

Preparation of culture media

Culture media (or growth media) are nutrient-rich liquid or solid or semisolid substances (like agar or broth) designed to support the growth of microorganisms in vitro.

OR

is a solid , liquid and semisolid preparation containing all the nutrients required by microorganisms for growth, survival, and reproduction.

Common ingredients of culture media :

- Peptone- source of carbon and nitrogen.
- Beef extract- source of amino acids.
- Yeast extract- source of, vitamins, minerals.
- Distilled water

- Agar- solidifying agent.

The importance of using culture media /Purposes for which it is used:

Isolation-1 عزل: To isolate pure culture

Enumeration -2 عدد

3- Identification تشخيص / Differentiation تمييز: colony type, color, shape,

To differentiate between different colonies .

4- To test antibiotic sensitivity.

Some examples of commercial culture media such as:

Nutrient Agar /Broth

MacConky Agar/broth

Mannitol salt agar

EMB Agar

Potato Dextrose Agar

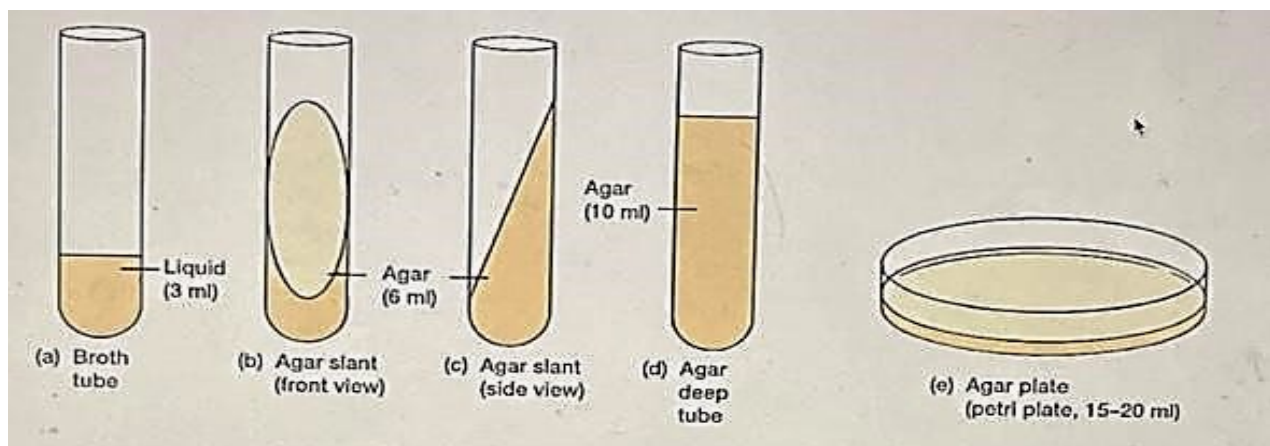
What is Agar-Agar?

a vegan alternative to gelatin ,but it differs from gelatin in that it cannot be biologically broken down .

Its a natural, odorless, and tasteless, often used in cooking as a thickener in food and a solidifying agent in scientific labs.

Common uses include : jellies, dairy products, lab petri dishes, and pharmaceutical capsules

Source: Extracted from seaweed, primarily red algae.



Different forms of culture media in (plane tube , petri dish)

How to prepare culture medium ?

Step-by-Step Preparation

1. **Calculate and Weigh:** Weigh the culture medium powder according to the instructions on the medium container (e.g., g/L).
2. **Dissolve Ingredients & Heating:** Add the powder to distilled or deionized water ,Heat the mixture to boiling and ensure the powder is completely dissolved.
3. **Adjust pH of the medium:** Check the pH using a pH meter and adjust to the required level (usually 6.5–7.5 for bacteria, 5.0–6.0 for fungi)
4. **Sterilization in an autoclave:** the medium in flasks, seal with cotton plugs or caps, and sterilize in an autoclave at 121°C for 15 min at 15lbs.
5. **Cool** the sterilized medium to 45°C.
6. **Pour** the medium into a Petri dish in sterile conditions and allow it to solidify.

7. **Storage:** Store solidified plates inverted in the fridge to prevent moisture. In the incubator, to minimize evaporation and prevent condensation on the surface.

جميع المعلومات اللازمة لتحضير الوسط تكون مكتوبة على العلبة الخاصة بالوسط وأيضاً طريقة التعقيم .

Dissolve 28g in 1 liter

28 g → 1 liter (1000 ml)
X g → 50 ml

مثلاً يكتب ونريد تحضير 50 ml فقط

$$X = 28 \times 50 \div 1000 = 1.4 \text{ g}$$

١. نقوم بوزن الكمية المطلوبة (مثلاً في المثال السابق 1.4 g) في كمية معلومة من الماء المقطر (مثلاً 50 ml)

٢. نقوم بإذابة الكمية في الماء المقطر ، ثم نقوم بتعقيم الوسط .

Q /Prepare a quarter liter of MacConkey agar; If you know that 51.5 g of the powder dissolves in 1 liter of distilled or deionized water?

The weight (g)

The volume (ml)

51.5 gm

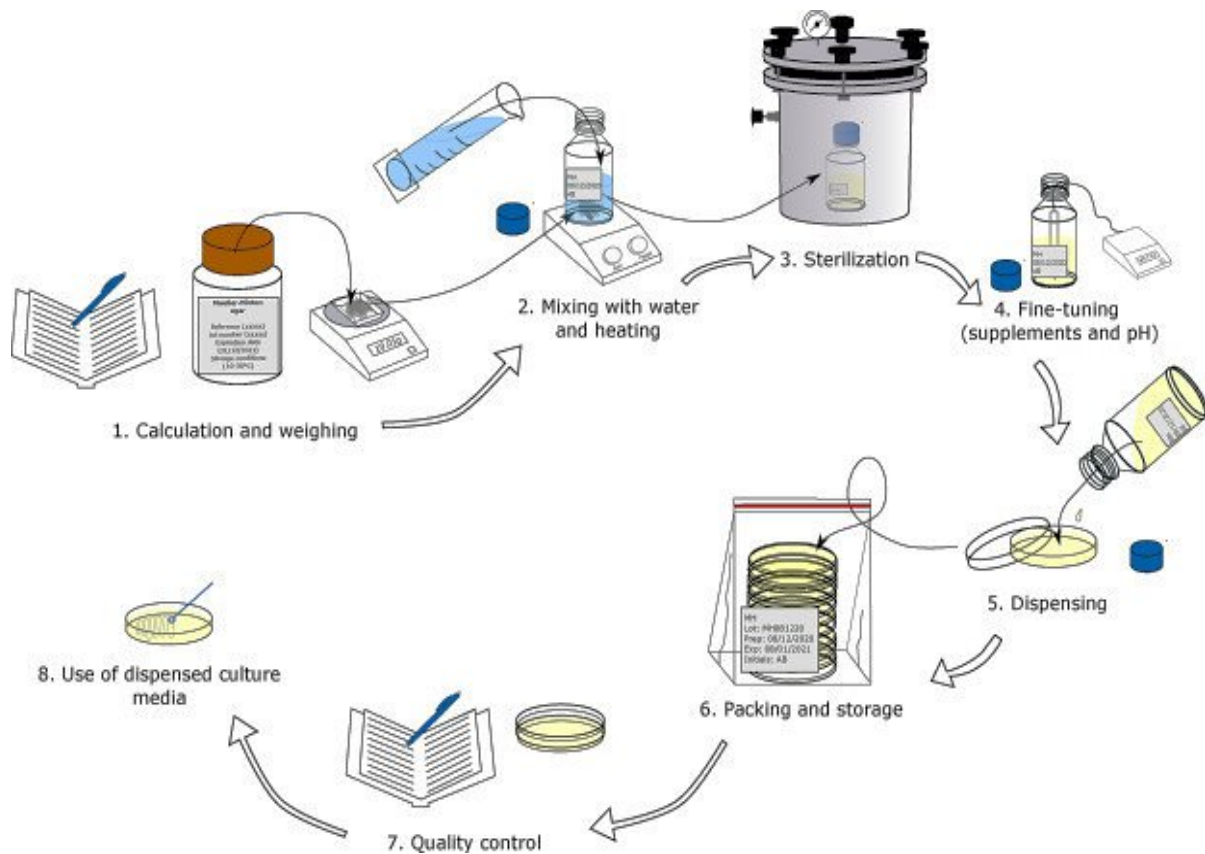
1000 ml

X

250 ml

$$X = \frac{51.5 \times 250}{1000} = 12.875 \text{ gm}$$

1000



Classification of Culture Media

A. Based on consistency (Physical forms):

1. Liquid media (Broth): Liquid medium is also called broth.

Characteristic: Does not contain any solidifying agents (Agar or Gelatin).

Put in tubes such as screw tube (A plain tube).

The Purpose: growth large number of bacterial cells.

Signs of growth: turbidity, gas, color, sediment, membrane & bubbles.

Examples: include (Nutrient broth, brain heart infusion broth.

2. Solid media (Agar) : solidify medium.

Characteristic: contains the solidifying agent (1.5 - 2 % agar).

Put in tubes & plates .

The Purpose: In Petri dishes

Isolation of colonies by forms

To study colony characteristics.

To get bacterial pure cultures.

Signs of growth: colonies

Examples: include (Nutrient agar, blood agar.)

3.Semisolid media:

Characteristic: contains a few agar 0.5% or less of agar.

Put in tubes such as screw cap tube (A plain tube)

The Purpose: Determining of microbial motility.

Determining the bacteria's oxygen requirement.

Signs of growth: observed via turbidity, spreading growth away from the stab line, or color changes in the media.

Examples: include Mannitol Motility Medium.

So what is a bacterial colony?

A bacterial colony is a group of bacteria derived from the same mother cell. This means that a single mother cell reproduces to make a group of identical cells, and this group of cells form a mass, which is known as a bacterial colony.

Diameter Measurement of colony : Use a ruler, to measure the diameter of colonies in millimeters.

