Determining toxin MICs

MATERIALS

Consumables

- Clear 96-well plates
- Frozen stock of strain(s) of interest
- Appropriate broth
- Stock solution of toxin(s)

Instrumentation

- Cuvette-format spectrophotometer
- Plate reader able to measure OD_{600}

METHODS

n is the number of technical replicates to be tested for a specific toxin/strain.

1a. FOR E. COLI: Preparation of diluted culture.

1.1a. Fill a test tube with 5 mL of LB and inoculate with the desired frozen stock. Incubate overnight at 37 C. Complete part 2 before proceeding to step 1.2.

1.2a. Combine 110 uL of overnight culture with 990 uL LB in a falcon tube and vortex to mix. Transfer 1 mL of the mixture to a cuvette and measure the absorbance at 600 nm using the spectrophotometer.

1.3a. Dilute the incubated culture with LB to a target OD_{600} of 0.1 (assuming 1 cm pathlength) in an appropriately sized falcon tube. Make a total volume of (n + 1)(90 uL). Calculate the volume of broth and culture as follows:

$$V_{culture} = \frac{OD_{target} \times V_{total}}{10 \times OD_{measured}}$$

$$V_{broth} = V_{total} - V_{culture}$$

1b. FOR B. DIAZOEFFICIENS: Preparation of diluted culture.

1.1b. Fill a test tube or flask with (n + 5)(90 uL) TY broth inoculate with the desired frozen stock. Incubate at 30 C for 4 days and 16 hours. Complete part 2 before proceeding to step 1.2.

1.2b. Transfer 1 mL of the incubated culture to a cuvette and measure the optical density at 600 nm using the spectrophotometer. Do not dilute.

1.3b. Check that the OD_{600} is between 0.4 and 0.6. This corresponds to mid-log phase growth, which is required for this assay. Do not dilute.

2. Preparation of broth with toxin.

2.1. Dilute the stock toxin solution with broth to the target concentration in an appropriately sized falcon tube. Make a total volume of (n + 2)(285 uL), where n is the number of replicates with that toxin. Calculate the volume of toxin stock and broth as follows:

$$V_{stock} = \frac{C_{target} \times V_{total}}{C_{stock}}$$
$$V_{broth} = V_{total} - V_{stock}$$

3. Well plate preparation.

NB: the volumes used here correspond to 3-fold serial dilutions. Other dilution factors may be used as needed.

3.1. Fill the wells according to figure 1, vortexing the broth with toxin before adding. Rows B through G are used to run toxicity tests. Rows B through G may each have a different toxin.

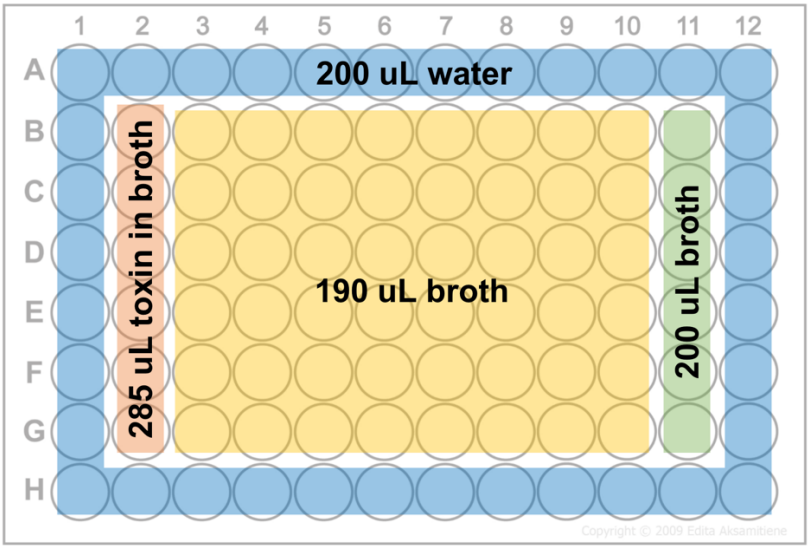

Figure 1. Step 3.1 well plate filling schematic.

3.2. For each row: Transfer 95 uL from column 2 to column 3 and pipette up and down 5 times in column 3. With a new pipette tip, transfer 95 uL from column 3 to column 4 and pipette up and 5 times in column 4. Repeat this until column 9. Remove 95 uL from column 9 and discard. Do not touch column 10 or 11.

3.3. Vortex the culture from part 1.3. Add 10 uL of the culture to each well in columns 2-10 rows B-G. This is shown in figure 2.

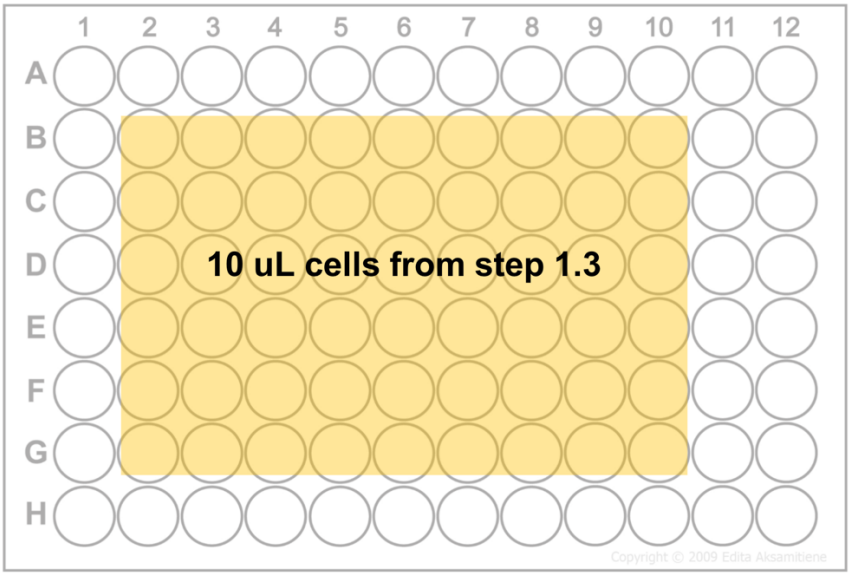

Figure 2. Step 3.3 well plate filling schematic.

3.4. Place the lid on the well plate. Label appropriately. Place in the appropriate incubator for the appropriate length of time.

4. Well plate reading.

4.1. Remove the well plate from the incubator at the appropriate timepoints. Read absorbance in the plate reader at 600 nm.