Dear Kate,

The antibiotic resistance lab this year is different. We are using a “citizen science” project (PARE), which has a standard set of protocols – as indicated in the instructor’s lab manual I sent.

We will be using the following supplies, with the numbers indicated for **TOTAL** of the two lab sessions per week. For the TOTAL counts I have added extra’s to cover dropped plates, etc.

**Week #1 TOTAL**

Sterile tubes with caps – plastic – large enough to hold 50 ml

1 tube per student 45 tubes

Plastic gloves – 45 pr gloves

**Week #2**

MacConkey plates – 1 set per student

* 5 MacConkey plates with antifungal 5 x 45 students = 225 plates
* 3 MacConkey plates with antifungal and tet3 3 x 45 students = 135 plates
* 3 MacConkey plates with antifungal and tet30 3 x 45 students = 135 plates

Dilution tubes – 1 set per student

* 6 sterile tubes that can contain 10 ml with caps 6 x 45 pairs = 125 tubes 270 tubes

Sterile water – 1 bottle per 2 students

* 60 ml sterile water in screw top bottle 1 x 345 students = 25 bottle

Pipettors and tips to measure 0.9 ml (1 set per 2 students)

Pippettors and tips to measure 0.2 ml (1 set per 2 students)

Spreaders - sterile glass beads – 1 250 ml bottle of beads per 2 students = 25 bottles

Scale to measure 1 gram soil

Weigh paper for scale

Markers to label tubes

Vortex

Timers with second hands

28 C incubator

parafilm

**Week 3**

Ethanol soluble markers for use in counting colonies

Cameras for recording plates

Printer for printing plate images

**Plate recipes:**

* MacConkey media and amphotericin B (10 ug/ml) label these plates NA – or put a black line on outside edge of plate
* MacConkey media and amphotericin B (10 ug/ml) and tetracycline (3 ug/ml) label these plates Tet3 – or put a red line on outside edge of plate
* MacConkey media and amphotericin B (10 ug/ml) and tetracycline (10 ug/ml) label these plates Tet30 or put 2 red lines on outside edge of plate

(line labeling plates – I create a stack of plates and then draw a marker straight up the column to create a line of some color on all of the plates in the stack. The line ends up on the top lid of each plate)

 **Media** (one liter of liquid agar yields about 30+ plates)

• MacConkey dehydrated medium (strongly preferred over other media) BD/Difco 220100

• Agar (15g/L) BD/Difco 214050

• Petri plates (100 x 15mm) VWR 25384-342

• Tetracycline hydrochlordide powder Sigma Aldrich T8032-10MG

• 70% ethanol (buy 95% and dilute)—for mixing tetracycline stock solution

• Amphotericin B powder Sigma Aldrich A4888, A2411

• DMSO (dimethyl sulfoxide)—for mixing amphotericin B stock solution Sigma Aldrich D8418

**Tetracycline Stock Solution**

Tetracycline is light sensitive so stock mix and plates should be kept in the dark. Tetracycline

stock should be mixed at a concentration of 15 mg/ml in methanol or 70% ethanol and stored at -20°C. Tetracycline is light-sensitive, so stock should be stored in a light-blocking container or one wrapped with foil. It is also temperature-sensitive so it must be added to medium after autoclaving, once the flask can be touched comfortably (45-55°C). It should be very warm, but not uncomfortably hot. Swirl to mix well and pour plates. Plates will remain stable for at least four weeks if kept at 4°C in the dark.

* 3 μg/ml medium: add 0.2 ml of tetracycline (15 mg/ml) stock solution to 1 L
* 30 μg/ml medium: add 2 ml of tetracycline (15 mg/ml) stock solution to 1 L

**Amphotericin B Stock Solution**

Stock solution should be mixed at a concentration of 10 mg/ml (1000x) in dimethyl sulfoxide

(DMSO) and stored at 4°C, protected from light. As with the tetracycline, add to medium after

autoclaving once it has cooled to 45-55°C (assessed by touching the flask).

* 10 μg/ml medium: Add 1 ml of 10 mg/ml stock to 1 L of medium.

**MacConkey + Amphotericin B**

Difco MacConkey powder (follow directions on bottle for amount—usually ~50g)

15 g agar

bring to 1L with water

Autoclave

Add 1 ml of 10 mg/ml Amphotericin B stock once flask has cooled enough to handle

Swirl to mix well before pouring

**Tet3** (culture medium + 3 μg/ml tetracycline)

23 g MacConkey powder (or nutrient broth or R2A medium powder)

15 g agar

bring to 1 L with water

Autoclave

1 ml of 10 mg/ml Amphotericin B stock (once flask has cooled slightly)

0.2 ml of 15 mg/ml tetracycline stock (once flask has cooled slightly)

Swirl to mix very well before pouring

**Tet30** (culture medium + 30 μg/ml tetracycline)

23 g MacConkey powder (or nutrient broth or R2A medium powder)

15 g agar

bring to 1 L with water

Autoclave

1 ml of 10 mg/ml Amphotericin B stock (once flask has cooled slightly)

2 ml of 15 mg/ml tetracycline stock (once flask has cooled slightly)

Swirl to mix very well before pouring