Goal: Figure out if the abx concentration in bd cultures are high enough to inhibit bacterial growth, relative to 0.5% tryptone and 1% tryptone control.

\*\* Using 0.5% and 1% tryptone as controls, because I suspect the “old” bd cultures will have depleted some nutrients in the broth. Want to make sure we’re not accidentally detecting nutrient depletion instead of ABX effects

PREP:

1. Inoculate bacteria into broth. Let grow.
2. Autoclave 7 small test tubes with 1mL tryptone broth in each. 2 additional test tubes with 1mL DI water in each. 6 additional sterile tubes as back-up.

EXPERIMENT, liquid:

1. Filter-sterilize 1mL of each abx Bd culture into tryptone-filled small test tube
   1. Full strength abx
   2. Diluted abx
   3. Plate wash flasks
   4. DI water (for 0.5% tryptone control)
   5. Nothing (for 1% tryptone control)
2. Aliquot 100uL each in triplicate into culture plate, plus 3 each sterile controls. (5treat\*3rep\*3bact=45 + 3rep\*5treat = 15; 60 wells. Borders are empty.)
3. Inoculate appropriate wells with 10uL of bacteria
4. Ensure border of plate is filled with liquid to prevent evaporation. Wrap carefully with parafilm
5. Wait 1-2 days (sooner better? Since nutrient depletion will occur quicker.)

EXPERIMENT, solid:

1. Spread 500mL zoospores onto a plate. Let dry a few hours.
   1. Plate with full abx
   2. Plate with diluted abx
   3. Plate with plate-washed abx (x2)
   4. Plate with water
   5. Will also include a positive control, where we spread nothing
2. Dot with a few bacteria. See if they grow or not.

ANALYSIS:

1. Use Schmidt lab plate reader to read OD. Subtract sterile controls as “zero” for turbidity, and assess whether bacteria grew less in certain cultures.
2. Assess growth of bacteria on abx plates compared to non-abx plates.