* Tuesday, May 26
  + Made mueller hinton agar/broth for H. influenzae
  + Poured plates (large and small)
  + Prepared liquid cultures
    - M. catarrhalis in tryptic soy broth
    - S. pneumoniae in tryptic soy broth
    - H influenzae in MH broth
* Wednesday
  + Only saw clear growth in liquid culture for S. pneumoniae
  + Prepared plates of all 3 species on agar that corresponded to their broth
* Thursday
  + Saw lots of growth on M. catarrhalis plate, not much on other two (yet)
  + Prepared inhibition base solutions:
    - 1. 50g garlic blended with 100g peanut oil
    - 2. 50g garlic blended with 100g mustard oil
    - 100g mustard oil
    - homogenized in waring commercial blender
    - MAYBE dilute to 200 mL for all?
* Tuesday, June 30th
  + Preparing stock solutions of inhibitors
    - Allow the purees to sit in a 4°C refrigerator until the chunks of garlic have settled to the bottom of the jar
    - Pipette out the top, clear portion of the fluid and use this as your original stock solution
  + Plate based paper disk inhibition:
    - Made bacterial lawns of H. influenzae on 5 plates
    - Prepared 10x dilutions of PO+G, MO, and MO+G into peanut oil
    - Soaked filter paper circles (milipore) in the inhibitors
      * Both at high and low concentrations
    - Left one lawn plate paper-diskless as positive control
    - For two plates (1 + duplicate), had 5 different disks: blank, PO, PO+G, MO, and MO+G, all at full concentration
    - For two plates (1 + duplicate), had 5 different disks: blank, PO, PO+G, MO, and MO+G, all at 10x dilution
  + Microwell based liquid inhibition:
    - Prepared bacterial stock solution by diluting base liquid culture 10x into MH broth
    - Microwell plate has 8 rows (A-H) by 12 columns (1-12)
    - Each column is 8 replicates of same conditions
    - Each well has 200 uL of fluid
      * For wells without inhibitor, there is 200 uL of either MH broth (M) or 200 uL of bacterial stock solution (B)
      * For wells with inhibitor, there is 190 uL of M or B with 10 uL of inhibitor
      * Inhibitor is original stock for all of these
    - Columns are prepared as follows:

1. M
2. M+B
3. M+PO
4. M+PO+B
5. M+PO+G
6. M+PO+G+B
7. M+MO
8. M+MO+B
9. M+MO+G
10. M+MO+G+B
11. M
12. M+B
    * + Microwell plate is left in incubator+shaker+reader for 24 hours with readings every half hour
        - 37°C, measuring OD at 600 nm wavelength
    * General notes
      + Only using one bug and only doing duplicates for now
        - If this all works out, we will use the other ones
      + Plate provides good visual, but analytical data from wells is more useful

* Wednesday
  + Making plates
    - Get petri dishes from Cargill
    - Make 500 mL of MH agar mix (follow procedure on box)
    - Pour ~25 plates with 15-20 mL of agar in each
  + Follow-up on microwell plate inhibition assay
    - Made growth curve
    - Garlic had very strong effect, mustard had basically no effect
    - Outer wells had most evaporation
  + Follow-up on agar plates
    - No inhibitory effect apparent
    - Will retry with filter paper disks that are made especially for this type of experiment to see if the issue is with the other filter disks
* Tuesday, July 7th
  + Plate based paper disk inhibition
    - Used GE life sciences paper disks (10 mm)
    - For each of the three bacteria, prepared 1 control plate with just lawn and 3 inhibition plates (triplicates), each with blank, PO, PO+G, MO, and MO+G
  + Microwell based liquid inhibition
    - Prepared bacterial stock solution by diluting base liquid culture 100x into MH broth
      * Should have done 10x, we’ll see what happens with results
    - Outermost layer of wells filled with water (on all 4 sides), so used rows B-G and columns 2-12
    - Each column was 6 replicates of same conditions
    - Wells are prepared as before (10 µL of inhibitor if present, 200 µL total)
    - Columns are prepared as follows:

1. M
2. M+B
3. M+PO
4. M+PO+B
5. M+PO+G
6. M+PO+G+B
7. M+MO
8. M+MO+B
9. M+MO+G
10. M+MO+G+B
    * + Microwell plate is left in incubator+shaker+reader for 16 hours with readings every half hour
        - 37°C, measuring OD at 600 nm wavelength

* Wednesday
  + Microwell plate shows contamination, data is thrown out
* Monday, August 3rd
  + Original medium had contamination
  + Remade MH broth
  + Made new liquid cultures for each bacteria by dipping inoculation loop in 3-4 colonies, then dipping in vial of MH broth
  + Made new garlic + water mix (50 g garlic in 100 mL water)
* Tuesday
  + Microwell plate inhibition
    - Filling outer two rows on all sides with water (leaving a 4x8 plate)
    - Each column is 4 replicates
    - Columns are: M ± B, M+PO±B, M+G±B, M+PO+G±B
    - Done with S. pneumoniae since it has the best liquid culture growth
  + Liquid culture update
    - H. influenzae has some growth, but not as good as S. pneumoniae
    - M. catarrhalis is looking dismal
  + Re plating from old plates
    - Only 2 extra plates, but since S. pneumoniae is already growing fine, just replated other two
    - Used inoculation loop to take a piece from the old plate and streak onto a new MHA plate
* Wednesday
  + Checking new plates + yesterday’s liquid cultures
    - H. influenzae liquid culture has grown more
    - H. influenzae plate has plenty of growth
    - M. catarrhalis liquid and plate both have zero growth
  + Checking yesterday’s microwell plate
    - Major growth in all bacterial rows except the garlic + water
    - No apparent contamination with new media
  + Microwell plate inhibition
    - Same as yesterday’s setup with full garlic assay
    - Using H. influenzae
* Thursday
  + Checking on M. catarrhalis
    - Liquid culture still didn’t grow
    - Plate grew!
    - Made new liquid culture based on plate
  + Microwell plate inhibition
    - Since the M. catarrhalis will need another day or two, used S. pneumoniae for mustard assay
    - Filling outer two rows on all sides with water (leaving a 4x8 plate)
    - Each column is 4 replicates
    - Columns are: M ± B, M+PO±B, M+MO±B, M+MO+G±B
    - NOTE: the S. pneumoniae 10% liquid culture grew really fast (at room temperature), so by today, it resembled the 100% solution. Not realizing, I used it without re-diluting, so the bacterial wells will start off with extra OD
* Friday
  + Microwell plate inhibition
    - Same as earlier garlic assay procedure
    - Using M. catarrhalis
    - Accidentally filled plate upside down (flipped within xy plane), so I put the plate in the reading machine upside down as well
* Saturday
  + Yesterday’s results
    - No growth, will put the M. catarrhalis back in the incubator to see if it needs more time
  + Microwell plate inhibition
    - Ran mustard analysis with H. inf
    - Used bottom two rows to figure out garlic concentration difference between water and oil mixes
    - (OD(G+W)-OD(W))/(OD(G+PO)-OD(PO)) = 3.4
* Monday, August 10th
  + Saturday’s results
    - No growth, made new H inf liquid culture
  + Making antibiotic base mixtures
    - Made 1 mg/mL solutions of amoxicillin (AX), vancomycin (V), and ampicillin (AP)
  + Microwell plate inhibition
    - Antibiotic assay (M±B, M+AX±B, M+V±B, M+AP±B) with S. pneumoniae
* Tuesday
  + Monday’s results
    - Growth on vancomycin, not on anything else
  + Garlic dilution
    - Using saturday’s side results, I found the garlic+water had about 3.4 times as much garlic as the garlic+peanut oil
    - Diluted garlic+water and will try to run all three garlic assays again
  + Microwell plate inhibition
    - Antibiotic assay (M±B, M+AX±B, M+V±B, M+AP±B) with M. catarrhalis
* Wednesday
  + Liquid culture status
    - H influenzae not growing very well, started another liquid culture from plate
  + Microwell plate inhibition
    - New garlic assay with diluted garlic water
    - M±B, M+G+PO±B, M+G+W±B, M+G+MO±B
    - Used S. pneumoniae
* Thursday morning
  + Microwell plate inhibition
    - H. influenzae with antibiotics
* Thursday night
  + Microwell plate inhibition
    - H. influenzae with full new garlic assay
* Friday morning
  + Liquid culture status
    - New M. catarrhalis cultures aren’t doing too well yet, so using old with for this test
  + Microwell plate inhibition
    - M. catarrhalis with old mustard assay
* Friday night
  + Gave up on M. catarrhalis
  + Microwell plate inhibition
    - H. influenzae with old mustard assay