Supplementary Material S2

Laboratory work instructions for carrying out susceptibility testing by the broth microdilution method

Materials and tools needed:

- 5 ml of sterile nutrient broth in a test tube
- 50 ml and 100 ml of nutrient broth in Erlenmeyer flasks
- stock solution of antimicrobial substance
- 8 pcs of 15 ml sterile test tubes
- stock solution of resazurin dye (0.1% w/v)
- sterile water
- sterile 96-well plate
- multichannel pipette
- sterile tips for multichannel pipette
- sterile serological pipettes
- sterile reagent reservoirs: one single-well and one multi-well reservoir

Performing the laboratory work:

Day 1:

1. Inoculate the bacteria into 5 ml of nutrient broth and incubate overnight at 37 °C.

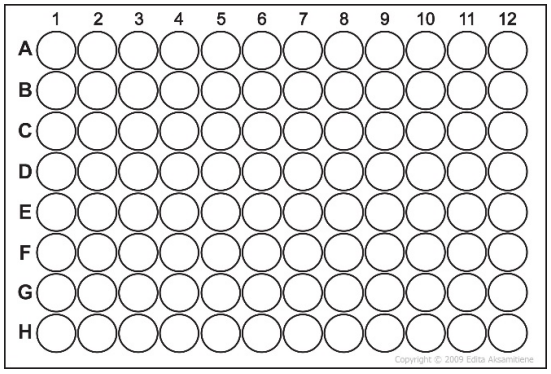
Day 2:

1. Prepare the dilutions of the antimicrobial substance into nutrient broth:

   Stock solution 1 mg/ml (ready-made), of which serial dilutions are made in the concentrations of 100 μg/ml, 10 μg/ml, 1 μg/ml, 0.1 μg/ml, 0.01 μg/ml, 0.001 μg/ml, and 0.0001 μg/ml. Prepare 5 ml of each dilution. Use the 50 ml of nutrient broth in an Erlenmeyer flask and the sterile test tubes.
2. Prepare 10 ml of 0.01% (w/v) resazurin dye dilution into sterile water. Use the 0.1% (w/v) resazurin dye stock.
3. Determine the bacterial growth in the overnight culture by visually examining the turbidity. Prepare a dilution of the overnight culture: add 0.1 ml of the culture into 100 ml of nutrient broth. Mix the dilution.
4. Plan the pipetting order of reagents and the bacterial culture dilution into the 96-well plate. Remember that you can use one single-well and one multi-well pipetting reservoir. The single-well pipetting reservoir can be reused during pipetting.
5. Pipette the reagents and the bacterial culture dilution into the 96-well plate.
6. Incubate the 96-well plate overnight at 37 °C.

Day 3:

1. Examine the resazurin dye color change and determine the MIC value of the antimicrobial substance. Calculate the final concentration of the antimicrobial substance in the well (μg/ml). Present the results to the teacher.



Order and volume of samples in the 96-well plate:

NB! Volumes of samples mean **µl/well**. One column consists of eight wells (A–H).

Columns 1 and 12: 230 µl of nutrient broth

Column 2: **background** (indicates the color of nutrient broth during susceptibility testing):

180 µl of nutrient broth (instead of bacterial suspension dilution and antimicrobial substance)

50 µl of sterile water (instead of resazurin dye)

Column 3: **negative control** (indicates the color if no bacterial growth occurs):

180 µl of nutrient broth (instead of bacterial suspension dilution and antimicrobial substance)

50 µl of resazurin dye

Column 4: **positive control** (indicates the color if bacterial growth does occur):

130 µl of bacterial culture dilution

50 µl of nutrient broth (instead of antimicrobial substance dilution)

50 µl of resazurin dye

Columns 5–11: **dilutions of antimicrobial substance:**

130 µl of bacterial culture dilution

50 µl of antimicrobial substance dilution (one column for each dilution)

50 µl of resazurin dye