**Preparation of Luria Bertani (LB) Agar infused with Tetracycline**

Tetracycline resistance breakpoints established by the CLSI

(https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf)

* Enterobacterales – 16 µg/mL
* *Pseudomonas aeruginosa* – N/A
* *Acinetobacter* spp. – 16 µg/mL
* *Burkholderia cepacia* complex – 16 µg/mL
* *Stenotrophomonas maltophilia* – 16 µg/mL
* Other Non-Enterobacterales – 16 µg/mL
* *Staphylococcus* spp. – 16 µg/mL
* *Enterococcus* spp. – 16 µg/mL
* *Haemophilus influenzae* and *Haemophilus parainfluenzae* – 8 µg/mL
* *Neisseria gonorrhoeae* – 2 µg/mL
* *Streptococcus pneumoniae* – 4 µg/mL
* *Streptococcus* spp. -Hemolytic Group – 8 µg/mL
* *Streptococcus* spp. Viridans Group – 8 µg/mL
* *Neisseria meningitidis* – N/A
* Anaerobes – 16 µg/mL

Summary

As the highest MIC breakpoint observed is 16 µg/mL, this concentration will be used. Tetracycline infused media is used to detect bacteria resistant to tetracycline in manure, soil, and water samples. As the highest resistance breakpoint observed is 16 µg/mL, the MIC breakpoint for this test will be 16 μg/mL.

Materials

* Distilled Water
* LB agar
* Tetracycline Stock Solution (16 μg/mL) - good for 3 months

or

* Tetracycline HCl powder - located in walk-in freezer immediately to the left upon entrance
* 50% Methanol - there is typically premade 50% methanol in the flammable cabinet in the WQRL Lab

Preparation of Tetracycline Stock Solution (if necessary)

1. Dissolve 160 mg Tetracycline HCl in 10 mL of 50% Methanol

Tips:

* Use a glass amber container to mix/store stock solution, these can be found in a box right outside the freezer among all the other boxes of bottles
* Make sure to label the glass container with contents, date, and name or initials
* Use a weigh boat to measure out 160 mg of powder, dump the majority into your container and then use the 10 mL of 50% Methanol to wash any residual powder on the weigh boat into the container

Preparation of LB Agar with Tetracycline

1. Prepare 1 L of LB agar following manufacturer’s instructions
2. Allow agar to cool to 50°C before adding tetracycline stock solution
3. Add 1 mL of the dissolved Tetracycline Stock Solution to 1 Liter of prepared LB agar (concentration will be at 16 μg/mL)
4. Aliquot into sterile petri dishes

Tips:

* Use a 2 L glass bottle to make agar
* As agar cools, position a suspended thermometer using tin foil so that you can watch when the temperature drops to 50 °C
* Use stir bar/stir plate for all agitating
* If pouring by hand is difficult you can always use a serologic pipette to add agar to petri dishes