Lab 3+4: Method of Organism Cultivation

Culture media is a special medium used in microbiological laboratories to grow different kinds of microorganisms. A growth or a culture medium is composed of different nutrients that are essential for microbial growth.

Since there are many types of microorganisms, each having unique properties and requiring specific nutrients for growth, there are many types based on what nutrients they contain and what function they play in the growth of microorganisms. The basic nutritional requirement including:

1. Energy source

2. Carbon source (contain carbon0

3. Nitrogen source

A. **organic** compound (amino acid)

B. **inorganic** compound NO_2, NO3, and NH_4).

4. Sulfur and phosphor (important for production of nucleic acid and proteins)

5. Mineral

A.**Macro** elements: require in small amount e.g. Mg, Fe, Ca, and K)

b. **Micro** elements just that they are required in different quantities and concentrations.

e.g. Zn, Cu, Mn, Co, Mo, and Ni.

❖ Classification of culture media

1. According to composition:

A. **Natural**: contain at least a compound of unknown chemical composition

B. **Synthetic**: the exit composition is defined

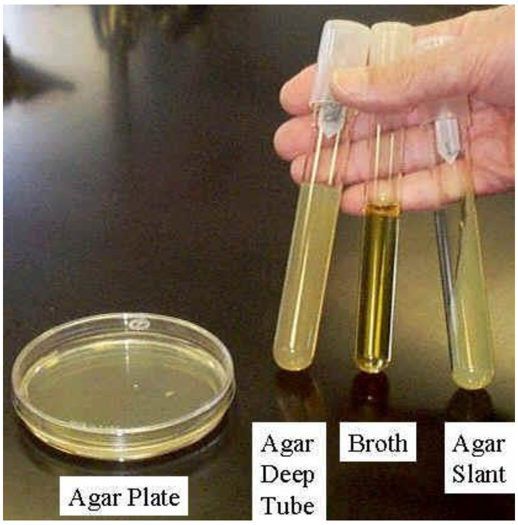
2. According to their physical state (solidity)

A. **Liquid**: for growth e.g. Nutrient broth, MacConky broth

B. Solid: Solid media are essential for isolating and separating bacteria growing together in a specimen to obtain the colony appearance and pure culture isolation of microorganism's e.g. Nutrient agar and blood agar

C. Semi solid media: for motility test ex. Semi solid manitol and semi-solid agar.

Solid media contain specific compound responsible for solidify state that contain extract from sea weed called agar that have not nutritional value for microorganisms that solidify in 42ºC and boiling at 90-100ºC .



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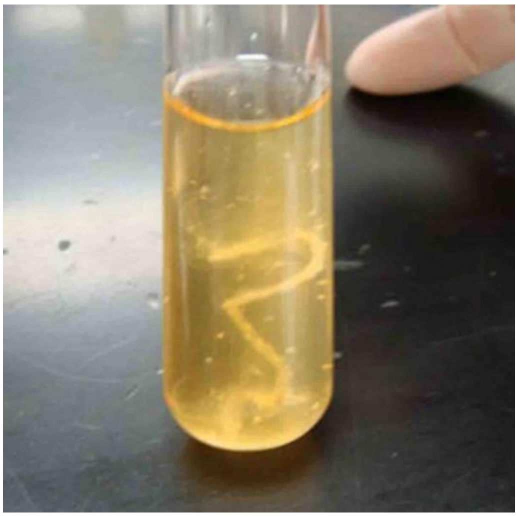

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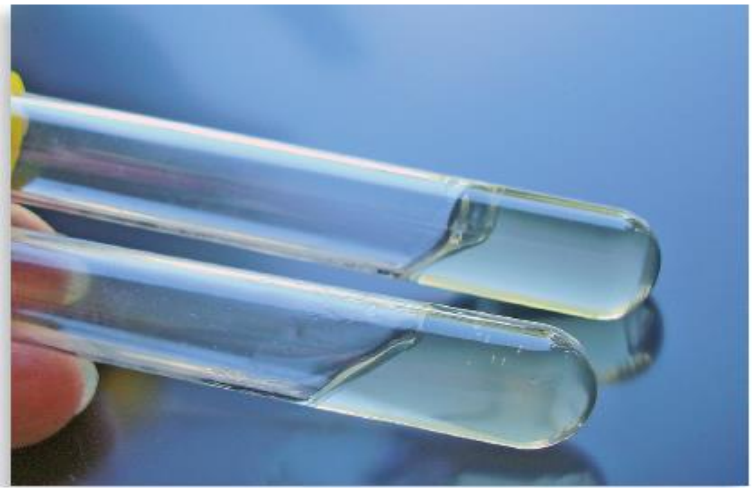

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Figure 1: Type of culture media depending of solidly.

3. According to function:

A. Differential media: contain reagent that allow differentiation between microorganisms e.g. **Eosin methylene blue agar** (EMP) for Entero-bacteriaceae

B. Selective media: that allow for one microorganisms to grow and prevent growth of anther microorganism e.g. **MacKay agar**

C. Specialized media: permit the growth of special microorganisms e.g. **Minitol salt agar** for staphylococcus sp.

D. Enriched media: for growth of fastidious microorganisms e.g. **Yeast extract agar** for clostridium.

E. Enrichment media: enhance the growth of pathogen and inhibit the growth of competitive microorganisms e.g. **Tetrathionet broth** for salmonella sp. **(see Appendix3)**

Figure 2: Type of culture media depending of function.

❖ Procedure of preparing media Agar Petri Plates

Procedure of media preparation as following: **1.** weighting media ingredients by analytical balance. **2.** Mixing ingredients by hot plate magnetic stirrer, **3.** Adjusting pH by pH meter devises **4.** Cover the beaker with cotton blug and foil, **5.** Autoclaving media by autoclave apparatuses. **6.** Aseptically pouring media in to petri plate **7**. When cooled, store upside-down in plastic bags in the refrigerator to prevent the agar from drying out, and labelled the data on it.

4. Inoculation and other aseptic procedures in Transferring Bacteria:

One of the first requirements to study specific microorganisms is to separate them from the mixed microbial populations in which they are found in the environment. To achieve this goal microbiologists use culture media and aseptic transfer techniques.

To start, aseptic technique is used to introduce a very small sample of cells (**the inoculum**) into a receptacle containing nutrient or culture medium. This process is called inoculation.

The **aseptic (sterile) technique** is a technique designed to keep the working environment as free of contaminants as possible. This is achieved first, by sterilizing all equipment and media that will be in contact with the microorganisms. This includes minimizing the air movement on the working area. Usually the work is done within the vicinity of a flame. Aseptic technique is required for the maintenance of pure cultures and the successful isolation of specific types of microorganisms.

A **pure culture** is a culture that contains only one species of bacteria. **A mixed culture** encompasses more than one species. When isolating bacteria from the environment the microbiologist always starts with a mixed culture. A pure culture can be obtained from the mixed culture by sub-culturing and streaking for isolation.

Asepsis can be defined as the absence of infectious microorganisms. However, the term is usually applied to any technique designed to keep unwanted microorganisms from contaminating sterile materials.

- ❖ **Essential point in bacteriology lab**
- ❖ **Use of the Loop/Stab Inoculator**:

Two different types of inoculators can be used depending on the purpose of the work. The **loop** is used to a) transfer cultures from one medium to another, b) to prepare bacterial smears, and c) to streak plates. The loop is the tool of choice for working with a liquid inoculum culture. The **stab** is used to prepare stab cultures and to pick single colonies from a plate.

❖ Sterilizing a wire loop

Wire loops are sterilized using red heat in a Bunsen flame before and after use. They must be heated to red hot to make sure that any contaminating bacterial spores are destroyed. The handle of the wire loop is held close to the top, as you would a pen, at an angle that is almost vertical. This leaves the little finger free to take hold of the cotton wool plug/screw cap of a test tube/bottle.

❖ Flaming procedure:

The flaming procedure is designed to heat the end of the loop gradually because after use it will contain culture, which may 'splutter' on rapid heating with the possibility of releasing small particles of culture and aerosol formation.

1. Position the handle end of the wire in the light blue cone of the flame. This is the cool area of the flame.

2. Draw the rest of the wire upwards slowly up into the hottest region of the flame, (immediately above the light blue cone).

3. Hold there until it is red hot.

4. Ensure the full length of the wire receives adequate heating.

5. Allow to cool then use immediately.

6. Do not put the loop down or wave it around.

7. Re-sterilise the loop immediately after use

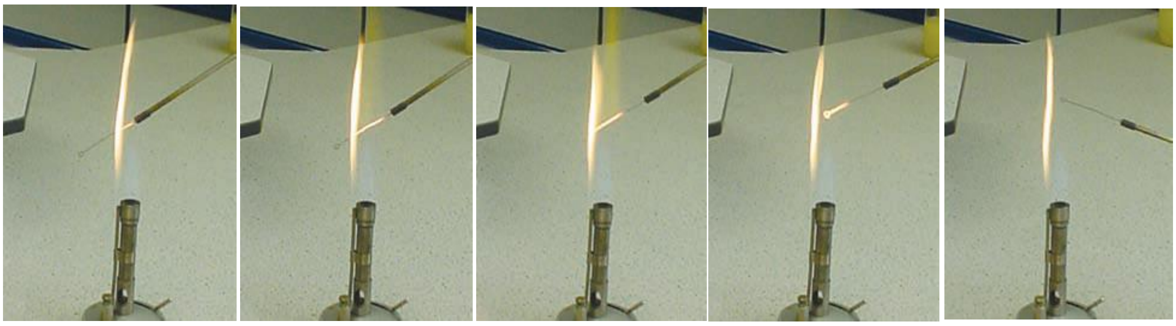


Figure 4: Using of loop in culturing methods.

❖ **Notes: if a loop does not hold any liquid the loop has not made a complete circle. To correct the problem, first ensure that the loop has been sterilised and then reshape the loop with forceps. Do not use your fingers because of the possibility of puncturing the skin.**

❖ Using a spreader

Sterile spreaders are used to distribute inoculum over the surface of already prepared agar plates.

- ❖ **Notes: It is advisable to use agar plates that have a well-dried surface so that the inoculum dries quickly. Dry the surface of agar plates by either incubating the plates for several hours, e.g. overnight, beforehand or put them in a hot air oven (55–60 °C) for 30–60 minutes with the two halves separated and the inner surfaces directed downwards. Wrapped glass spreaders may be sterilized in a hot air oven (They can also be sterilized by flaming with alcohol.**

❖ Sterilization using alcohol

1. Dip the lower end of the spreader into a small volume of alcohol

- ❖ **Notes: (70 % IDA) contained in a vessel with a lid (either a screw cap or aluminum foil). Ensure that the spreader is pointing downwards when and after igniting the**

2. Pass quickly through a Bunsen burner flame to ignite the alcohol; alcohol to avoid burning yourself. The alcohol will burn and sterilize the glass. Keep the alcohol beaker covered and away.

3. Remove the spreader from the flame and allow the alcohol to burn off. From the Bunsen flame.

4. Do not put the spreader down on the bench.

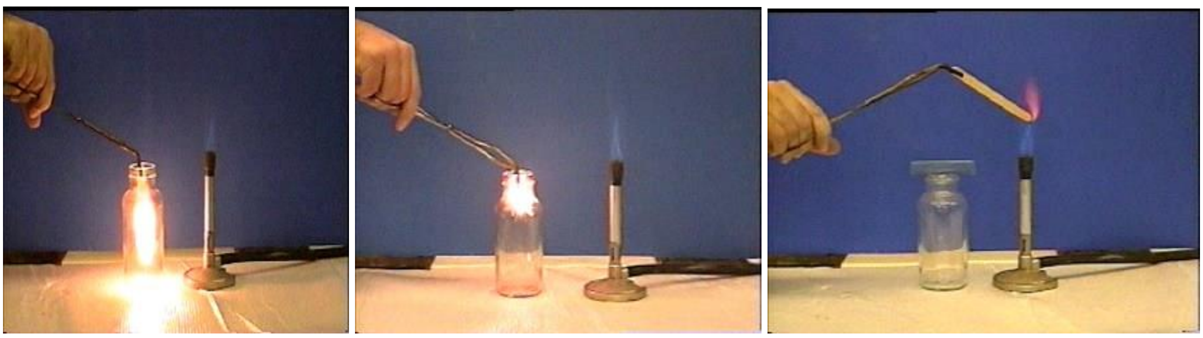


Figure 5: Using of spreader in culturing methods.

❖ Flaming the neck of bottles and test tubes

1. Loosen the cap of the bottle so that it can be removed easily.

2. Lift the bottle/test tube with the left hand.

3. Remove the cap of the bottle/cotton wool plug with the little finger of the right hand. (Turn the bottle, not the cap.)

4. Do not put down the cap/cotton wool plug.

5. Flame the neck of the bottle/test tube by passing the neck forwards and back through a hot Bunsen flame.

6. After carrying out the procedure required, e.g. withdrawing culture, Replace the cap on the bottle/cotton wool plug using the little finger. (Turn the bottle, not the cap.)

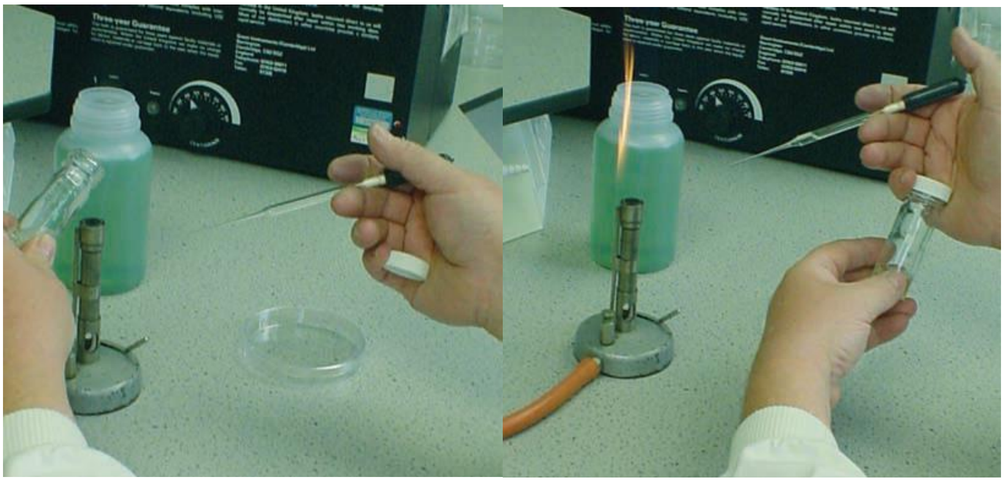


Figure 6: Flaming the neck of bottles and test tubes.

✓ **Notes :**

- **Label tubes and bottles in a position that will not rub off during handling. Either marker pens or self-adhesive labels are suitable.**
- **Occasionally cotton wool plugs accidentally catch fire. Douse the flames by immediately covering with a dry cloth, not by blowing or soaking in water.**

❖ Inoculating a Liquid Media

There are several essential precautions that must be taken during inoculation procedures to control the opportunities for the contamination of cultures, people or the environment.

- Operations must not be started until all requirements are within immediate reach and must be completed as quickly as possible.
- Vessels must be open for the minimum amount of time possible and while they are open all work must be done close to the Bunsen burner flame where air currents are drawn upwards.
- On being opened, the neck of a test tube or bottle must be immediately warmed by flaming so that any air movement is outwards and the vessel held as near as possible to the horizontal.
- During manipulations involving a Petri dish, exposure of the sterile inner surfaces to contamination from the air must be limited to the absolute minimum.
- The parts of sterile pipettes that will be put into cultures or sterile vessels must not be touched or allowed to come in contact with other non-sterile surfaces, e.g. clothing, the surface of the working area, the outside of test tubes/bottles.

EXERCISE:

For this exercise a tube containing a liquid culture will be used to provide the source of the inoculum for broth and agar slants. Work close to the flame!

1. Hold the source culture tube with your non-dominant hand and hold the inoculating loop with your dominant hand. Carefully shake the culture to make sure the cells are suspended
2. Sterilize the inoculator by first passing the entire wire through the flame, starting at the handle end. Wait until the whole wire becomes red hot. Allow the wire to cool (without waving it on the air) for about 30 seconds.
3. Remove the cap from the source tube using the pinkie finger of the same hand that is holding the inoculator. You will hold the cap in your finger until it is time to put it back on the source tube.
4. Pass the mouth of the source tube through the flame once.
5. Without touching the walls of the tube, put the sterile inoculator into the source tube containing the culture and dip the loop into the liquid.

- ✓ **Note: If you were using a slant or plate as a source you will simply touch the surface of the agar where the bacteria are growing with the inoculator. We will do this in the near future.**

6. Withdraw the inoculator from the source tube, and replace the cap.

7. Remove the cap of the tube to be inoculated (using the pinkie finger as before) and insert the inoculator into the tube. Don't stir or shake the loop excessively. Flame the top of the tube and replace the cape

8. Flame the inoculator as before but, heat it slowly so that any material remaining on the loop does not spatter.

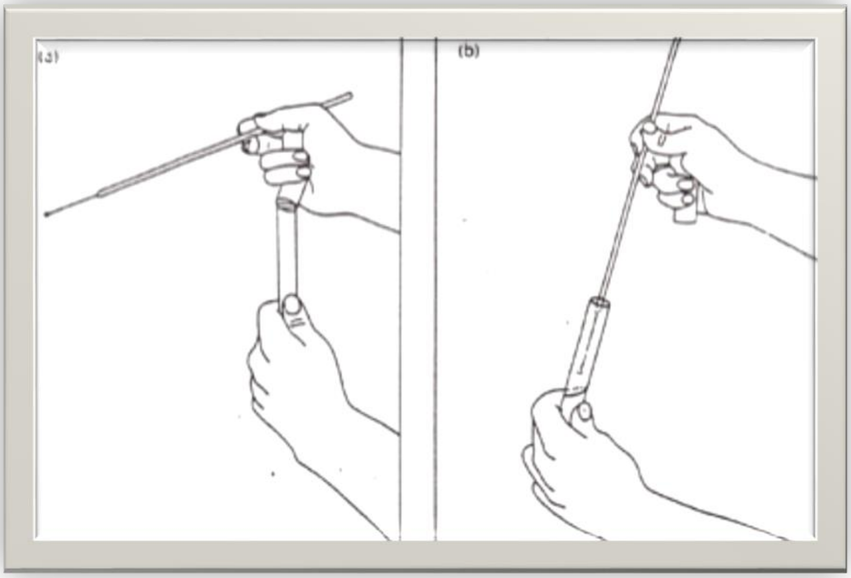
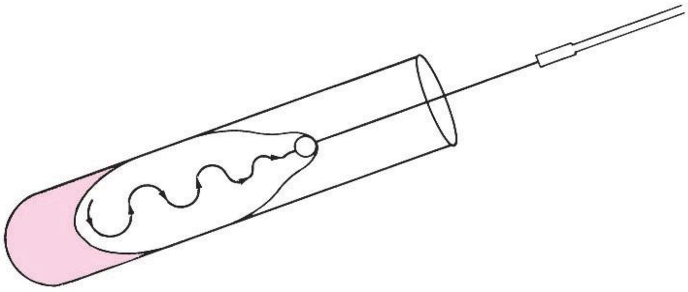


Figure 7: Inoculating a Liquid_Media.

❖ Inoculation of an agar slant **EXERCISE:**

Rest the inoculator gently at the lower end of the slant and withdraw it slowly upwards moving it from side to side (the surface of the agar should not be broken). This should leave a streak on the surface of the slant (In some specific experiments you may be require to stab the slant just under the agar surface, if that is the case it will be clearly specified in the instructions).



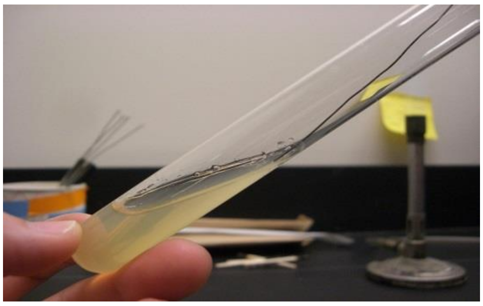
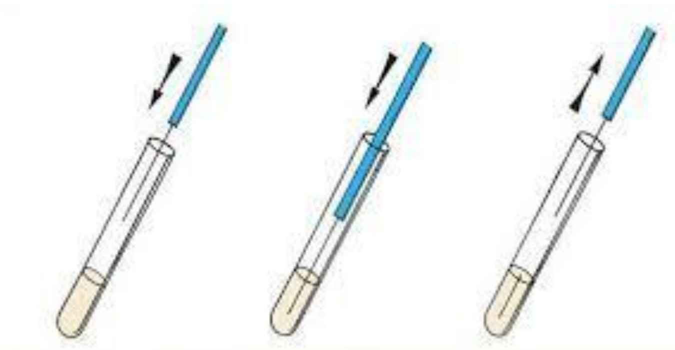


Figure 8: Inoculation of an agar slant:

❖ Inoculation of an agar stab **EXERCISE:**

Using aseptic technique pick a single well isolated colony with a sterile inoculating stab needle and stab the needle several times through the agar to the bottom of the vial or tube. Replace and tighten the cap. Make sure the tube and cap are well labeled. Give the stab to your instructor for storage.



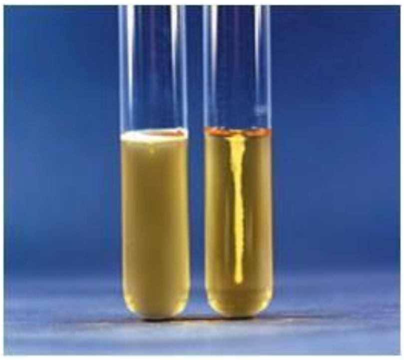


Figure 9: Inoculation of an agar stab.

❖ Inoculation of an agar plate

Working with agar plates is bit different than working with media in tubes in that you have a wide lid instead of narrow cap. This means there is a greater surface area of sterile media that can be exposed to contaminations in the atmosphere. The key is to keep as much of the lid over (covering) the open agar plate as possible. **Never set the lid down on the lab bench when in an open contaminating environment.** Who the agar in a plate is inoculated depends on the goal. The methods of culturing are discussed later in type of streaking method.

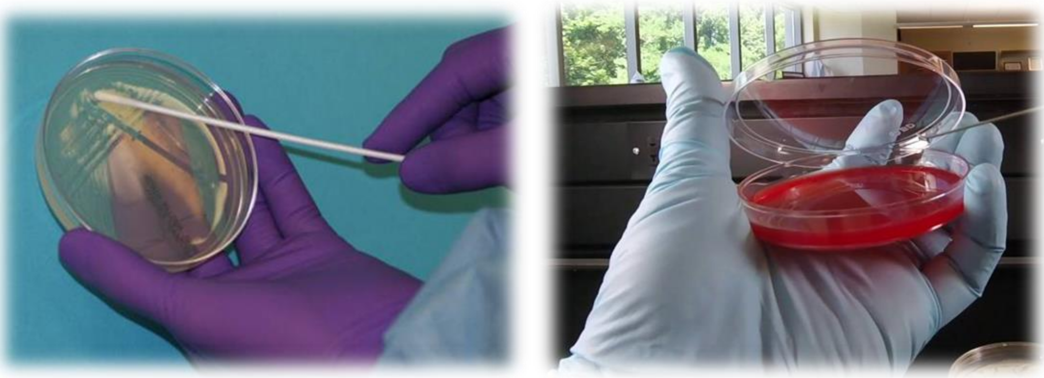


Figure 10: Inoculation of an agar plate

❖ Type of culturing methods

Simpler methods for isolation of a pure culture include (1). **Standard plate count** method that include **poured plate** method and **spread plating** on solid agar medium with a glass spreader and (2). **Streak plat** in with a loop. The purpose of spread plating and streak plating is to **isolate** individual bacterial cells on a nutrient medium.

❖ Standard methods cultivation

A. pours plate cultivation

EXERCISE:

1. Collect one bottle of sterile molten agar from the water bath.

2. Hold the bottle in the right hand; remove the cap with the little finger of the left hand.

3. Flame the neck of the bottle.

4. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the lid.

5. Flame the neck of the bottle and replace the cap.

6. Gently rotate the dish to ensure that the medium covers the plate evenly.

7. Allow the plate to solidify.

8. Seal and incubate the plate in an inverted Position.

✓ **Notes:**

1-The base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles.

2- The plates should be used as soon as possible after pouring. If they are not going to be used straight away they need to be stored inside sealed plastic bags to prevent the agar from drying out.

3- Use a water bath at 50 °C to store bottles of molten agar.
4- Ensure that the temperature of the molten agar is cool enough for mixing with the culture.

5- Take care not to contaminate the molten agar in the bottles with water from the water bath.

6- To avoid contamination ensure: that the water in the water bath is at the right depth the bottles are kept an upright position that the outsides of the bottles are wiped before they are use

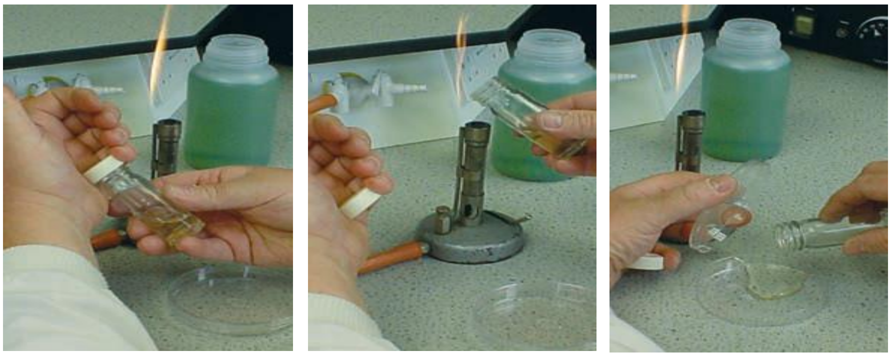


Figure 11: pours plate cultivation

B. Spread Plate Technique

Spread plates, also known as lawn plates, should result in a heavy, often confluent growth of culture spread evenly over the surface of the growth medium. This means that they can be used to test the sensitivity of bacteria to many antimicrobial substances, for example mouthwashes, garlic, disinfectants and antibiotics. The spread plate can be used for quantitative work (colony counts). If 0.1 cm^3, the dilution and volume of the inoculum, usually are known, the viable count of the sample, i.e. the number of bacteria or clump cm3, of bacteria per can be determined. The dilutions chosen must be appropriate to produce between 30 and 100 separate countable colonies.

In this technique, the number of bacteria per unit volume of sample is reduced by serial dilution before the sample is spread on the surface of an agar plate.

EXERCISE:

1. Dilute the bacterial sample with serial saline or BPS **(Phosphate Buffer Saline)** (for isolation of bacteria from environment) or broth until the bacteria are dilute enough to count accurately to make dilute 10^{-1}.

2. With marker pencil, label the bottom of six petri plates with the following dilution 10^4, 10^{-5}, 10^{-6}, 10^7, 10^{-8}, 10^{-9}.

3. Using aseptic technique, the initial dilution is made by transferring 1ml of liquid sample 10^{-1} in to 9ml of serial saline this dilation is 1\100 or 10^{-2}

4. The 10^{-2} blank is then shaken vigroiselly, this serves to distribute the Bactria and break any clumps of bacteria that may be present. Then add 1ml of dilution 10^{-2} to tube 10^{-3} and shaken, repeat this process until reach to tube 9 (10^{-9}).

5. Prepare serial dilutions of the broth culture as shown below. Be sure to mix the nutrient broth tubes before each serial transfer. Transfer **0.1 ml** of the final three dilutions (10-5, 10-6, and 10-7) to each of three nutrient agar plates, and label the plates.

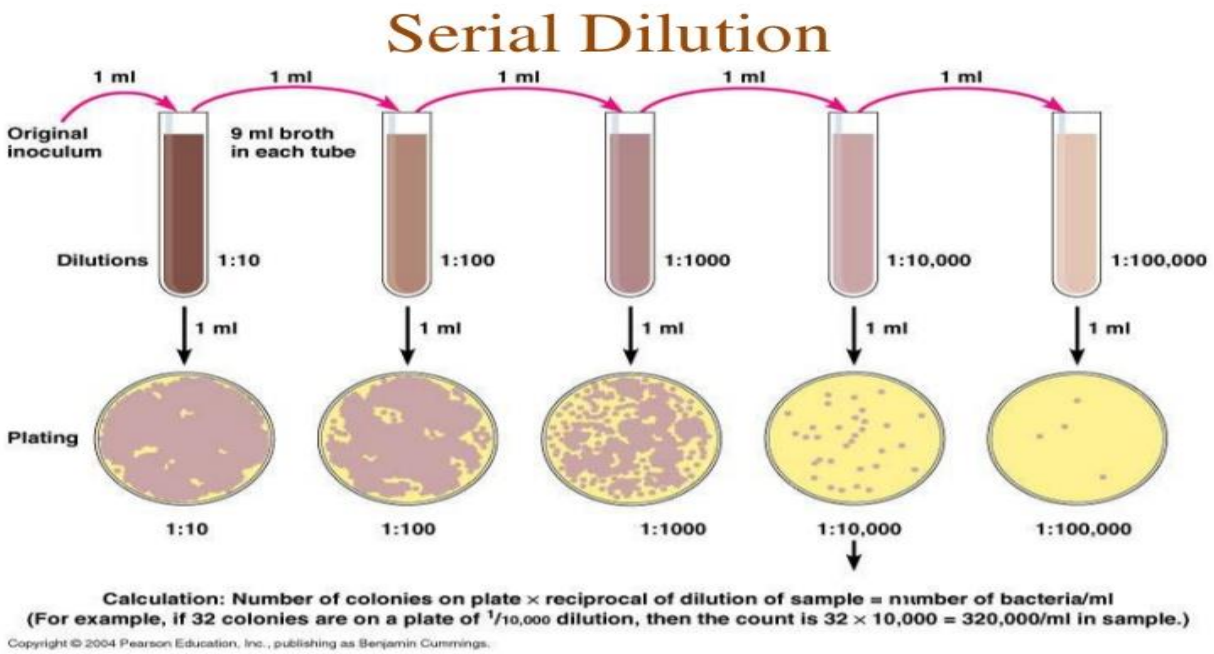


Figure 12: Serial dilution method.

- ✓ **Note: Position the beaker of alcohol containing the glass spreader away from the flame. Remove the spreader and very carefully pass it over the flame just once (lab instructor will demonstrate). This will ignite the excess alcohol on the spreader and effectively sterilize it.**

6. Spread the 0.1 ml inoculum evenly over the entire surface of one of the nutrient agar plates until the medium no longer appears moist. Return the spreader to the alcohol.

7. Repeat the flaming and spreading for each of the remaining two plates.

8. Invert the three plates and incubate at room temperature until the next lab period.

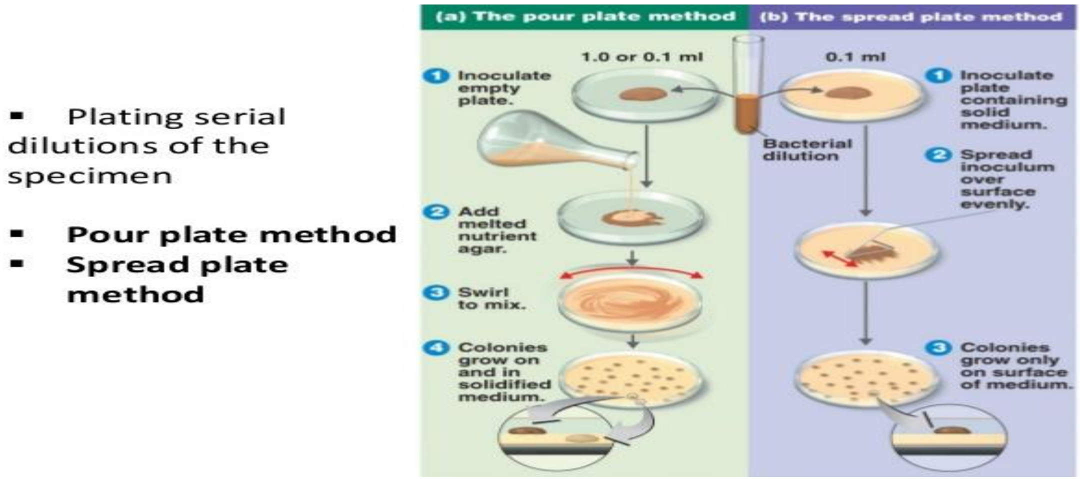


Figure 13: Type of standard plate count methods cultivation.

❖ Streaking plate Technique

Colonies of several different species are regularly present on the same agar plate when certain patient specimens are inoculated onto them. Work with pure cultures permits the microbiologist to study the properties of individual species without interference from other species. This practice of streaking plates to obtain pure cultures is critical in the hospital laboratory because it allows the microbiologist to determine how many types of bacteria are present, to identify those likely to be causing the patient's disease, and to test which antimicrobial agents will be effective for treatment.

❖ Type of streaking methods

There are different types of streaking methods to obtain pure culture of bacteria but the most used type: (T streak, Quadrant streak, Radiant streak, and continuous streak): that discus as follow:

For make procedure of every type of streaking methods we need to this material:

1. Culture of bacterial isolates:

-*Escherichia coli*

-*Bacillus subtilis*

-*Streptomyces sp.*

2. Nutrient agar plate

A. T streak methods (triple streak method)

EXERCISE:

1. Label your plate divided your plate in three areas by drawing on outsides bottom of plate.
2. Inculcate one areas using single continuous motion with any of culture
3. Flame your loop and allow it to cool
4. Take loop full from 1 area by cross streak tree to four times and continue streak in to second adjacent area.
5. Flame your loop and allow it to cool
6. Repeat your procedure in step 4 and 5 to carry microorganisms from the second area to the third area. Sterilized your loop
7. Place your plate inverted in 37ºC in incubator for 24-48h
8. At next laboratory session observe your streak plate observe where your find well isolated colonies. Describe your results

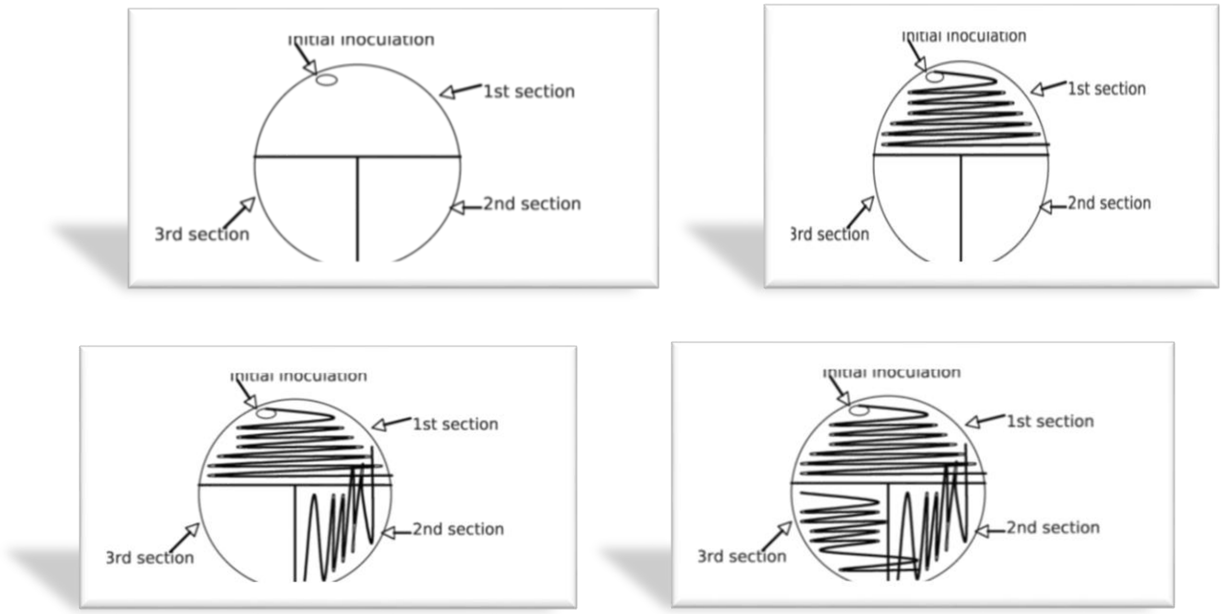


Figure 14: Triple streaking method.

B- Quadrant streaking methods

EXERCISE:

1. Label your plate, drown – Quadrant on outside bottom of un agar petri-plate

2. Concentrate the culture in first area of the plate using continuous streaking method

3. Flame your loop allow it to cool and cross streak the previous area to produce second quadrant

4. Repeat this same procedure for each succeeding area, until you have inoculated the four guardant

5. Place your plate inverted in 37C in incubator for 24-48h

6. At next laboratory session observe your streak plate observe where your find well isolated colonies, describe your results

Figure 15: Quadrant streaking method.

C. Radiant streaking method

EXERCISE:

1. Label your plate.

2. Aseptically pick up heavy culture inoculum with an insulation loop and streak within small area at the edges of plate.

3. Sterilizes your loop and allow cooling it.

4. Turin your plate 90 and make series of dis continuous streak from the first area in radial fashion.

5. Sterilizes your loop and allow cooling it.

6. Turn your plates 80 and make second series of discontinuous radiant streak at right angles to the first series sterilized your loop.

7. Place your plate inverted in 37C in incubator for 24-48h.

8. At next laboratory session observe your streak plate observe where your find well isolated colonies, describe your results.

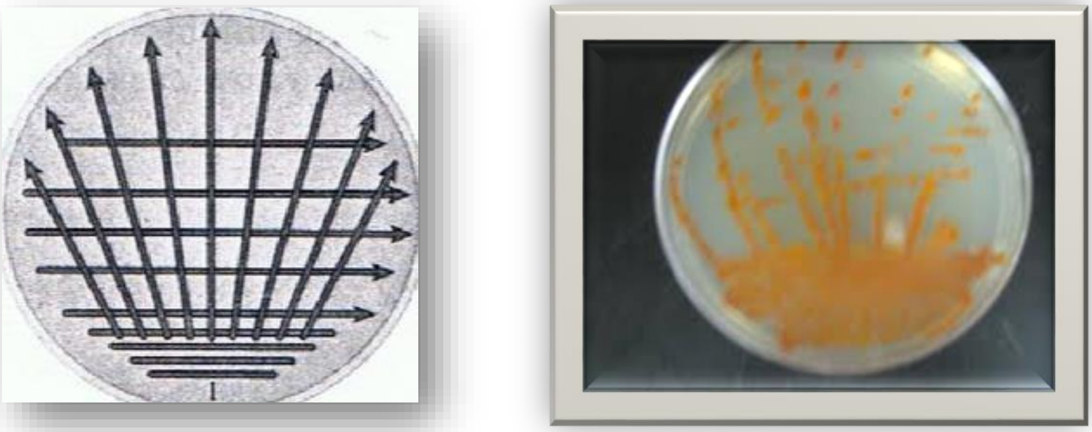


Figure 16: Radiant streaking method.

D. Continuous streaking method

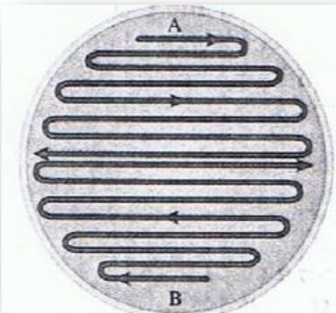
EXERCISE:

1. Take a small amount of culture inoculation on your loop and spread it in a single continuous back and forth motions over halve of your plate.

2. Without flaming your loop and using the same face of inculcating loop, turn your plates 180 and continue the streaking procedure as you did in initial area.

3. Place your plate inverted in 37C in incubator for 24-48h.

4. At next laboratory session observe your streak plate observe where your find well isolated colonies, describe your results.



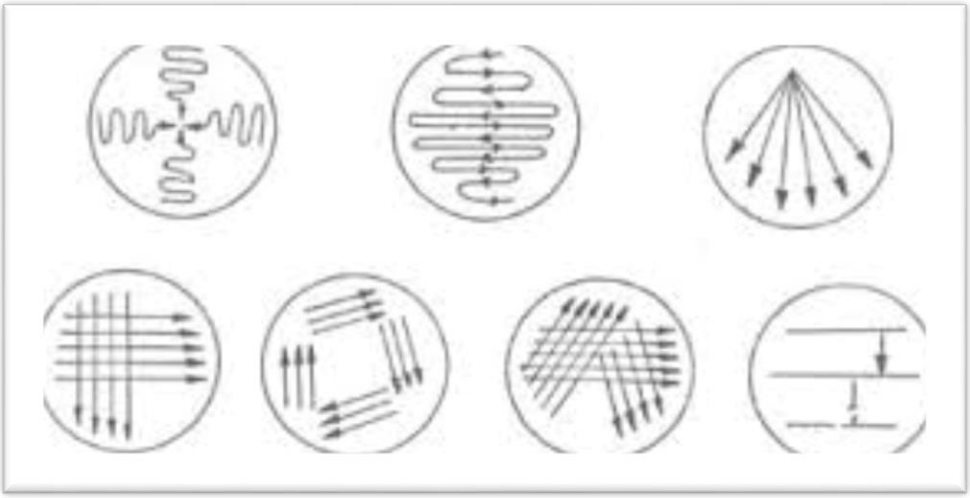



Figure 17: Continuous streaking method.

Sub culture, maintenance and preserving of bacterial isolates

Pure culture theoretically contains a single bacterial species. There are a number of procedures available for the isolation of pure cultures from mixed populations. A pure culture may be isolated by the use of special media with specific chemical or physical agents that allow the enrichment or selection of one organism over another.

Sub culturing: is a process of transferring of bacteria cell from one growth medium container, such as broth or agar, to another, and allowing the bacterial cell to grow. Sub culturing is also useful in keeping strains alive by transferring them to fresh growth medium. As follow some types or cases of sub culturing.

Procedures

A. Transfer of a Slant Culture to a Nutrient Broth

1. The procedure will be demonstrated. Watch carefully and then do it yourself, following directions given.
2. Take up the inoculating loop by the handle and hold it as you would a pencil, loop down. Hold the wire in the flame of the Bunsen burner or in the bacterial incinerator until it glows red

Remove loop and hold it steady a few Moments until cool. *Do not wave it around, put it down, or touch it to anything.*

3. Pick up the slant culture of *Escherichia coli* with your left hand. Still holding the loop like a pencil, but more horizontally, in your right hand, use the little finger of the loop hand to remove the closure (cotton plug, slip-on, or screw cap) of the culture tube. Keep your little finger curled around this closure when it is free—*do not place it on the table.* Insert the loop into the open tube (holding both horizontally). Touch the loop (*not the handle!*) to the growth on the slant and remove a loopful of culture. Don't dig the loop into the agar; merely scrape a small surface area gently.

4. Withdraw the loop slowly and steadily, being careful not to touch it to the mouth of the tube. Keep it steady, *and do not touch it to anything* (it's loaded!) while you replace the tube closure and put the tube back in the rack.

5. Still holding the loop steady in one hand, use the other hand to pick up a tube of sterile nutrient broth from the rack. Now remove the tube closure, as you did before, with the little finger of the loop hand (don't wave or jar the loop). Insert the loop into the tube and down into the broth. Gently rub the loop against the wall of the tube (don't agitate or splash the broth), making sure the liquid covers the area but does not touch the loop handle.

6. As you withdraw the loop, touch it to the inside wall of the tube (not the tube's mouth) to remove excess fluid from it. Pull it out without touching it again, replace the closure, and put the tube back in the rack.

7. Now carefully sterilize the loop. If you are using a Bunsen burner, hold it first in the coolest part of the flame (yellow), then in the hot blue cone until it glows. Be sure all of the wire is sterilized, but do not burn the handle. When the wire has cooled, the loop can be placed on the bench top.

8. Label the tube you have just inoculated with your name, the name of the organism, and the date.

9. Repeat steps 2 through 9 with each of the other two slant cultures (*Bacillus subtilis* and *Serratia marcescens*).

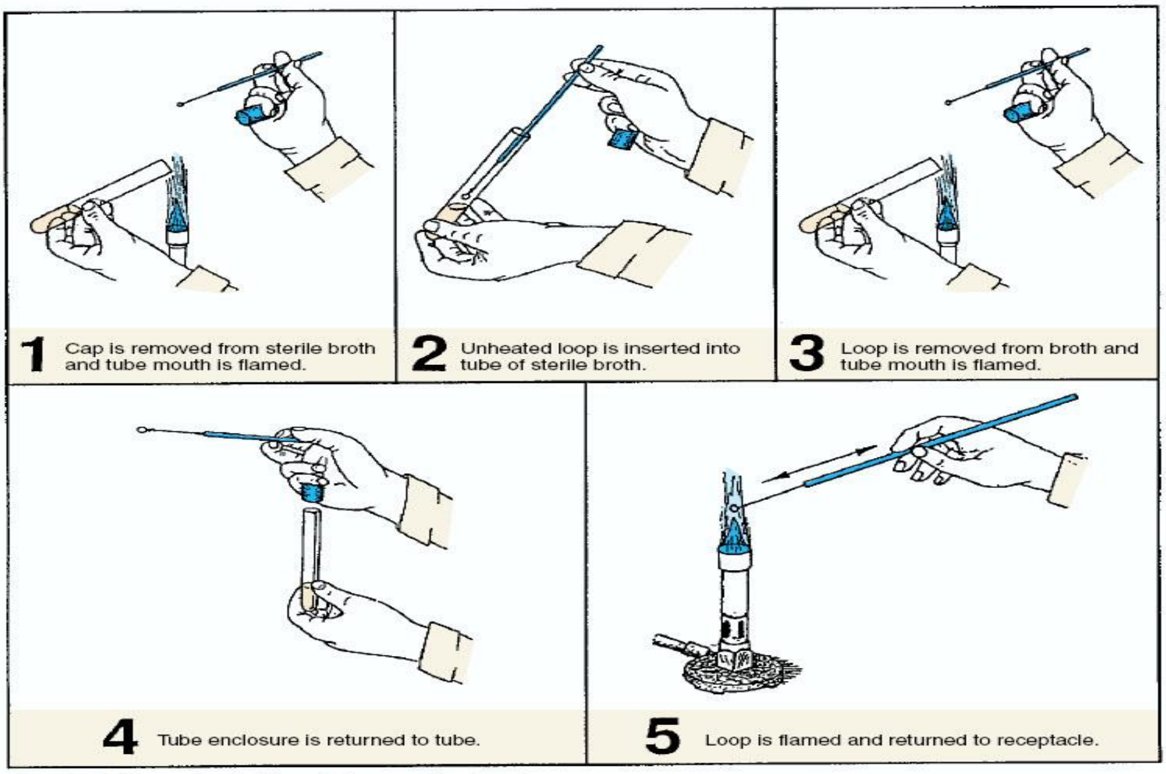


Figure 2 Procedure for inoculating a nutrient broth

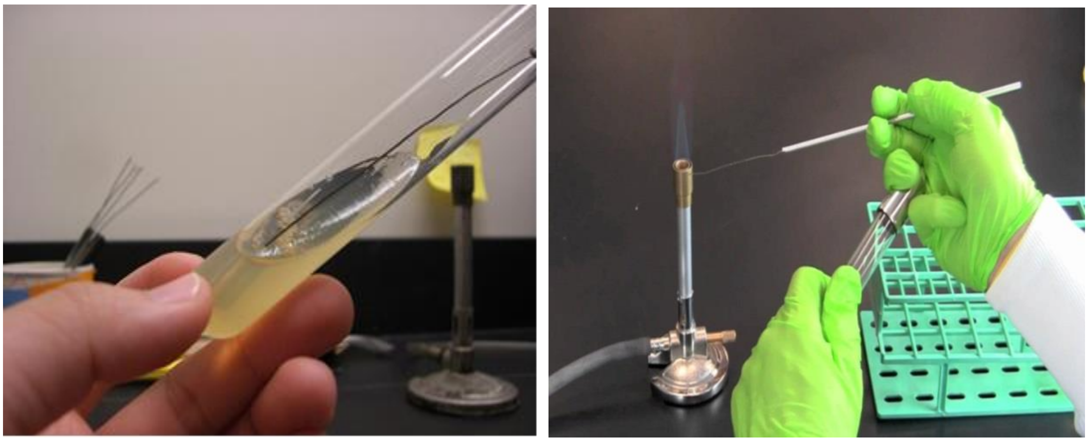


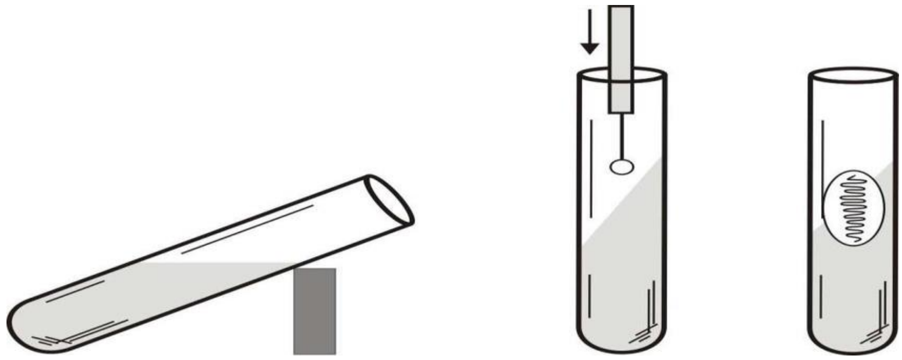
Figure 1: Transfer of a Slant Culture to a Nutrient Broth

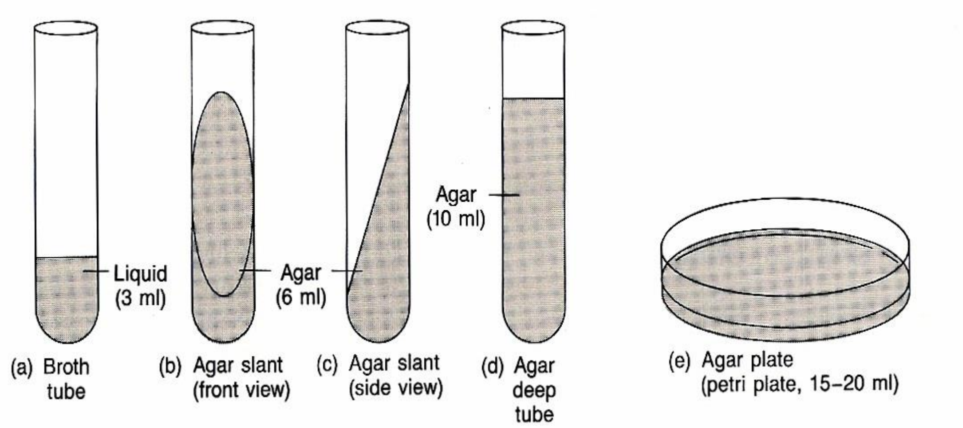
B. Transfer of a Slant Culture to a Nutrient Agar Slant

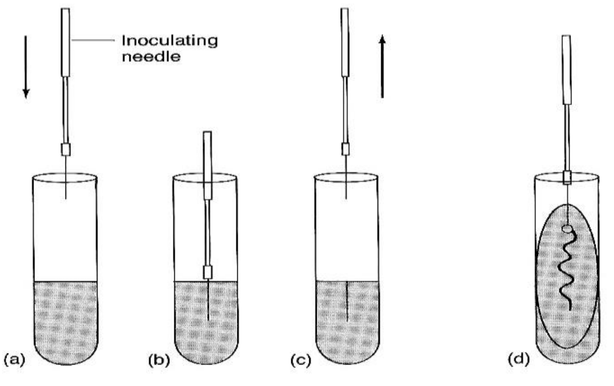
1. Start again with sterilizing the loop.
2. Pick up the slant culture of *E. coli,* open it, and take up some growth on the sterile loop.
3. Recap the culture tube carefully and replace it in the rack. Pick up and open a sterile nutrient agar slant (keep the charged loop steady meantime).
4. Introduce the charged loop into the fresh tube of agar, and without touching any surface, pass it down the tube to the *deep* end of the slant. Streak the agar slant by lightly touching the loop

to the surface of the agar, swishing it back and forth two or three times (don’t dig up the agar), then zigzagging it upward to the top of the slant. Lift the loop from the agar surface and withdraw it from the tube without touching the tube surfaces (fig. 2.4).

5. Close and replace the inoculated tube in the rack; then sterilize the loop as before.
6. Label the freshly inoculated tube with your name, the name of the organism, and the date.
7. Repeat steps 1 through 6 of procedure B with each of the other two slant cultures provided (*B. subtilis* and *S. marcescens*).







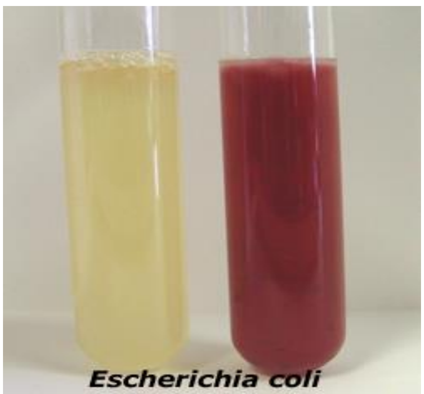


Figure 2: Transfer of a Slant Culture to a Nutrient Agar Slant

Transfer of a Single Bacterial Colony on a Plate Culture to a Nutrient Broth and a Nutrient Agar Slant

1. Start again with sterilizing the loop.
2. Hold the sterile, cooling loop in one hand and with the other hand turn the assigned plate culture of *Serratia marcescens* so that it is positioned with the bottom (smaller) part of the dish up. Lift this part of the dish with your free hand (fig. 2.5) and turn it so that you can clearly see isolated colonies of *S. marcescens* growing on the surface of the plated agar.
3. With the sterile, cool loop, touch the *surface* of one isolated bacterial colony (fig. 2.6). Withdraw the loop and replace the bottom part of the dish into the inverted top lying open on the table.
4. Now inoculate a sterile nutrient broth with the charged loop, as in procedure A, steps 6 through 9.
5. Sterilize the loop again, open the plate, pick another colony, close the plate, and inoculate a sterile agar slant as in procedure B, steps 4 through 6.

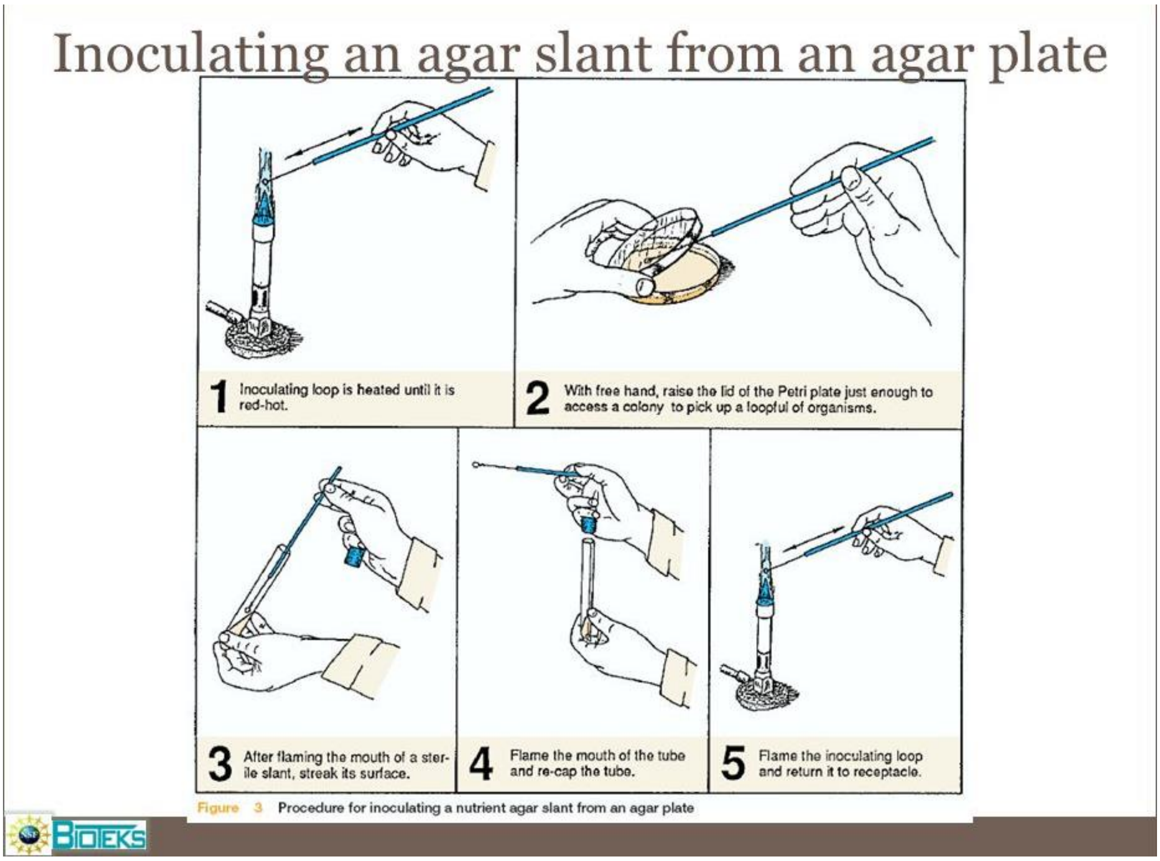


Figure 3: inoculating agar slant from agar plate.

❖ Maintaining stock cultures

- It may be convenient to maintain a stock of a pure culture instead of re-purchasing it when needed. Most of those considered suitable for use are also relatively easy to maintain by sub-culturing on the medium appropriate for growth but maintenance of stock cultures needs to be well organised with attention to detail.
- Be prepared to transfer cultures four times a year to maintain viability.
- Cultures on streak plates are not suitable as stock cultures. Slope cultures in screw cap bottles are preferred because the screw cap reduces evaporation and drying out and cannot be accidentally knocked off (cf. a streak plate culture). Slope cultures are preferred to broth (i.e. liquid medium) cultures because the first sign of contamination is much more readily noticed on an agar surface.
- Two stock cultures should be prepared; one is the ‘working’ stock for taking sub-cultures for classes, the other is the ‘permanent’ stock which is opened only once for preparing the next two stock cultures.
- Incubate at an appropriate Temperature until there is good growth. For growing strict aerobes it may be necessary to slightly loosen the cap for incubation (but close securely before storage) if there is insufficient air in the headspace.
- As soon as there is adequate growth, the cultures may be stored in a refrigerator, but never one in which human foodstuffs are kept? However, they will remain viable at room temperature in either a cupboard or drawer. Keep on the lookout for contamination.

❖ Checking cultures for contamination

- Evidence for a culture being pure or otherwise is given by the appearance of colonies on a streak plates and of cells in a stained microscopically preparation.
- There should be uniformity of colony form and cell form (and consistency with the appearance of the original culture!).

- It is sensible to check purity on suspicion of contamination of the working stock culture from time to time and of the permanent stock when preparing new stock cultures.
- If a culture becomes contaminated, it is not advisable to try to remedy the situation by taking an inoculum from a single colony from a streak plate of the mixed culture because of the possibility of (1) not being able to distinguish between the colony forms of the contaminant and the original culture, and (2) culturing a variant of the original culture that does not behave as the original culture did. Instead, go back to the working (or permanent) stock cultures; that are what they are for!

❖ Preventing contamination of cultures and the environment

❖ Cotton wool plugs

- Plugs made of **non-absorbent** cotton wool are used in test tubes and pipettes to prevent micro-organisms from passing in or out and contaminating either the culture or the environment.
- The necessary movements of air in and gaseous products out are not prevented and the gaps between the cotton wool fibres are even wide enough for Micro-organisms to pass through. However, this does not happen because micro-organisms (negatively charged) are ‘filtered’ out by being attracted to and adsorbed on the oppositely charged cotton wool.
- The cotton wool must remain dry because this filtration property is lost if the cotton wool becomes moist – hence the use of **non-absorbent** cotton wool.
- For use in test tubes a plug should be properly made to ensure that it can be held comfortably without being dropped and its shape and form are retained while being removed from and returned to a test tube several times.
- Aseptic technique cannot be maintained with poorly made plugs; working surfaces, floors and cultures may become contaminated and students may become understandably (but avoidably) frustrated and lose interest.

❖ Aseptic transfer of cultures and sterile solutions

- Regular practice is necessary to ensure that the manipulations involved in aseptic transfer of cultures and sterile solutions become second nature.
- Making a **streak plate** is a basic procedure that tests several skills and serves several purposes.
- During the inoculation procedure, the agar surface is protected from contamination by micro-organisms that are carried in the air by keeping the time that the Petri dish is open to a minimum.
- The choice of **loop or pipette** for transfers between test tubes and screw cap bottles depends on whether they contain agar slopes, liquid media or sterile solutions.
- The wire loop is usually satisfactory for inoculating a tube or bottle from a separate colony on a plate but a straight wire is occasionally needed for dealing with very small colonies such as occur with pure cultures of some bacteria, e.g. species of *Streptococcus* and *Lactobacillus*, and on plates that are being used for isolating cultures from natural samples.

Figure 4: Opening a petri plate culture. The bottom is lifted out of the top, and the top is left lying face up on the bench.

❖ Preparing cultures for class use

- Microbial cultures cannot be taken from a shelf and instantly be ready for use.
- It is necessary to begin to prepare cultures well in advance otherwise the outcome might not be as expected.
- The key is to transfer cultures several times in advance to ensure that they are growing well and are presented as young, fully active cultures on the day of the practical class.

- For most cultures of **bacteria** this will be after incubation for 1 or 2 days; progress of growth can be followed by observation with the naked eye, looking for growth on an agar surface or turbidity in a broth culture.
- The main points to observe are use of an adequate amount of inoculum, an appropriate culture medium and incubation temperature and, if it is necessary to grow a strictly aerobic organism in a single large volume of liquid culture (i.e. more than cm 20 cm3), provision of adequate aeration.
- It will save time in preparing large numbers of cultures of bacteria and yeast for the class if the inoculum is taken by Pasteur pipette from a well-growing (i.e. turbid) broth culture.
- A line of growth on a slope culture inoculated by wire loop is easy for students to observe but almost the same effect can be achieved with a pipette.

❖ Methods of preserving culture bacteria

1. Refrigeration:

Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms. This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped.

2. Paraffin Method:

This is a simple and most economical method of maintaining pure cultures of bacteria and fungi. In this method, sterile liquid paraffin in poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture is preserved for several years.

3. Cryopreservation:

Cryopreservation (i.e., freezing in liquid nitrogen at-196°C) helps survival of pure cultures for long storage times. In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol that prevent the formation of ice crystals and promote cell survival.

4. Lyophilisation (Freeze-Drying):

In this method, the culture is rapidly frozen at a very low temperature (-70°C) and then dehydrated by vacuum. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators. Freeze- drying method is the most frequently used technique by culture collection centres.