التجربة رح تكون عن :-
1) media preparation
2) Sources of microbial contamination

Experiment 2

Preparation of culture media under aseptic conditions. Quality assurance –microbial monitoring of environment

➢ **Introduction:**

Bacterial taxonomy طريقة تسمية البكتيريا

عنا حرفين بالتسمية :-
1] genus → capital letter
2] species → small

each species has to be assigned to a genus (binary nomenclature). There are two parts of the name, one defining the genus and the other the species e.g. *Streptococcus pyogenes*. The genus in this case is "*Streptococcus*" and the species is "*pyogenes*" (genus / species). The genus is normally written with an upper-case initial letter and the species with a lower-case initial letter, e.g., *Staphylococcus aureus* or *Escherichia coli*. These names are printed in *italics* to designate their status as proper names (in old books: underlined).

- عادة الاسماء تنكتب بشكل مائل Italics حتى اميز انها اسم علمي
- بالكتب القديمة كانوا الكتبة على ورقة يكتبوا الاسم ويحطوا تحته خط.

Normal microbial flora

The term "normal microbial flora" denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. For a healthy human, the internal tissues, e.g. blood, brain, muscle, etc, are normally free of microorganisms. However, the surface tissues, i.e., skin and mucous membranes are constantly in contact with environmental organisms and become readily colonized by various microbial species. The mixture of organisms regularly found at any anatomical site is referred to as the normal flora.

هاي الأماكن خالية من M.O

normal micro flora
non pathogenic حتى ما تمرض انها
mucous membrane بتكون موجودة مع الجلد او
environment الأماكن اللي بتكون مع اتصال مباشر مع »

Factors Influencing Normal Flora

عوامل تؤثر مع Normal flora

1. Local Environment (pH, temperature, redox potential, O2, H_2O, and nutrient levels…). — H_2O عم تشكل رطوبة
2. Diet
3. Age
4. Health condition (immune activity…)
5. Antibiotics,…..etc

Classification of microbial flora

أنواع Micro Flora

1. **The resident flora** موجودة بمكان محدد وبتراكيز محددة.

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. (مثال عليها)

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.

مثلاً E. coli مكانها جال intestine ولكن اذا استقت لمكان آخر مثل UT ممكن تسبب UT infection.

* تشكل عام هاي Flora غير مؤذية بمكانها الأصلي فقط

موجودة على outer surface. جاي من environment.

2. The 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.

هي ليست harmful بوجود الـ resident flora بمنع هاد النوع انه يعمل disease
* او تغير ع resident
ممكن تعطي disease.

Beneficial Effects of the Normal Flora

Some of the characteristics of a germ-free animals that are thought to be due to lack of exposure to a normal flora are:

micro flora ماعندهم germ free animal *
ف وحدا عندهم هاي الأعراض

1. Vitamin deficiencies, especially vitamin K and vitamin B12
2. Increased susceptibility to infectious disease
3. Poorly developed immune system, especially in the gastrointestinal tract
4. Lack of "natural antibody" or natural immunity to bacterial infection

The overall beneficial effects of microbes are summarized below:

1. Normal flora synthesizes and excretes vitamins in excess of their own needs, which can be absorbed as nutrients by their host. For example, in humans, enteric bacteria secrete Vitamin K and Vitamin B12.

Vitmins تنتج أكثر من حاجتها الزيادة بتمتصه الجسم مثل vit K / B12

2. Normal flora prevents colonization by pathogens by competing for attachment sites or for essential nutrients.

بتنافس مع M.O

3. Normal flora may antagonize other bacteria through the production of substances which inhibit or kill non indigenous species.

جزء من intestine

4. Normal flora stimulates the development of certain tissues, i.e., the caecum and certain lymphatic tissues (peyer's patches) in the GI tract. The caecum of germ-free animals is enlarged, thin-walled, and fluid-filled, compared to that organ in conventional animals. Also, based on the ability to undergo immunological stimulation, the intestinal lymphatic tissues of germ-free animals are poorly-developed compared to conventional animals.

5. Normal flora stimulates the production of natural antibodies. Since the normal flora behaves as antigens in an animal, they induce an immunological response.

↳ antibodies بتحفز انتاج ← Immuni System هي جزء من

➢ **Objective**:

The aim of this experiment is to:

1. Prepare the Culture Media under Aseptic Conditions
2. Conduct a quality assurance tests microbial monitoring of environment

حتى اشوف environment اللي بشتغل فيها قديش فيها M.O

Preparation of Culture Media under Aseptic Conditions

الزيادة بتكون بالعدد الخلايا وليس بالحجم

Microbial Growth: Refers to an increase in cell number, not in cell size. Bacteria grow and divide by binary fission, a rapid and relatively simple process.

The requirements for microbial growth are both physical and chemical

A. Physical requirements:

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

الحرارة اللي بحتاجها حتى تنمو

اقل حرارة بقدر يعيش فيها

اعلى حرارة

افضل درجة للنمو ≈37 °C

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.

pH(5.6) . اللي هم فطريات

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

all cells in general prefere isotonic soln.

shrinkage — Swelling.

4. **Other factors:** Like moisture, light and time.

B. Chemical requirements:

احفظوا . elements كل دين موجود وش مصادره .

1. **Carbon**: It is a structural backbone of all organic compounds. Its sources are lipids, proteins, carbohydrates and carbon dioxide.

مصادره .

2. **Nitrogen**: Used to form amino acids, DNA, and RNA. Its sources are ① Protein, ② ammonium and nitrogen gas (N_2).

organic source

non organic source

3. **Sulfur**: Used to form proteins and some vitamins (thiamin and biotin). Its sources are protein, hydrogen sulfide and sulfates.

4. **Phosphorus**: Used to form DNA, RNA, ATP, and phospholipids. Its sources are mainly inorganic phosphate salts and buffers.

5. **Oxygen:** Organisms that use oxygen produce more energy from nutrients than those do not use it. i.e., aerobic microorganisms produce more energy than anaerobic microorganisms.

M.O اللي بتحتاج O_2 ← (هوائيين) بتعطينا طاقة أكثر من اللاهوائية

6. **Other Elements:** Potassium, magnesium, and calcium are often required as enzyme cofactors.
Calcium is required for cell wall synthesis in Gram positive bacteria.

عبارة عن خليط فيه كل اللي بتحتاجه البكتيريا للنمو.

Bacterial culture media

To grow bacteria, we should provide them with suitable environmental conditions and suitable media. The mixture (in which the nutrients are supplied) is referred to as the growth medium or culture medium. تعريفه

A growth medium or culture medium is a liquid or gel designed to support the growth of **microorganisms** or **cells**, or small **plants** like the **moss *Physcomitrella patens***. Generally, the growth medium contains:

media مكونات الـ

1. Moisture (water)
2. An energy source, for example: glucose, amino acids, nitrite and nitrate
3. Nutritionally suitable sources of carbons, nitrogen, sulfur and oxygen ← chemical requirment
4. Organic growth factors, for example: amino acids. بتساعد في تصنيع DNA و RNA

Vitamins and nucleosides are other ingredients that may be added to the medium in order to grow the desired microorganisms.

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.

media لازم تكون معقمة (Sterile) ✳ مهم

قبل عملية الزراعة حتى يضمن فقط البكتيريا اللي أنا زرعتها

Classification of culture media:

A. Classification based on consistency

1. 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. eg. Sugar fermentation tests, MR-VP broth.
2. 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.
3. 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.

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
- An agarose medium is porous
- Fluid agar solutions set (solidify) at approximately 40°C, but do not reliquefy on heating until the temperature is in excess of 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: 3 forms

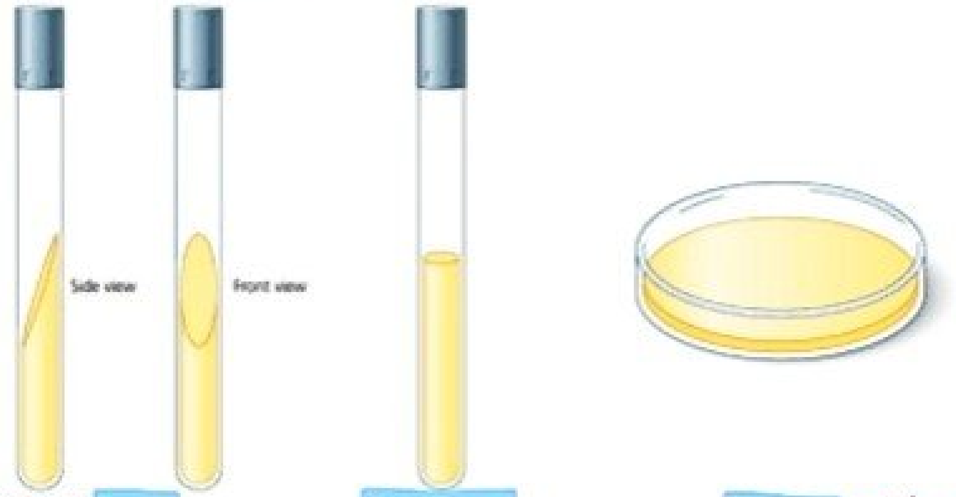
A. Slant (test tube):

- 1/3 full
- Solidified in a slant position

B. Deep (test tube):

- 1/3 – 1/2 full
- Solidified in an upright position
- For reduced O2 environment
- Or for observation of in-media growth

C. Plates (Petri-dish)



يجمد وهو مائل (a) Agar slants

not flat surface.
large surface له ميزته عندي
* more exposure to oxygen.

(b) Agar deep tube

1/3 tube
يجمد بطريقة عمودية.
حتى اشوف growth
best tube

(c) Agar plate

mainly used to see the colony
حجمها وحفافها.

B. Classification based on the basis of purpose/ functional use/ application

1. General purpose media/ Basic media/ Minimum Media

- Contains minimal nutritional requirements for microorganisms
- Supports the growth of only a relatively narrow range of bacteria
- Growth is slow
- Generally used for the primary isolation of microorganisms.

2. Enriched medium (All purpose-medium):

- Contains a wide range of nutrients (sugar, amino acids, fatty acids, vitamins, salts, organic products from animals and plants)
- Supports the growth of a wide range of microorganisms
- Growth is fast
- Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basic medium makes them enriched media.

3. Selective medium: For specific type of M.O. + suppression for specific type

- It is designed to suppress the growth of some microorganisms while allowing the growth of others (i.e., they select for certain microbes).
- Uses certain dyes, sugars, high salt concentration, or pH to achieve the selectivity.
- Examples: MacConkey agar: Enterobacteriaceae members contains Bile salt that inhibits most gram positive bacteria, EMB (Eosin Methylene Blue) agar For gram -ve only.

4. Differential medium (indicator medium):

- Allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies i.e they Support the growth of different microorganisms but in different ways.
- Example: Incorporation of 0.5% mannitol + phenol red (a pH indicator that changes from red to yellow in acidic medium) into a medium that supports the growth of both Staphylococcus aureus and Staphylococcus epidermidis, to differentiate between them.
- Since Staphylococcus aureus can only ferment mannitol (unlike Staphylococcus epidermidis) and produce acidic degradation products that would change the pH of the medium to acidic and the color to yellow. In the case of Staphylococcus epidermidis, there is no color change.

Source of microbial contamination:

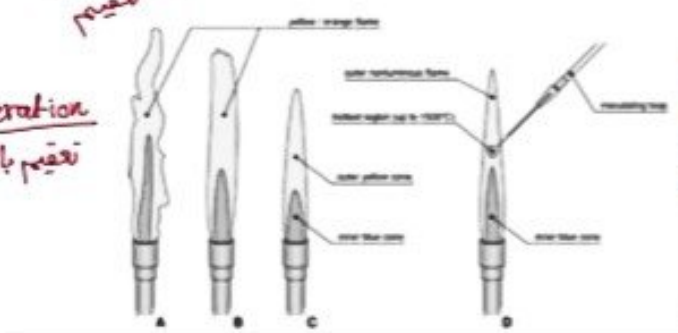
1. Personal contamination
   a. contamination from hands
      Your fingers are NOT clean. The surface of your skin is home to thousands of bacteria. These bacteria are essential for our health. However, they will easily contaminate media, plates and glassware in the lab. Therefore, you need to be carful 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
2. Contamination from the air
3. Contamination from the water
4. Contamination from the surfaces

Aseptic techniques applied to prepare a 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
2. Hand hygiene "as **described previously**"
3. Decontaminate the surface of your bench at least one time using 70% alcohol
4. 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)
5. 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
6. 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.
7. Sterilize all needed glassware's in autoclave prior to use.



عبارة عن عملية تعقيم*

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 cocker. 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).

بشتغل لـ 15 min كافية لقتل M.O اي

بتقاوم وقت اكتر.

تشكله مثل طنجرة الضغط //

جواته ماء بشغله ع حرارة 121°c بتكون Steam

by steam Sterilization

"الجزء العملي"

Practical part

Part 1: Preparation of Culture Media under Aseptic Conditions

Preparation and preservation of media

Most culture media are sterilized by autoclaving. Certain media that contain heat liable 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.

I. Making Liquid Media

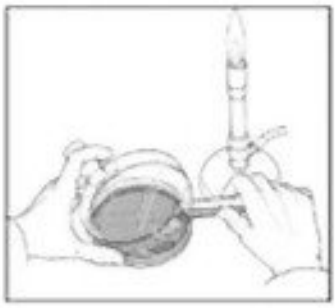
1. Read the label on a bottle of nutrient broth. It specifies the amount of dehydrated powder required to make 1 litter (1000 ml) of medium. Then calculate the amount needed to prepare ____ ml and weight this quantity.
2. Place the required amount of distilled water in Erlenmeyer flask. Add the weighed powder and shake to dissolve.
3. Use a pipette to dispense 5 ml aliquots of the broth media into sterile test tubes.
4. Close it with the cotton plug or cap then place the flask in autoclave (121°C, for 15 min) to sterilize broth media.
5. After that, tubes are ready for culturing.

II. Making Solid media:

1. Read the label on a bottle of nutrient agar. It specifies the amount of dehydrated powder required to make 1 litter of medium. Then calculate the amount needed to prepare ___ ml and weight this quantity.
2. Place the required amount of distilled water in Erlenmeyer flask. Add the weighed dehydrated agar while stirring; use a glass rod to prevent lumping, if needed.
3. Set the flask over a hot plate and start heating agar till boiling (solution will become transparent when agar completely liquefies). While heating keeps shaking the flask to prevent charring of the precipitated agar.
4. After that remove the flask from the hot plate, close it with the cotton plug or cap.
5. Place in the autoclave (121°C, for 15 min).
6. After sterilizing agar allow it to cool to about 50 °C (the agar should be warm and melted, 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.

بصورة بغطي
بس اخلص .



- 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 happened the medium could be destroyed.

بعد ما الـ agar جمد بقلبه
حتى امنع تجمع تقران اللي فيه (الرطوبة)
وبعمله storage وهو مقلوب.

- 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.

حتى اتأكد انه steril
بحطه وهو مقلوب جوا incubator ع حرارة 37
لـ 24 ← 48 ما حار عليه growth sterile يعني هو

* شوفوا فيديو شرح
عند دقيقة 36:47 فيه فيديو توضيحي
لطريقة عمل الـ agar.

Part 2: Quality assurance- Microbial monitoring of environment

Source of microbial contamination?

A. Personal contamination

A.1 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 finger print (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 finger prints firmly from the same fingers in the second section of the plate
6. Rub disinfectant into the hands, leave hands to dry in air and again take finger prints (the same fingers) on the fourth section 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

A.2 Contamination from respiratory and mouth

1. Divide the nutrient agar plate into 2 halves.
2. Swab the mouth cavity or teeth using a cotton swab. Then streak on the surface of the first half of petri dish.
3. Use a mouth wash for 1 min.
4. Swab the mouth cavity using a new sterile cotton swab. Then streak it on the surface of the second half of petri dish.
5. Examine your plates and describe some of the colonies observed

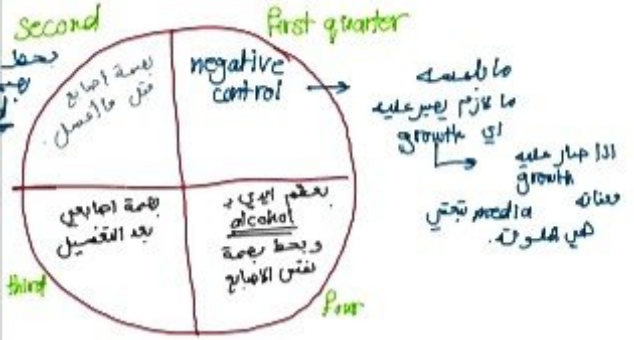
B. Contamination from the air

1. Expose the agar plate to air by placing it over the bench for 30 minutes
2. Expose another agar plate to the air by placing it into the laminar air flow for 30 minutes
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 the water

1. Transfer 1 ml of tap water to a nutrient broth test tube and mix it will.
2. Transfer 1 ml of sterile water to another nutrient broth test tube and mix it will.
3. Incubate the tubes for 24 hours at 37 °C
4. Describe the general appearance of the broth.

بقسم الـ agar plate الى أربع أقسام



بعدين بسكرد ويحطه incubation for 24 hours at 37°c

mouth

بقسمه لجزئين

* جاخذ من mouth cavility cotton swab

mouth wash ① مرة قبل ما استعمل
" " " ② مرة بعد

عندي مجموعتين ؟

- observation ← وصف للي شوفته جوا عندي growth
جمعها وعدها ...
- discussion ← تفسير للي شفته
سبب الـ growth ....

D. Contamination from the surfaces

one agar plate

1. Divide the nutrient agar plate into 4 sections and label 1 through 4.
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
4. Swab the clean bench surface using new sterile cotton swab. then streak on the surface of the second section of petri dish
5. Use sterile cotton swab to swab an area from the environment such as a door knob, floor or water knob
6. Streak each cotton swab into the surface of the remained two section of the plate (section 3 and 4) and label it correctly.
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

* مرة باخد swap قبل ما انظف bench
* ومرة swap بعد ما انظف bench
* ومرة من أماكن أخرى من ال environment مثل ايد الباب.

وموفقين جميعًا.... ♡

« Artesy Academy »

by Yasmine Robin