still holding the cover over the Petri-dish, pour approximately 20 mL of agar solution into the dish. Cover the dish.



- Flame the surface of agar solution to remove any bubbles that formed during pouring and to make the surface smooth. Cover the dish
- When the plates are cool (agar solidified), invert them to prevent condensing moisture from accumulating on the agar surface. If it happened the medium could be destroyed.
- To test the sterility of broth and agar plates. Place the inverted agar plates and tubes of sterilized nutrient broth in the incubator at 37 °C. They should be incubated for at least 24 hours to ensure they are sterile (free of contaminating bacteria) before using them.

Part 2: Quality assurance- Microbial monitoring of environment

A. Personal contamination: Contamination from hands

1. Divide the nutrient agar plate into 4 sections and label 1 through 4.
2. Section 1 is your negative control. Don't touch it.
3. Take fingerprint (3 fingers) firmly from unwashed fingers on section 2
4. Wash your hand thoroughly with detergent and water
5. Allow the hands to dry in the air and take fingerprints firmly from the same fingers in the section 3 of the plate
6. Rub disinfectant into the hands, leave hands to dry in air and again take fingerprints (the same fingers) on the section 4 of the plate.
7. Incubate the plate inverted for 24 hours at 37 °C
8. Draw the general appearance of the plate and describe a few types of colonies observed

B. Contamination from air

1. Expose the agar plate to air by placing it over the bench for 2 hours
2. Expose another agar plate to the air by placing it into the laminar air flow for 2 hours
3. Replace the lid and incubate the plate inverted at 37 °C for 24 hours
4. Examine your plates and describe some of the colonies observed

C. Contamination from water

1. Divide an agar plate into two sections and label as tap water and sterile water
2. Soak a sterile swab with tap water and then streak on the surface of the first section of the agar plate.
3. Soak a sterile swab with sterile water and then streak on the surface of the second

section of the agar plate

4. Incubate the plate for 24 hours at 37 °C
5. Describe the general appearance of the agar plate.

D. Contamination from surfaces

1. Divide the nutrient agar plate into 3 sections and label 1 through 3.
2. Use sterile cotton swab to swab a bench surface before cleaning with disinfectant then streak on the surface of the first section of petri dish
3. Clean your bench using disinfectant and repeat step 2 with a new sterile cotton swab on the second section of the agar plate
4. Use sterile cotton swab to swab an area from the environment such as a doorknob, floor or water knob, then streak on the surface of the third section of petri dish
5. label correctly each section with its nominated sample.
6. Incubate the plate inverted for 2
7. Draw the general appearance of the plate and describe a few types of colonies observed



بالنسبة للجزء العملي احضروه من الفيديو لانه بخير. صياحه
ربح مسأله ان شاء الله