Department of biology

((Microbiology))

stage 2

7th lecture

Types of dilutions used in microbial experiments

By

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Minimal Inhibitory Concentration (MIC):

The lowest concentration of antibiotic that inhibit growth of bacteria also called tube dilution method, The lowest concentration (highest dilution) of test agent preventing appearance of turbidity (growth) is considered to be the minimal / minimum inhibitory concentration (MIC). At this dilution, the test agent is bacteriostatic, and this Achieved by

1. Tube dilution methods.
2. Agar dilution method.

Advantages of MIC:

1- Dose to inhibit MIC is a better predictor of appropriate drug.

2- Use a lower concentration which reach to the body fluid, able to use a less expensive but still effective antibody

3- Decrease chance of toxic effects on the patient's systems/organs.

Tube dilution methods:

1- In this method, we use sterile Mueller Hinton broth.

2- We make 2-folds dilution of antibiotics in the broth i.e. 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml and so on.

3- Then we add broth culture (0.1ml) of test organism to the prepared dilutions.

Procedure:

1- Arrange sterile test tubes in a rack and dispense 1 ml of sterile Mueller-Hinton broth into each tube.

2- Prepare a stock solution of the antimicrobial agent and diluted according to your serial dilution to your test. The value of MIC according to CLSI is : (0.5,1,2,4,8,16,32,64,128,256,512,1024).

3- Start to do the serial dilution according to your value. Make twofold dilution of the antimicrobial agent by transferring 1 ml of the solution from the solution from the first tube to the second one.

4- Continue to make serial dilution till the entire range is covered. The last tube concentration is 0.5µg/ml and let two tube one of them without add anything as it act as growth control (positive control). In addition, the second just broth and antibiotic without inoculated by bacteria (negative controle).

5- Adjust the turbidity of bacterial suspension overnight growth by 0.5 McFarland standard.

6- Unify the sizes in all the tube according to the less size of tubes.

7- Add 0.1 mL of this suspension to each dilution and control tube.

8- Incubate the tubes in 16-18hr at 37 ºC.

9- Results by compare with the control tube read all tubes for visible growth record the result. The lowest concentration with no visible growth is the MIC for the test organism.