

Microbiology Practical 02

Preparation of Culture Media and

Techniques for Sub-Culturing and Transfer of Microbial Cultures

Objective:

This practical session aims to teach students the preparation of various culture media, ensuring their sterility throughout the process, and understanding their specific applications. Additionally, students will learn and apply aseptic techniques for sub-culturing and transferring microorganisms to different media, including broth, slant agar, deep agar, and Petri dishes, using sterile tools and methods to prevent contamination.

1- Introduction:

The preparation of culture media is essential for microbial growth and study, requiring proper composition and sterilization. Sub-culturing techniques help maintain and analyze microbial strains while preventing contamination through aseptic practices. In this practical session, students will transfer *Escherichia coli* onto various media (broth, slant agar, deep agar, Petri dishes) using different inoculation methods to observe growth patterns and isolate colonies.

2- Principle:

Microorganisms need specific nutrients, energy sources, and environmental conditions to grow. A culture medium serves to meet these requirements and is typically prepared as either a liquid medium (broth) or a solid medium. Solid media are prepared by adding agar, a natural polysaccharide derived from marine algae. The sterility of the medium is paramount during preparation to prevent contamination.

3- Types of media

Table .1: Types of Media and Their Use

Type of Media	Purpose
Complex	Used to grow most heterotrophic organisms. Contains water-soluble extracts of plant or animal tissue (e.g., peptone, tryptone), but the exact composition is unknown.
Defined	Used to grow specific heterotrophs and is often required for chemoautotrophs, photoautotrophs, and microbiological assays. The exact chemical composition is known.
Selective	Suppresses unwanted microbes or encourages the growth of desired microbes by adding selective agents (e.g., inhibitors or specific substrates).
Differential	Distinguishes colonies of specific microbes from others by adding indicators or substrates.
Enrichment	Similar to selective media but designed to increase the number of desired microorganisms to a detectable level without stimulating the rest of the bacterial population.
Reducing	Promotes the growth of obligate anaerobes by removing oxygen.

4- Aseptic Technique and Media Forms

Aseptic technique is essential for transferring microorganisms from a pure culture to a sterile medium without introducing contaminants. For solid media, such as agar, microorganisms can be cultured using the following methods:

1- Slants: Liquefied media is poured into test tubes and allowed to solidify at an angle, providing a larger surface area for growth.

2- Stabs: Agar is solidified in test tubes in an upright position, useful for studying microbial growth under different oxygen conditions.

3- Petri Dishes: Agar is poured into sterile Petri dishes to solidify, offering a large surface area for isolation and study of microorganisms. Plates should be stored inverted to prevent condensation.

5- Preparation and Sterilization of Nutrient Medium

Table. 2: Composition of Nutrient Medium

Component	Quantity (g/l)
Beef extract	3
Peptone	5
NaCl ₂	3
Distilled water	1000 ml
Agar	15 g (for nutrient agar)
pH	7.0 ± 0.2

5.1. Preparation of Liquid and Solid Media

Materials Required

- **Reagents for Nutrient Medium:**
 - Beef extract (Lab Lemco)
 - Peptone
 - NaCl₂
 - Distilled water
 - Agar (for solid media)
- **Equipment and Glassware:**
 - Pipettes with pipette aids
 - Petri dishes (for solid medium)
 - Conical flasks
 - Autoclave

5.2. Procedure for Preparing Liquid Medium

1. Weigh and dissolve the following ingredients in **800 ml of distilled water**:
 - Beef extract: 3 g
 - Peptone: 5 g
 - NaCl: 3 g
2. Gently heat the mixture to dissolve the components fully.
3. After dissolution, cool the solution and **adjust the pH to 7.0 ± 0.2** using an appropriate acid or base.
4. Adjust the final volume to **1000 ml** with distilled water.
5. Dispense the liquid medium into **sterilized conical flasks or test tubes**.
6. **Autoclave at 15 psi for 15-20 minutes** to sterilize the medium.

5.3. Procedure for Preparing Solid Medium

1. Prepare the liquid nutrient medium as described in the above procedure.
2. To prepare a **solid medium**, add **agar** to the liquid nutrient medium:
 - Agar: 15 g (for nutrient agar)
3. Stir the mixture and **heat gently** until the agar dissolves completely.
4. After the agar has dissolved, allow the medium to cool to approximately **45°C**.
5. Pour the molten medium into **sterilized Petri dishes** (glass or plastic, 15 x 90 mm).
6. Allow the agar to **solidify** at room temperature.
7. Once solidified, the plates should be **inverted** for storage to prevent condensation from dropping onto the agar surface.

Part 2: Transfer of Microbial Cultures

Required Materials

1. Cultures:

- **E. coli** cultures (18-24 hours old) grown in nutrient broth or slant agar.

2. Reagents:

- Sterile nutrient agar plates.
- Sterile nutrient broth tubes (10 mL).
- Sterile slant and deep agar tubes.

3. Equipment and Glassware:

- Sterile inoculation loops and needles.
- Bunsen burner or laminar flow hood.
- Sterile pipettes and test tubes.
- 70% ethanol (for disinfection).

Experimental Procedure

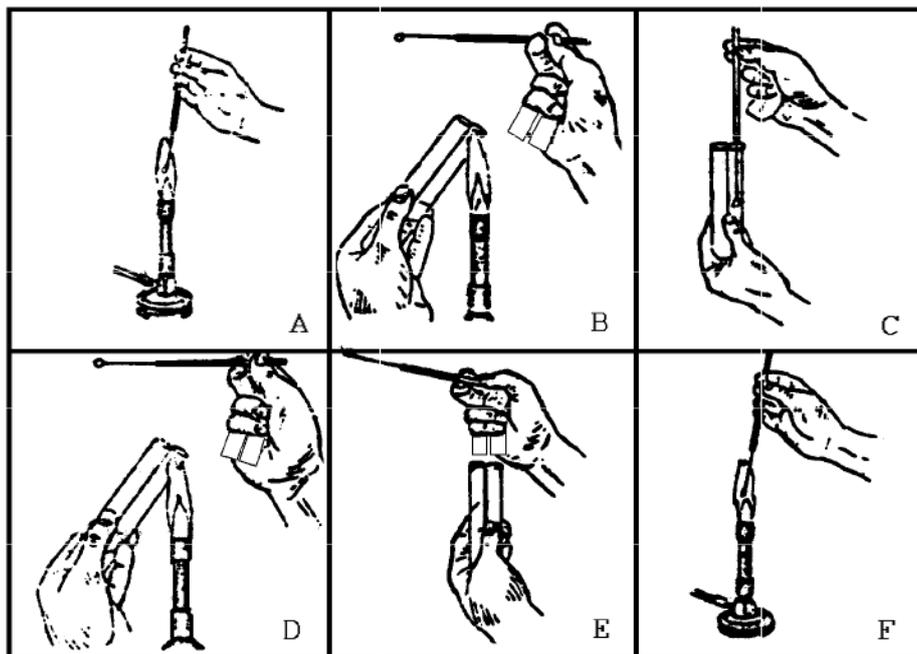
1. Sterilization of the Inoculation Loop

1. Light a Bunsen burner and adjust the flame.
2. Pass the inoculation loop through the flame until it glows orange.
3. Allow the loop to cool for a few seconds before using it.

2. Inoculum Collection

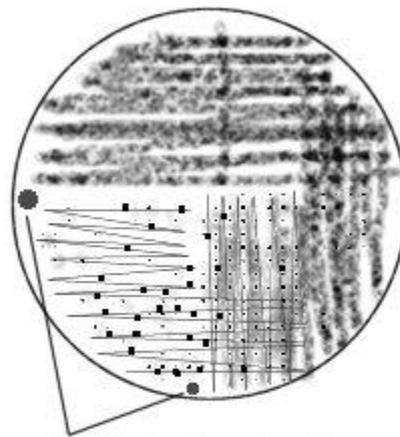
a. From nutrient broth:

- Hold the culture tube in one hand and remove the cap using the little finger of the other hand holding the sterile loop.
- Briefly flame the tube's opening to prevent contamination.
- Insert the loop into the broth to collect a small amount of culture.
- Reflame the tube's opening and replace the cap.



b. From Petri dishes:

- Slightly lift the lid of the Petri dish to insert the sterile loop.
- Cool the loop by touching an area of the agar without growth.
- Gently scrape a small colony from the agar surface.
- Close the Petri dish immediately after sampling.



contaminants not on streak lines

well-isolated colonies in 3rd section

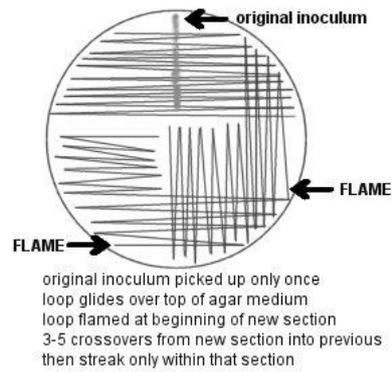
3. Transferring the Inoculum to a Sterile Medium

a. Nutrient broth:

1. Hold a sterile nutrient broth tube and remove its cap as previously described.
2. Briefly flame the tube's rim.
3. Dip the inoculation loop containing the inoculum into the broth and gently swirl.
4. Flame the tube's rim again, replace the cap, and sterilize the loop.

b. Agar plates (streaking):

1. Slightly lift the lid of the sterile agar plate.
2. Perform **quadrant streaking** by dragging the loop in four successive sections on the agar surface.
3. Sterilize the loop after streaking each quadrant.

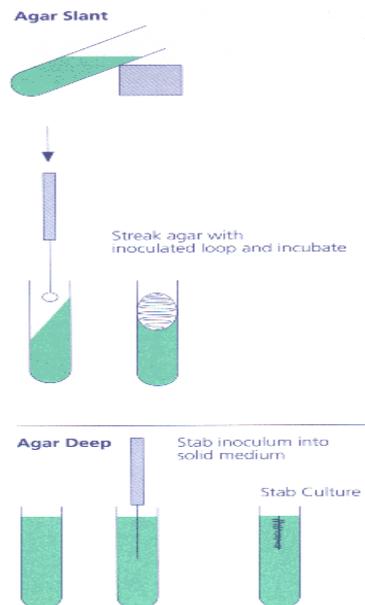


c. Slant agar:

1. Insert the inoculation loop into the sterile slant tube.
2. Gently streak the agar surface in an upward motion.

d. Deep agar (stabbing):

1. Use a sterile inoculation needle.
2. Insert the needle vertically into the center of the agar until halfway through the depth.



4. Incubation

- Place the inoculated tubes and plates in an incubator at the optimal temperature for **E. coli** (37°C).
- Incubate for 24 hours.

Questions :

1. What is the primary objective of preparing a culture medium in microbiology? Why is it essential to prepare specific media for different types of microorganisms?
2. List the components used to prepare the liquid nutrient medium and explain their respective roles in microorganism growth.
3. What is the fundamental difference between solid and liquid culture media? Why would you choose a solid medium over a liquid one for a specific culture?
4. What is the role of agar in preparing solid media? Why is agar preferred over other gelling agents?
5. Describe the process of preparing both a liquid medium and a solid medium. What are the common steps between the two methods?
6. Explain the role of the autoclave in the preparation of culture media. Why is it necessary to sterilize the media before using them to culture microorganisms?
7. Why is it important to adjust the pH of culture media? How would you adjust the pH of the prepared nutrient medium?
8. What is the difference between a slant, stab, and Petri plate preparation? In what case would you use each type of preparation?
9. What is the effect of the position of the tubes (inclined for slants and upright for stabs) on microorganism growth, particularly regarding oxygen requirements?
10. What are the main precautions to take during the preparation and sterilization of culture media to avoid contamination and ensure sterility?
11. Why is it important to sterilize the inoculation loop after each step?
12. What is the difference between quadrant streaking and slant agar inoculation?
13. What happens if the loop is not cooled before collecting the inoculum?
14. What are the risks of contamination during transfers, and how can they be avoided?
15. Why do we use different techniques (streaking, stabbing, etc.) for different media?
16. What is the optimal growth temperature for *E. coli*, and why?
17. What growth patterns can be observed in nutrient broth?
18. What does "pure culture" mean, and how can you verify it?
19. Why is it necessary to flame the opening of the tubes before and after use?
20. Which technique would you use to isolate a bacterial strain from a mixed sample, and why?