

Antibiotic Technology Laboratory

Lab 3: Prepare antibiotics discs

Antibiotics are typically supplied as powders or tablets. Pure antibiotics should be obtained from commercial sources, and injectable formulations should not be used. The powdered antibiotics must be precisely weighed and dissolved in appropriate diluents to achieve the required concentrations, using sterile glassware.

For the preparation of dried filter paper discs, Whatman No. 3 filter paper is used to produce discs approximately 5–8 mm in diameter. The discs are placed in Petri dishes and sterilized in a hot-air oven. Antibiotics are applied to the discs using a 20-gauge wire loop with a 2 mm diameter, which delivers 0.01 mL (10 μ L) of antibiotic solution to each disc.

Procedure:

- 1- Prepare discs were then autoclaved at 15lbs pressure for 30 minutes.
- 2- Standard antibiotic powders were obtained commercially. Known weight of antibiotic powder was dissolved in sterile distilled water to obtain the stock solution.
- 3- The stock solution was diluted at the time of disc preparation to obtain the working solution.
- 4- A paper disc of 6mm diameter can absorb 0.001ml or 10 μ l of solutions. The concentrations of antibiotic solutions were expressed in μ g/ μ l.
- 5- **Impregnation of the discs:** Sterile discs were placed in petri dishes approximately 5mm apart. Using a mechanical pipette, a fixed volume of 10 μ l was loaded on each disc one by one, taking precautions that the tip was in slight contact with the disc.
- 6- **Drying and Storage:** Without covering the petri dishes, the discs were allowed to dry in a clean incubator at 37°C for 10-15 min. Now it's ready to use in sensitivity test. This method ensures uniform antibiotic loading and reliable results in antimicrobial susceptibility testing.

Key Considerations:

- **Sterility:** All materials must be sterile to avoid contamination. Use forceps to handle discs.
- **QC:** Store in the dark and allow to warm to room temperature before opening to avoid moisture.

Preparation of Antibiotic Discs

1. Antibiotic Preparation



Dissolve antibiotic powder in sterile diluent.

2. Filter Paper Discs



Sterilize 5~8 mm discs in Petri dish.

3. Impregnation of Discs



Apply 10 µL of antibiotic per disc.

4. Drying of Discs



Let discs dry at room temperature.

5. Storage

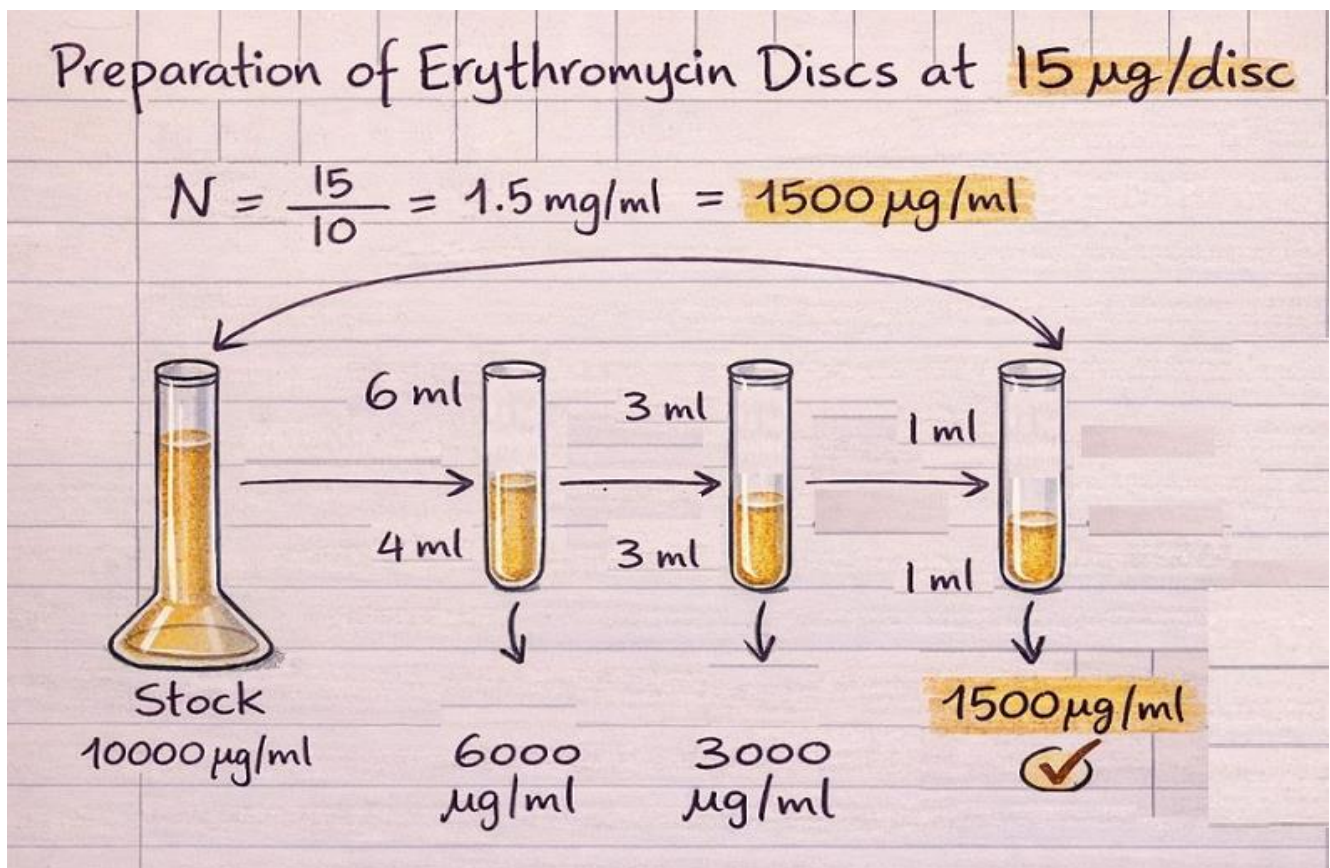


Store in airtight container at low temperature.

Calculate of concentrations of antibiotic:

required concentration = Drop volume $\times$ Target concentration

1) Prepare 15 μg /disc = Erythromycin



So 1500 $\mu\text{g/ml}$ gives disc from erythromycin with concentration 15 μg /disc by adding 10 μl from 1500 $\mu\text{g/ml}$ on to filter paper disc.

2) Ampicillin = 10 µg \ disk

1- Ampicillin = 10 µg \ disk

$$10 = N \times \frac{10}{10000}$$

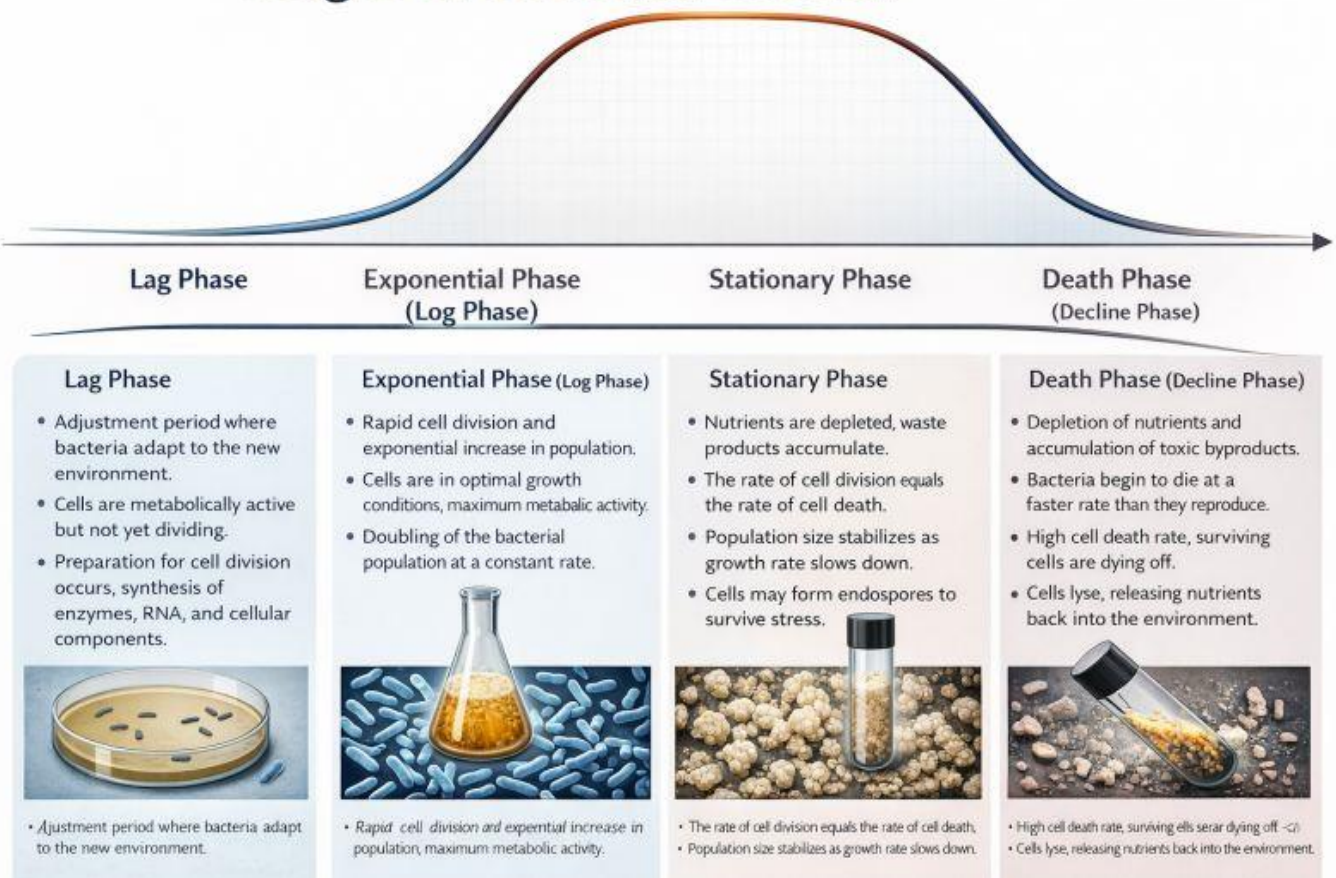
$$N = \frac{10}{0.01} = \mu\text{g/mL } 1000$$



Homework:

- 1) Prepare Amikacin with concentration 35 µg \ disk
- 2) Prepare tetracycline with concentration 45 µg \ disk by using stock solution 500 mg/ml.
- 3) Prepare Gentamycin with concentration 70 µg \ disk by using stock solution 500 mg/ml.

Stages of Bacterial Growth



Linking Bacterial Growth Phases to Antibiotic Susceptibility

- **Lag phase**
 - Bacteria are metabolically adjusting
 - Limited cell division
 - Low susceptibility to most antibiotics
- **Log (Exponential) phase**
 - Rapid cell division and high metabolic activity
 - Highest susceptibility to antibiotics
 - Most antibiotics (e.g., β -lactams, aminoglycosides) act best here
 - Antibiotic Susceptibility Testing (AST) is performed using bacteria in this phase
- **Stationary phase**
 - Nutrient depletion and waste accumulation
 - Reduced metabolic activity

- Decreased antibiotic effectiveness
- Presence of tolerant and persister cells
- **Death phase**
 - Decline in viable bacterial cells
 - Antibiotic activity is no longer clinically meaningful

Note: Antibiotic susceptibility is not fixed—it depends on the bacterial growth phase. Understanding this relationship is essential for correct laboratory testing and clinical interpretation

The Circle That Changed Microbiology: Story of Agar Plates

🔬 Ever noticed that agar plates are always circular and never rectangular? There's a fascinating scientific reason behind this design choice!



The circular shape allows agar to spread evenly during pouring, ensuring a smooth and uniform surface for microbial growth.

🌍 Unlike rectangles with corners that dry out quickly, circles maintain **consistent moisture** and prevent **uneven results**.



➔ **Diffusion** of microbes, antibiotics, or chemicals happens uniformly in all directions, giving more reliable data.

🧪 The round edges also make plates easier to **stack, store, and sterilize**—boosting lab efficiency.



🧪 In essence, the simple circle is not just a shape, but a smart design that ensures accuracy, reproducibility, and convenience in microbiology. Sometimes, science proves that the simplest shapes create the most powerful impact.