

Lab 1: Preparation of Culture Media and Physiological Water

Culture media are biological or chemical preparations that allow the growth, isolation, purification, and identification of microorganisms. They must provide all the necessary nutrients (sources of carbon, energy, minerals, growth factors) and offer favorable physicochemical conditions (pH, isotonicity, redox potential).

Several types of culture media can be distinguished:

- **According to composition:** complex (natural), semi-synthetic, or synthetic media.
- **According to use:** basic, enriched, enrichment, selective, elective, identification, preservation, and transport media.
- **According to consistency:** liquid, solid (agar), or semi-solid media.

These different types of media make it possible to adapt the culture conditions to the specific needs of the microorganisms studied.

1. Purpose of the Practical Work

- To learn about the different types of culture media used in microbiology.
- To give an example of the preparation of a culture medium and a sterile diluent: physiological water.

2. Steps for Preparing a Culture Medium

The preparation of a culture medium in microbiology follows a precise procedure aimed at obtaining a sterile product suitable for microbial growth.

2.1. Equipment and Materials Used

The equipment includes glassware (Erlenmeyer flasks, graduated cylinders, Petri dishes, screw tubes, racks, watch glass, spatula), a magnetic stirrer with heating, magnetic bars, a precision balance, and an autoclave.

The dehydrated media used are **nutrient broth (BN)** and **nutrient agar (GN)**, prepared with distilled water.

2.2. Main Steps of the Protocol

1. **Weighing:** Measure the amount of dehydrated medium corresponding to 250 mL of distilled water according to the manufacturer's instructions.
2. **Dissolution:** Add the powder to distilled water, stir vigorously, and gently heat with a magnetic stirrer until completely dissolved.
3. **Light Boiling:** Maintain a light boil for about one minute while controlling the temperature to prevent overflow.
4. **Sterilization:** Place the preparations in the autoclave at 121 °C for 20 minutes.
5. **Pouring:** After cooling below 60 °C, pour the media in front of a Bunsen burner into sterile Petri dishes and screw tubes, then allow the agar to solidify.
6. **Labeling:** Indicate the name of the medium, date of preparation, and other necessary information.
7. **Storage:** Store the prepared media at low temperature to ensure their stability and shelf life.

3. Preparation of the Diluent (Physiological Water)

Physiological water, or **0.9% saline solution**, is a solution containing 9 g of sodium chloride (NaCl) dissolved in 1 liter of sterile water, corresponding to a 0.9% concentration.

It is called *physiological* because its salt concentration is similar to that of human body fluids, such as blood plasma. This isotonicity prevents dehydration or osmotic shock of cells, which is why it is frequently used in medicine and biology.

Main Uses of Physiological Water:

1. Intravenous rehydration
2. Wound washing
3. Eye or nasal rinsing
4. Dilution of drugs before injection
5. Maintenance medium for cultured cells

In the microbiology laboratory, it is mainly used as a **sterile diluent** for microbial suspensions or sample washing, as it preserves the osmotic balance of cells.

3.1. Materials and Reagents Required

- Pure sodium chloride (NaCl) (analytical grade)
- Distilled or demineralized water
- Precision balance
- Beaker or graduated flask (1 L)
- Magnetic stirrer (optional)
- Sterile bottles for storage
- Autoclave (for sterilization if necessary)

3.2. Preparation Protocol for Physiological Water (0.9% Saline Solution for 1 L)

1. **Weighing NaCl**
Weigh 9 g of NaCl using a precision balance.
2. **Dissolution**
Pour about 800 mL of distilled water into a clean beaker, then add the NaCl.
3. **Stirring**
Mix until the salt is completely dissolved (magnetic stirring may be used).
4. **Volume Adjustment**
Add distilled water until the total volume reaches 1 L.
5. **Sterilization (if necessary)**
If the solution is intended for biological or medical use, sterilize it in an autoclave at 121 °C for 15–20 minutes.
6. **Storage**
Store in clean or sterile, tightly closed bottles.
 - If sterilized: store at room temperature or in a refrigerator.
 - If not sterilized: use immediately after preparation.