

## Script to prepare video clip(s)

Manuscript Title:	Microwave assisted synthesis, biological evaluation and molecular docking studies of 4-nitrocinnamide analogues		
Video title	Minimum inhibitory concentration (Broth dilution method)		
ID: 32116			
Material:	Duration:	Date:	Location: Your Lab

### Position of your smart phone



Take the video using your smart phone in horizontal position instead of vertical

Smart phone on the tripod reduces the shaking of picture

### Preparation of Culture Media

Materials and Reagents

Laminar hood; Electronic balance; Glass bottle; Mueller-Hinton agar; Distilled water; Autoclave; Petri dish; Calcium; Magnesium; Aminoglycoside; Tetracycline

Action

Subtitle

- 1** Show the Mueller-Hinton broth. Close view
- 2** Add calcium (20 to 25 mg/L) and magnesium (10 to 12.5 mg/L) to the broth
- 3** Aminoglycoside is used when *Pseudomonas aeruginosa* isolate is tested. In case of other bacteria, tetracycline is used
- 4** To minimize evaporation and deterioration of antimicrobial agents, tubes should be tightly capped and stored at 4 to 8°C until needed
- 5** With most agents, the dilutions should be used within 5 days of preparation or as long as quality control ranges are maintained

### Inoculation Procedures

Materials and Reagents

Laminar hood; Colonies; Mueller-Hinton broth; Ciprofloxacin; Test compound; Test tubes

Action

- 1** Show the isolated colonies of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* or *Staphylococcus aureus* to test Close view
- 2** Combine 4 to 5 colonies and culture overnight in Mueller-Hinton broth
- 3** Add Mueller-Hinton broth with appropriate dilution series of either ciprofloxacin or test compound (200, 100, 50, 25, 12.5, 6.25, 3.125, and 0 µg/mL) to sterile 13- x 100-mm test tube
- 4** The final inoculum for broth dilution testing is  $5 \times 10^5$  CFU/mL
- 5** Isolates are inoculated into a broth that will support good growth and incubated until turbid

<b>6</b>	The turbidity is adjusted to match that of a 0.5 McFarland standard (approximately $10^8$ CFU/mL)
<b>7</b>	A portion of the standardized suspension is diluted approximately 1:100 (to $10^6$ CFU/mL) with broth or saline
<b>8</b>	When 1 mL of this dilution is added to each tube containing 1 ml of CAMHB containing the 2× concentrated drug dilutions, a final inoculum of $5 \times 10^5$ CFU/mL is achieved.
<b>9</b>	Broth not containing an antimicrobial agent is inoculated as a control for organism viability (growth control).
<b>1</b>	All tubes should be inoculated within 30 min of inoculum preparation, and an aliquot of the inoculum should be plated to check for purity.

### Incubation

Materials  
and  
Reagents

[Laminar hood; Test tubes](#)

Action

Tubes are incubated in ambient air at 35°C for 16 to 20 hours before MICs are determined.

Some tube shows visible growth whereas others do not

[Close  
view](#)

### Interpretation

Materials  
and  
Reagents

Action

Compare the amount of growth in the wells or tubes containing the antimicrobial agent with the amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set of tests when determining the growth end points

For a test to be considered valid, acceptable growth ( $\geq 2$  mm button or definite turbidity) must occur in the growth-control well