

Testing Antibiotics

Introduction

Bacteria are prokaryotic cells may cause disease in certain organisms. Antibiotics are compounds that specifically target prokaryotic cell features and hence treat bacterial infections without harming the host organism. A Mastring is a circular device consisting of several antibiotic susceptibility discs that is used for testing the sensitivity of any bacterial strain against a variety of antibiotic compounds.

When the Mastring is placed on an agar plate covered with bacteria, the antibiotics in each disc will diffuse into the agar. If an antibiotic is effective against the bacteria, it creates a clear circular area of no growth around that lobe, known as the zone of inhibition (ZOI). The size of the zone of inhibition will indicate the relative susceptibility of the bacteria to the agent (larger ZOI means more susceptibility).

Aim

To determine the susceptibility of a non-pathogenic strain of bacteria (*E. coli*) to a variety of different antibiotics contained within a Mastring device.

Materials

- Mastring disc
- Nutrient agar plate
- *E. coli* broth
- Bench paper
- 1 ml syringe
- Spreader
- Sterile forceps
- Disinfectant
- Incubator

Method

1. Place the bench paper and materials on the workspace
2. Using the syringe, add 0.5ml of bacteria to the agar plate
3. Use the spreader to create a uniform bacterial 'lawn'
4. Using the forceps, place the Mastring device on the agar
5. Place the lid on the agar plate and seal sides with tape
6. Place plate in the incubator at 25°C (agar side facing up)
7. Place all utensils that contacted bacteria in disinfectant
8. After 72 hours, measure the annular radius of the zone of inhibition with a ruler or a digital calliper (see figure 1)

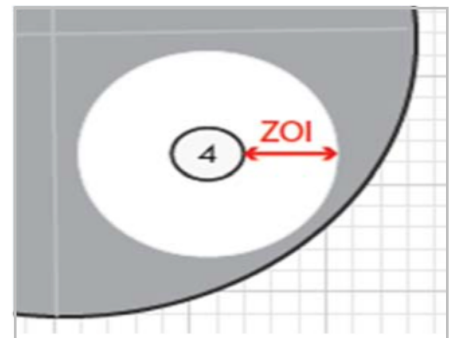


Figure 1: Annular radius

Results

Antibiotic	Annular radius	Antibiotic	Annular radius
Ampicillin (AP = 10µg/disc)		Streptomycin (S = 10µg/disc)	
Chloramphenicol (C = 25µg/disc)		Sulphatriad (ST = 200µg/disc)	
Penicillin (PG = 1 unit/disc)		Tetracycline (T = 25µg/disc)	

Discussion

1. Describe the susceptibility of *E. coli* to the different antibiotics.

2. Suggest one weakness of using annular radius to measure antibiotic susceptibility.

3. Outline a limitation of the experimental design that impacts the validity of any conclusions.

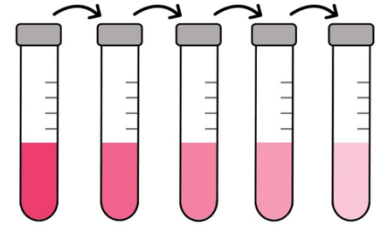
4. Discuss the benefits of using a digital calliper instead of a ruler to measure bacterial growth.

5. Identify one prokaryotic feature that could be targeted by an antibiotic.

Extension

A minimum inhibitory concentration (MIC) is the lowest antibiotic concentration considered effective against a bacterium. In standardised microbiology testing (the CDS Antibiotic Susceptibility method) the criterion for effectiveness is set at a ZOI of 6mm or more. A ZOI below 6mm is not inhibitory. The MIC can be determined by plotting the ZOI against antibiotic concentration and interpolating a value.

Different antibiotic concentrations can be created via serial dilutions of a stock solution. If half of the stock solution is transferred to a new test tube and combined with an equal amount of water, the concentration is halved (50%). Repeating the process with the diluted stock creates further dilutions – each with half the concentration of the last dilution.



Design a methodology to determine the MIC for ampicillin susceptibility. You have 1ml of ampicillin.