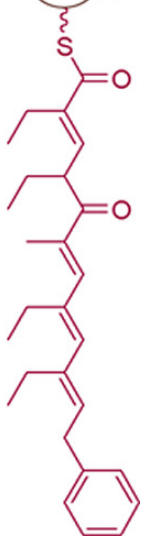
MIC Determination by Microtitre Broth Dilution Method



Materials

Suitable growth liquid cultures (A_{600} 0.07 to 0.1 or 0.5 Macfarland)
Sterile 96-well microtitre plates
Sterile petri dishes
Test antibiotic
Sterile Müeller Hinton Broth
Sterile saline solution (for bacteria dilution)

- Grow the chosen strains to the right A600 or Macfarland standard.
- Prepare the desired test antibiotic solution then dilute in Müeller Hinton broth to 2X the top concentration desired in the test (e.g. if the highest test concentration is 256µg/mL, dilute to 512µg/mL).
- Dispense 100µL of Müeller Hinton broth into all wells of the microtitre plate.
- Pipette 100µL of 2X antibiotic solution into the wells of column 1.
- Using the pipette, mix the antibiotics by sucking up and down 5-8 times.
- Withdraw 100µL from column 1 and add to column 2. Mix by sucking and transfer to column 3. Repeat the procedure to column 10.
- Discard 100µL from column 10.
- Prepare the bacteria inoculum to the size of 10^4 to 10^5 CFU/mL, by diluting it using broth or saline solution.
- Pour 5µL of bacteria into wells in columns 1 to 11. Do not add bacteria into column 12 (this will be the broth sterility control and blank for reading plates in a scanner)
- Incubate the plates in 37°C or other desired temperature for 12-18 hours.
- The reading of results could be made manually using a black card or electronically with an ELISA reader.

NOTE: different drugs could be tested in different rows of the same plate, but avoid putting bacteria together to prevent cross-contamination.