

Experiment 2: Determination of minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC) and bacterial susceptibility to antibiotics

Task A: Minimal Inhibitory Concentration (MIC) (Broth Tube Dilution Method)

Protocol 2.1 (Week 4)

1. Number sterile universal tubes 1 to 9.

All of the following steps are carried out using aseptic technique.

2. Add 2 mL of antibiotic X solution (100 µg/mL) to the first tube

3. Add 1 mL of sterile broth to all other tubes

4. Transfer 1 mL from the first tube to the second tube

5. Using a new pipette tip, mix the contents of this tube and transfer 1 mL to the third tube

6. Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette

7. Remove 1 mL from tube 8 and discard it. The ninth tube, which serves as a control, receives no antibiotic

8. Dilute the *E. coli* and *Staphylococcus aureus* cultures (OD ~0.5) by aseptically pipetting 125 µL of each into 10 mL of Mueller-Hinton broth (separately).

9. Add 1 mL of the diluted culture suspension to each of the tubes. The final concentration of antibiotic is now one-half of the original concentration in each tube

10. Incubate all tubes at 35 °C overnight

Protocol 2.2 - In the next laboratory session (Week 6)

11. Examine tubes for visible signs of bacterial growth. Record your observations in Table 2.1. The highest dilution without growth is the minimal inhibitory concentration (MIC). (See the section for Lab 3 Data Analysis.)

12. Plate 500 µL of the cultures that showed no or very little growth on Mueller-Hinton plates and incubate as above.

Table 2.1. MIC of Antibiotic X. + indicates growth of *E. coli* or *S. aureus*; 0 indicates no growth.

| Tube Number | [Antibiotic X] | <i>E. coli</i> growth? | <i>S. aureus</i> growth? |
|-------------|----------------|------------------------|--------------------------|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |

Protocol 2.3 - In the following laboratory session (Week 8)

13. Count the colonies on your Mueller-Hinton plates and record your observations in Table 2.2. Calculate the MBC values. (See the section for Lab 3 Data Analysis.)

Table 2.2. MBC of Antibiotic X. TNTC = Too Numerous To Count; TFTC = Too Few To Count

| Tube Number | <i>E. coli</i> colonies | <i>S. aureus</i> colonies |
|-------------|-------------------------|---------------------------|
| | | |
| | | |
| | | |
| | | |
| | | |

Task B: Antibiotic Disk Susceptibilities (Kirby-Bauer Disk-Diffusion Method)

Protocol 2.4

1. Place a sterile cotton swab in the *E. coli* or *Staphylococcus aureus* suspension (OD ~0.5) and remove the excess fluid by pressing and rotating the cotton against the inside of the tube above the fluid level. The swab is streaked in at least three directions over the surface of the Mueller-Hinton agar to obtain uniform growth. A final sweep is made around the rim of the agar. Be sure to streak for confluency. Prepare 3 plates per pair of students for each organism. This experiment will be performed in triplicate (6 Petri dishes in total – 3 for each organism)

2. Allow the plates to dry for five minutes

3. Using sterile forceps, place the pre-made (commercial) disks containing the known antibiotics on the plate (Figure 1). Add 20 μL of antibiotic X to a paper disk and add this to the plate as well. Be sure to press the disks firmly into the plate so that they will remain in place when the plates are inverted.

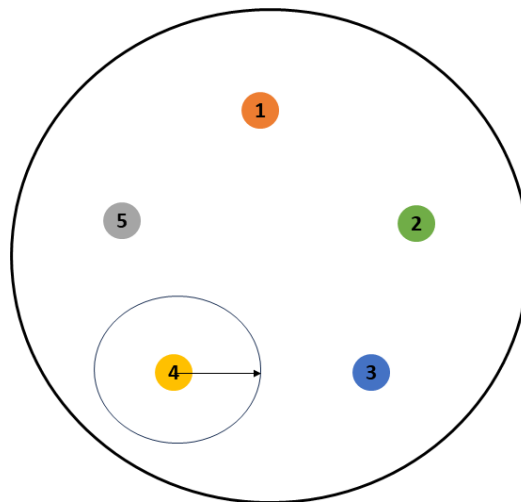


Figure 1. Schematic showing placement of antibiotic disks on a Petri dish (top view). 5 disks (small coloured circles; the different colours represent different antibiotics) impregnated with antibiotics are placed roughly equidistant to one another on an agar plate spread with a lawn of bacterial cells. As shown by the prospective zone of inhibition surrounding disk 4 (circle with radius), disks should not be placed too close to the edge of the plate.

Protocol 2.5 - In the next laboratory session (Week 6)

4. After the plates have been incubated overnight at 35 °C, measure the diameter of the zone of growth inhibition around each disk to the nearest whole mm. Examine the plates carefully for well-developed colonies within the zone of inhibition (spontaneous resistant mutants).

5. Determine if the strain is resistant, intermediate, or susceptible to the antibiotics tested