

## **Lab 2 : Dilution Techniques and Preparation of Solutions under Aseptic Conditions**

### **1. Objective :**

Students will learn how to perform serial dilutions and prepare sterile solutions using methods that ensure the absence of contamination.

### **2. Materials:**

#### **• Equipment:**

- Precision balance
- Magnetic stirrer or vortex
- Bunsen burner
- Graduated pipette (10 mL)
- Micropipettes (1 mL) with sterile tips
- Wash bottles containing sterile distilled water, bleach, and 70% alcohol
- Paper towels

#### **• Glassware and Tubes:**

- Test tubes containing 9 mL of sterile physiological water
- Empty sterile test tubes

#### **• Chemicals and Solutions:**

- Ampicillin (100 mg/mL solution)

#### **• Samples:**

- Raw milk
- Baker's yeast (1 g of dry yeast / 10 mL sterile water)

### **3. Serial Logarithmic Dilution Techniques:**

Serial dilutions are used to progressively reduce the concentration of a sample in a controlled manner.

#### **3.1. Procedure:**

##### **• Preparation of dilution tubes:**

Fill several sterile test tubes with 9 mL of sterile diluent (physiological water).

##### **• Aseptic transfer:**

Use a micropipette with a sterile tip to take 1 mL of the initial sample (e.g., raw milk) and add it to the first tube containing 9 mL of diluent.

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This results in a  $10^{-1}$  dilution.

Mix gently by aspirating and expelling the content using the micropipette.

• **Successive dilutions:**

Take 1 mL from the  $10^{-1}$  dilution and add it to the second tube containing 9 mL of diluent to obtain a  $10^{-2}$  dilution.

Repeat this process to obtain  $10^{-3}$ ,  $10^{-4}$ , etc.

Use a new sterile tip for each transfer to avoid contamination.

#### 4. Dilution Calculations and Solution Preparation:

Ampicillin is a widely used antibiotic in microbiological control to assess bacterial sensitivity or resistance.

##### 4.1. Preparation of Ampicillin Solution:

To prepare 200  $\mu$ L of a 25 mg/mL solution from a 100 mg/mL stock, use the dilution formula:

$$C_1 \times V_1 = C_2 \times V_2$$

$$V_1 = (C_2 \times V_2) / C_1 = (25 \text{ mg/mL} \times 0.2 \text{ mL}) / 100 \text{ mg/mL} = 0.05 \text{ mL (or 50 } \mu\text{L)}$$

• **Preparation:**

- Take 50  $\mu$ L of the concentrated ampicillin solution using a sterile micropipette.
- Add 150  $\mu$ L of sterile solution (distilled water or physiological saline) into a sterile test tube.
- Mix gently, avoiding air bubbles.

• **Storage:**

- Label the tube with concentration, date, and initials.
- Store the ampicillin solution according to recommendations (usually at 2–8 °C).

#### 5. Waste Management:

• **Non-contaminated materials:**

Dispose of paper and other non-contaminated waste in the regular trash bin.

• **Contaminated materials (tips, tubes):**

Use special containers for biological waste.

Disinfect all materials before disposal according to laboratory protocols (e.g., by soaking in bleach).