تفريغ لاب مايكرو

محاضرة: Exp 3

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لجان الدُّفعات

Soul Academy

Experiment 3
Culturing Methods and Plating Techniques

❖ **Objectives:**

The aim of this experiment is to:

1. Know and differentiate between different Culturing Methods and Plating Techniques.
2. Plating using streak plate technique.
3. Subculturing from liquid culture to liquid broth

❖ **Introduction:**

Culture methods: (purpose) بناءً على إنو شو محتاج اعمل بختار الـ method

• Culture methods employed depend on the purpose for which they are intended.

• **The indications for culture are:**

✓ To isolate bacteria in pure cultures. Microorganism أي احضر نوع واحد معين من الـ

✓ To obtain sufficient growth for the preparation of antigens and for other tests. ← احضر الـ antigen لأعمل cure لنوع من الـ Microorganism اللي بسبب disease

✓ To determine sensitivity to antibiotics. Microorganism الـ المختلفة

✓ To estimate viable counts. → عشان اقدر اقرر كمية الـ microorganism في عينة بدائية قبل الزراعة. Colonies بحدده حسب الـ اللي طلعت

✓ Maintain stock cultures. → محتاجه أكبر عدد معين من البكتيريا عشان استخدمه بـ test تاني

• **Culture methods include:**

Colony = group of microorganism cell

✓ Streak culture

✓ Lawn culture agar الـ Microorganism واضيف كمية ضيئة منه على الـ (agar) Plate مثلاً بعمله توزيع على سطح الـ

✓ Stroke culture

✓ Stab culture agar الـ Surface الـ incubation بكون الـ

✓ Pour plate method

✓ Liquid culture سائل microorganism ممكن افحص الـ فيه يتحرك او لأ

✓ Anaerobic culture methods
Anaerobic microorganism لـ

✓ Spread plate method.

The common plating techniques employed in microbiology are Streak Plate Method, Spread Plate Method and Pour Plate Method.

1) Streak Plate Method

هاي الطريقه اكثر طريقه بستخدمها لما يكون في Mixture من الـ microorganism
وانا مهتم بنوع واحد منهم شو اعمل؟ رح استخدم هاي الطريقة لاعمل isolation

لازم اعمل Isolation عشان Microorganism اعرف ادرسه

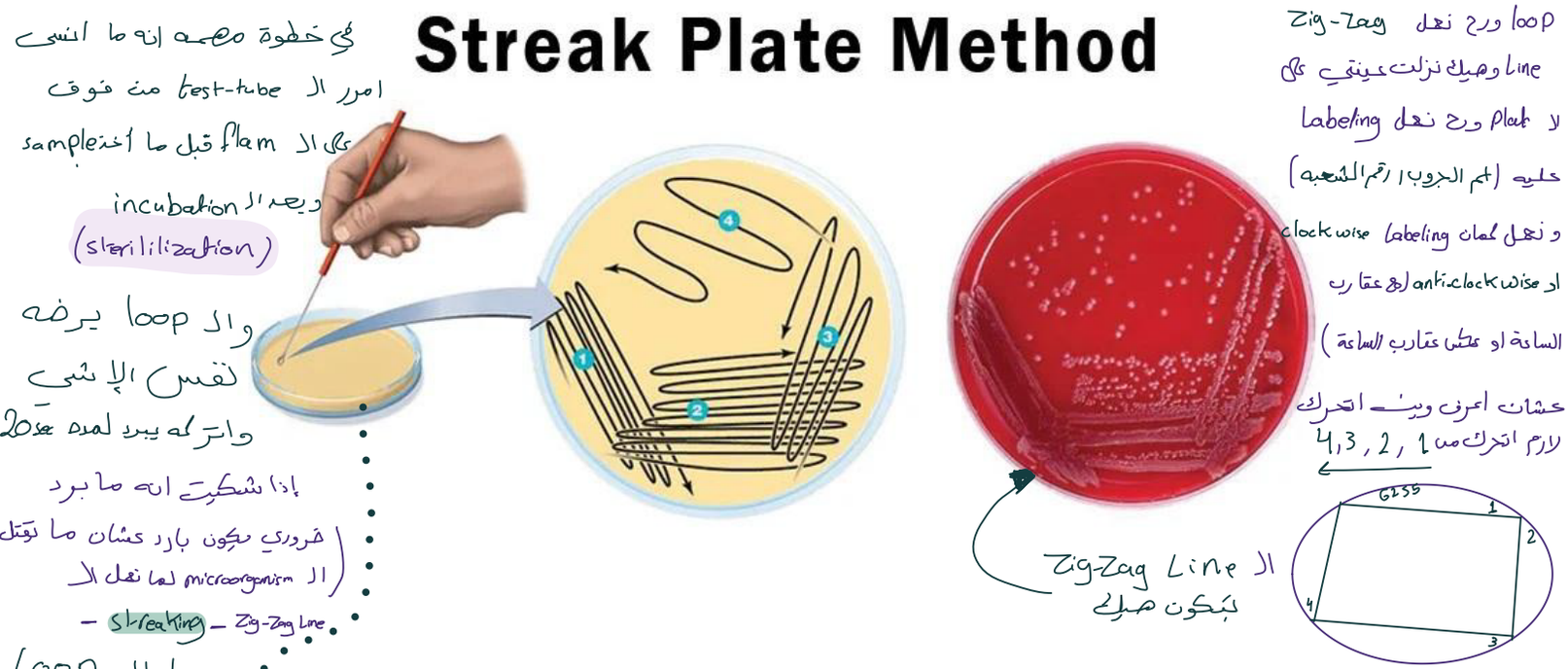
The streak plate technique is the most widely used method of obtaining isolated colonies from a mix of cultures (pure cultures). Why? To characterize, identify, differentiate and study or perform antimicrobial susceptibility testing on a microorganism; one must first isolate the targeted microorganism from the other species to which it does not belong.

لاعرف الـ antimicrobial الـ susceptibility لـ antibiotic المختلفين

A sterilized inoculating needle with a loop made up of either platinum or nichrome wire is used for streaking. One loopful of specimen is transferred onto the surface of the agar plate in a sterile petri dish and streaked across the surface in the form of a zig-zag line. This process is repeated to streak out the bacteria on the agar plate so that some individual bacteria are separated from each other. The first streak will contain more organisms than the second and the second more than the third and so on. The last streaks should thin out the culture sufficiently to give isolate colonies. The successful isolation depends on spatial separation of single cells. Each colony usually represents the growth from a single organism when such a plate is incubated colonies will appear on the surface of the medium. Pure colonies can be obtained from well isolated colonies by transferring a small portion of each to separate culture media.

لما بدي اخذ الـ Microorganism بقدر اخذها من Suspension او من Plate حتى من Test-tube
لما بدي اخذ من Suspension بكون موجود بـ Test-tube فلازم اهز الـ Test-tube هز لانه الـ Segamentation للبكتيريا
الـ Shaking لازم يكون horizontal

بعد الـ Shaking رح ناخذ عينه الـ inoculation ورح نعمل loop Zig-Zag line وهيك نزلت عينتي على الـ Plate ورح نعمل labeling عليه (اسم الجروب، رقم الشعبه) و نعمل labeling عمان clockwise او anti-clockwise زي عقارب الساعة او عكس عقارب الساعة)
عشان اعرف وين اتحرك لازم اتحرك من 1, 2, 3, 4



في خطوة مهمه انه ما انسى امرر الـ test-tube فوق الـ flam قبل ما اخذ sample وبعد الـ incubation (sterilization)
والـ loop يرضه نفس الإشي واتركه يبرد لمده 20sec
إذا شكيت انه ما برد ضروري يكون بارد عشان ما تقتل الـ microorganism لما نعمل الـ Streaking – Zig-Zag Line

2) Spread Plate Method

ليش بدي استخدم الـ spread

The spread plate technique is used for the separation of a dilute, mixed population of the microorganisms so that individual colonies can be isolated.

وممكن استخدمها لما بدي اعد الـ Colony-Count بالـ initial suspension الي فيه البكتيريا

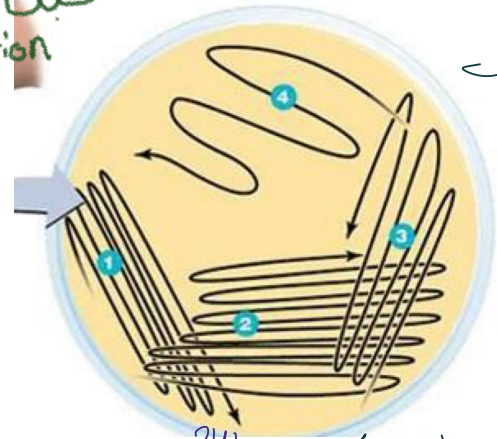
In this technique, a small volume of dilute microbial mixture is transferred to the center of an agar plate and spread evenly over the surface with a sterile L-shaped bent glass rod, while the petri dish is spun, at some stage, single cells will be deposited with the bent glass rod on the agar surface. Incubate the agar plate at 37ºC for 24 hours, in the inverted position.

The dispersed cells will develop into isolated colonies. Because the number of colonies will be equal to the number of viable organisms in the sample spread plates can be used to count the microbial population.

الـ loop بحطه على طرف الـ Plate لازم يعمل صوت شبيه بريش بلماء على النار Sizzling Sound

Streak Method

قبل الـ incubation



بس اسحب Sample و استنى الـ loop يبرد بيلش ارسم الـ line برقم 1 وبس اخلص

برجع اعقم الـ loop على الـ flam برسم الـ line من رقم 2 باتجاه

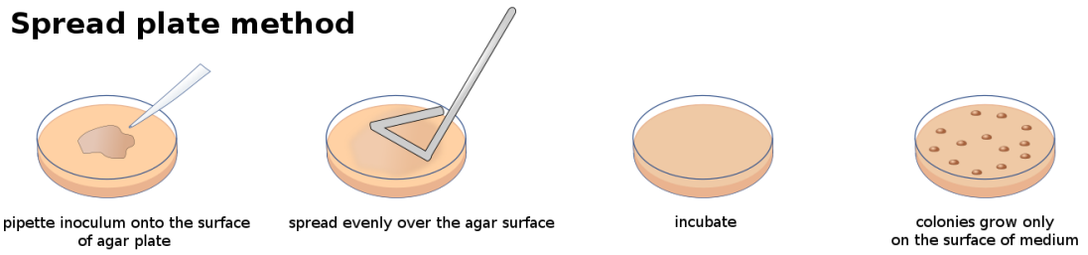
رقم 3 وبرجع اعقم الـ loop كمان مرة وبسحب خط واحد من جهه

الـ line إلي رسمتها برقم 3 بطريقة Zig-Zag باتجاه رقم 4 وال incubation ح يكون بعد 24hr

بعد ما ارسم الـ line عند رقم 4 اتأكد من تعقيم الـ Incubating loop قبل ما اي MO معلقة على الـ loop

بعد الـ incubation

وين اعلى تركيز للعينة عند 1 و بعدين التركيز بلش يقل ليش؟؟ لانه بكل خطوة بعمل MO dilution وعلى رقم 4 بلشت اشوف isolation colony



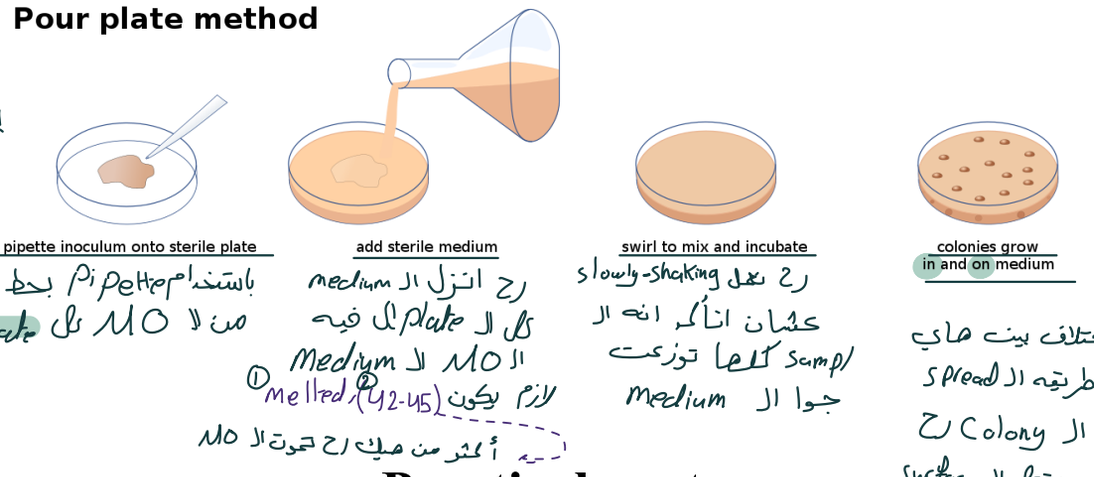
استخدمات الـ Pour plate Method

3) Pour Plate Method

① dilution of inculum الـ استخدمنا برضه هون ← isolation Colony على لاحصل ولحق

② Colony اعد

In pour plate method, successive dilutions of the inoculum are added into sterile petri plate to which is poured melted and cooled (42ºC - 45ºC) agar medium and thoroughly mixed by rotating the plates which is then allowed to solidify.
After incubation, the plates are examined for the presence of individual colonies. The pure colonies may be isolated and transferred into test tube culture media for making pure cultures. This technique is employed to estimate the viable bacterial count in a suspension.



من البدايه في اختلاف في طريقه incubation

① sample بحط باستخدام Pipette من الـ MO على الـ sterile Plate

medium رح انزل الـ على الـ Plate اللي فيه الـ MO الـ Medium

① Melted (42-45) لازم يكون

أي أكثر من هيك رح تموت الـ MO

slowly-shaking رح نعمل عشان انا كمان انه الـ sample كلها توزعت جوا الـ Medium

لانه في اختلاف بين هاي الطريقه وطريقه الـ Spread هو انه الـ Colony رح تكون موجودة على الـ Surface و برضه جوا الـ Medium

Practical part

عشان انا كمان انه ما في اي MO رح يدخل على الـ Vessel او يطلع منها

i Flam the neck of the bottles

This ensures that no microorganisms enter the mouth of the vessel to contaminate the culture or the medium.

1. Loosen the cap of the bottle so that it can be removed easily.
2. Lift the bottle/ test tube with your left hand.
3. Remove the cap/ cotton wool plug of the bottle/ test tube with the little finger curled towards the palm of your right hand. (Turn the bottle, not the cap.)
4. Do not put down the cap/ cotton wool plug. ← flamming لازم نشيلها قبل ما اعمل
5. Flame the neck of the bottle/ test tube by passing the neck forwards and back through a hot Bunsen burner flame.
6. After carrying out the procedure required, for example, withdrawing culture, replace the cap/ cotton wool plug on the bottle/ test tube using your little finger. Take care! The bottle will be hot. (Turn the bottle, not the cap.)

i Loop sterilization

1. The air-control of the Bunsen burner should be adjusted to give a flame with a short, blue, central cone

2. With the aluminum handle held as nearly vertical as possible without burning the fingers
3. The loop should be placed in the hottest part of the flame (just above the blue cone) until the whole length of the wire is heated to redness thus ensuring sterilization of the wire and the chuck of the handle
4. The loop should now be allowed to cool before use, or the bacteria that it touches will be killed (cooling takes approximately 20 seconds)

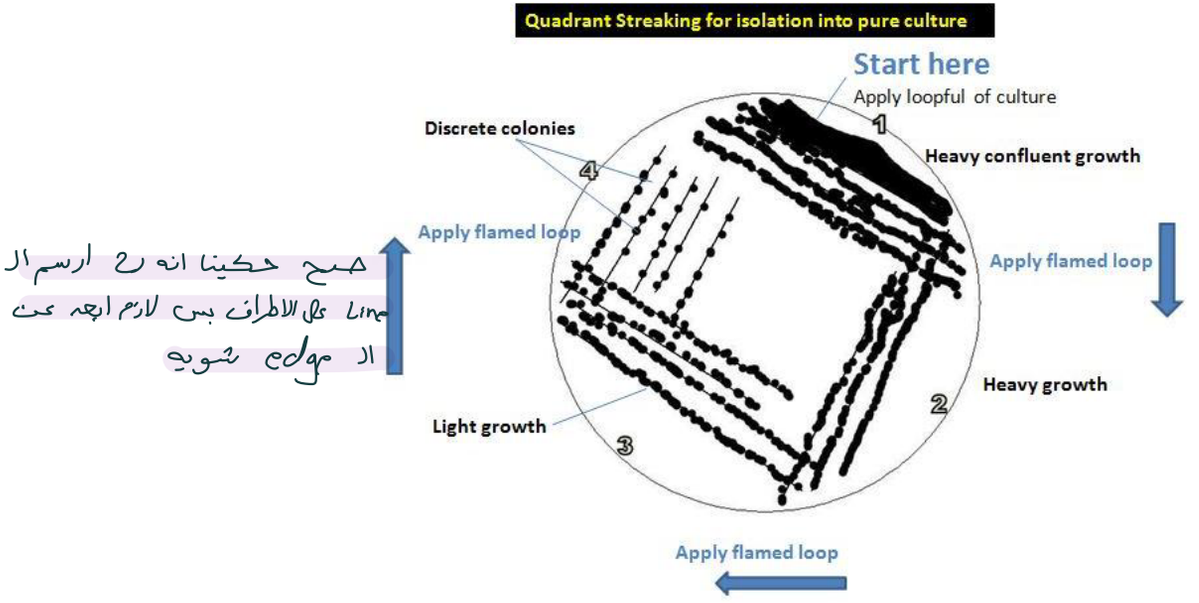
- هاي الخطوة ضرورية ب
- اعمل الـ test الـ اطراف
الـ agar عشان انه برد

Practical part ادرسوا الـ
لانه ممكن تجيب كل
نمط الكويزات .

معلومة: باك streak method احنا رح نعمل
incubation مرة واحده فقط

i **The plating out procedure**

1. Label a culture plate on its base with initials, sample and date
2. Sterilize the inoculating loop in the Bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.
3. Pick up the suspension provided in the left hand
4. Remove the plug with the little finger (with a twisting motion) of the right hand and flame the mouth of the tube
5. Dip the sterilized loop into the tube/culture bottle, withdraw a sample without touching the wall of the tube
6. Immediately streak the inoculating loop very gently over a quarter of the plate using a back-and-forth motion (see area 1 in the figure above).
7. Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2).
8. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3).
9. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate (area 4).
10. Flame your loop once more.
11. Replace plate in its lid
12. Incubate the plate inverted for 24 hours at 37 oC
13. Examine the colonies grown in the plate carefully. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate to obtain a pure culture.



صح حكينا انه ح ارسم ال Line على الاطراف بس لازم اجعد عند ال edge شوية

كيف ممكن اتأكد من الي فيه بكتيريا والي ما فيه ؟؟ اي فيه Turbidity بدل ما فيه growth للبكتيريا

Subculturing from liquid culture to liquid broth

هذا اي فيه بكتيريا — ح يكون sterile

Test :1

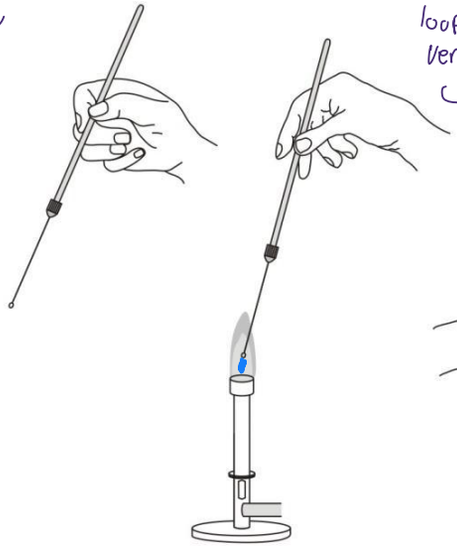
1. Label a tube containing the sterile nutrient broth
2. Place both test tubes (the sterile nutrient broth and that of containing the culture) in the palm of your hand in a V-like shape and stabilize them with your thumb. They should be held at an angle and thus not directly exposed to airborne laboratory contaminants.
3. Take the inoculating loop in the other hand and hold it like a pencil. Flame the inoculating loop along its full length over a Bunsen burner until the wire becomes red-hot.
4. Using the same hand that is holding the inoculating loop, remove the cap from the test tubes, hold it between your fingers, and briefly flame the neck of the tubes over a Bunsen burner
5. Insert the loop into the test tubes that containing the culture without touching the sides of the tube, and then remove it, carrying a loopful of bacterial cells.
6. Insert the loop containing the culture into the destination tube of sterile broth, swirl gently and remove.
7. Flame the necks of the tubes and close them with their caps.
8. Re-sterilize the loop before putting it down by inserting the loop into the flame *very slowly*! Doing this slowly allows any liquid remaining on the loop to evaporate rather than boil and avoid splattering live bacterial cells all over the bench and you.
9. Place the tubes and the inoculating loop into the rack.
10. Incubate at 37°C for one week.
11. Check the growth of the culture.

لازم اعمل labeling

بمسك الـ 2 test-tube على شكل حرف "V" وبعديني بعمل shaking مش horizontal Vertical

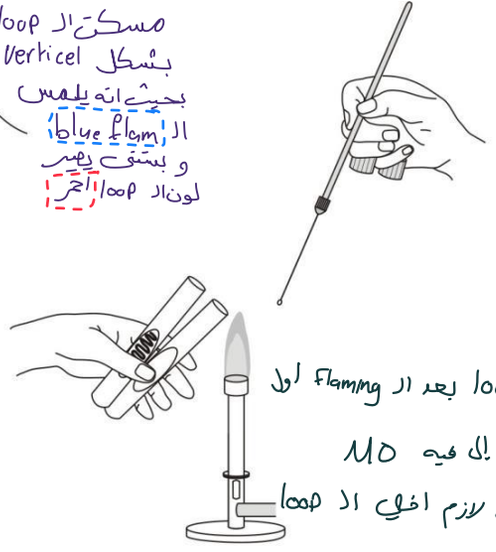
عشان البكتيريا ما توصل الـ tip الـ test-tube

(a)



(b)

مسكت الـ loop Vertical بشكل بحيث انه يلمس الـ blue flam و بشتغ يبين لون الـ loop الجر

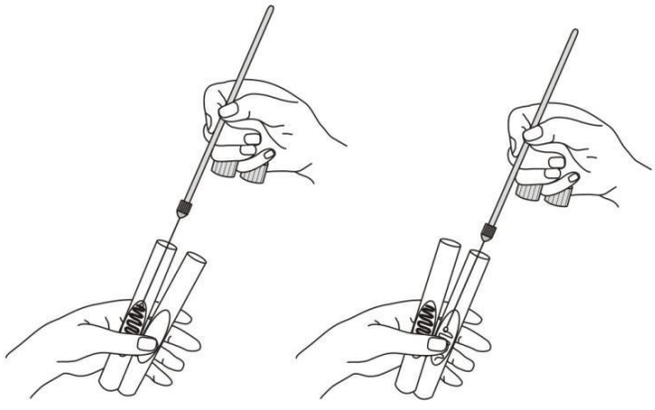


(c)

بدخل الـ loop بعد الـ Flaming اول مره بال test-tube اللي فيه MO

هون برضه لازم اخلي الـ loop يبرد

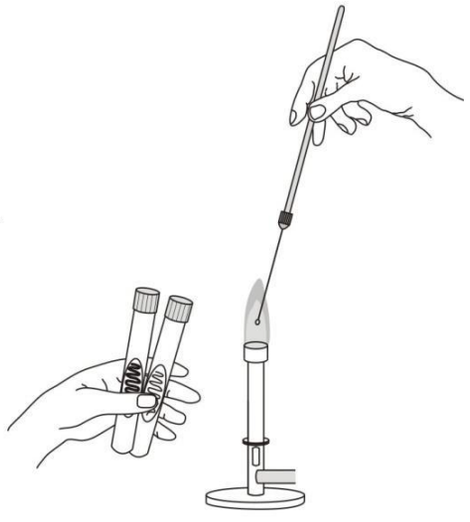
لساما كملت Flaming



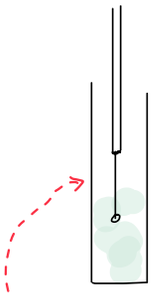
(d)

(e)

رح ادخل الـ loop على الـ broth عشان اعمل incubation



(f)



ما طلعت الـ loop من الـ test-tube طلعته من الـ suspension

اذا طلع من الـ test-tube لازم اعمل Flaming مره ثانيه

كيف انا اتأكد انه الـ inoculated-loop أخد sample رح ادخل الـ loop واطلعه من الـ suspension أكثر من مره او اعمله shaking وهو جوا الـ test tube

لازم اتأكد انه الـ inculated-loop بارد عشان هيك بدخله اكثر من مرة لانه اول مره ممكن ما يكون بارد وهيك رح اقتل البكتيريا ومارح يطلع نتيجه صح

اي مكان ذكرت فيه كلمه الـ loop قصدي فيها inoculated loop

الـ MO = Microorganism