Minimal Inhibitory Concentration (MIC)

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Abstract

This protocol is modified from Wiegand and colleagues protocol[1] to fit the conditions of our experiments.

[1]Wiegand, Irith, Kai Hilpert and Robert E W Hancock (2008). \Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". I: Nat Protoc 3.2, s. 163{75

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Protocol

Prepare medium

Step 1.

Müller-Hinton Agar and Broth

Plate the agar on plates.

Prepare the bacterial isolates

Step 2.

Streak the bacteria onto agar plates without inhibitor.

Step 3.

Incubate plates for 18-24h at 37ºC

Prepare the bacterial isolates

Step 4.

For each isolate, select three to five morphologically similar colonies from the agar plates and transfer them to a glass tube with 5ml MH-Broth.

Incubate for 18-24h at 37ºC

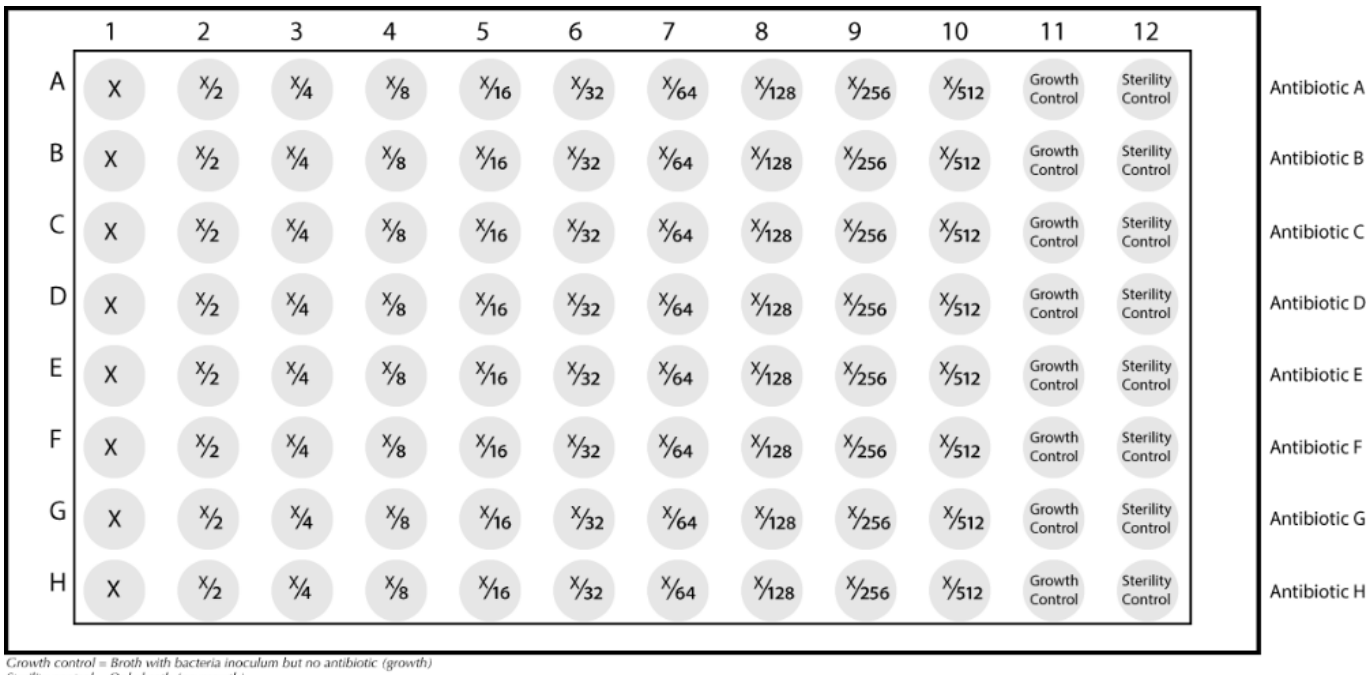
Step 5.

Measure OD450 on cultures from step 4, and make dilutions in x mL MHB to OD450 = 0,01

Prepare the ELISA microplate

Step 6.

Use the setup shown in the following figure.



Growth control = Broth with bacteria inoculum but no antibiotic (growth)
Sterility control = Only broth (no growth)

Step 7.

Pipette 50µl of MHB into column 2-11

Step 8.

Add 100µl MBH into column 12

Step 9.

Add 100µl of the antibiotic into column 1

Step 10.

Withdraw 50µl from each well in column 1, add this to the corresponding wells in column 2. Mix by pipetting up and down 4-6 times.

(this makes column 2 a twofold dilution of column 1)

Step 11.

Repeat step 10 down to column 10 - discard the withdrawn solution from column 10.

Inoculate the ELISA microplate

Step 12.

Vortex the bacteria suspension from step 5.

Add 50µl of the suspension to each well in columns 1-11.

Incubate the plates

Step 13.

Cover the plates with film, make sure to create a tight seal to prevent any evaporation.

Repeat

Step 14.

To exclude any errors repeat every plate setup three times (triplicates).

Incubate the plates

Step 15.

Incubate the plate at 37ºC for 16-24h.

Results

Step 16.

Measure the growth by determinating $OD_{450.}$

Compare the data from the three triplicates, and exclude any deviations