

# Notebook Week 6 (July 2-7)

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**Project:** iGEM 2017

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**Dates:** 2017-07-02 to 2017-07-07

SUNDAY, 7/2

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Researched other methods of lead water testing and cost, also found a Massachusetts site about lead in water:

<http://www.mass.gov/eea/agencies/massdep/water/drinking/is-there-lead-in-my-tap-water.html>

<http://www.mwra.com/04water/html/testinglabs.html>

Found that lead testing can cost between \$12-\$80, and take about 4-14 days. They appear to use Flame Atomic Absorption Spectrometer (AA), and/or Inductively Coupled Plasma Spectrometer (ICP). One of the previously posted links has a list of places that test in Massachusetts that could be helpful to contact for more specific details.

MONDAY, 7/3

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Data Analysis for MRS & LB Standard Curves

 Standard Curve.xlsx

THURSDAY, 7/6

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Plan:

- Skype Call with BU @ 10am
- Contact Lead water testing companies in Massachusetts
  - Make Questionnaire Sheet for Calls: [Questionnaire](#)
- Go over responses from FDA
  - Our probiotic would be considered a recombinant LBP, and therefore a IND
  - Used to start filling in "Questions" Page
  - <https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/General/UCM292704.pdf>
  - <https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm153222.pdf>
- Research more into the Lead Growth Curves
  - How to isolate lactobacillus colonies on MRS agar?
    - Dilute to an OD 0.1 and then plate liquid cultures on MRS agar to try and get isolated colonies to use for growth curves instead of Culturelle pill
  - Grow the bacteria in lead overnight? Why are they not dying with exposure to lead within 8 hours?
  - Alter pH? Paper ([here](#)) says that B.subtilis absorbed 97.68% within 48h of incubated at an optimum pH 4.5 and 40 degrees celsius
- Go over lead assay results, try to figure out what to do next
  - Try assay row by row on 7/7/17, start with 5,000ppb then decrease to 2,000ppb, 1,500ppb, and 1,000ppb, make sure to time how long Pb and AuNP's are together before GSH is added.
- Go over questions/ start answering them
- Look at:

<https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations>

<https://www.epa.gov/dwreginfo/strategic-plan-targeted-outreach-populations-affected-lead>

<https://www.epa.gov/dwlabcert/contact-information-certification-programs-and-certified-laboratories-drinking-water>

- Work on presentation
- Overnight cultures for Growth Curve
  - All lead spiked comes from large bottle 300 ppm stock
  - Lactobacillus
    - L(1) 1 parent culture (20 mL MRS & Culturelle pill)
    - L(2) 1 culture with (20 mL MRS, Culturelle pill, 6.68 mL of DI H2O)
    - L(3) 1 culture with 50 ppm lead ( 20mL MRS, Culturelle pill, 3.34 mL h2o, 3.34 mL Lead spiked)
    - L(4) 1 culture with 100 ppm lead ( 20mL MRS, Culturelle pill, 6.68 mL lead spiked water)

- B.subtilis
  - B(1) 1 parent culture (20mL Lb & 3 colonies)
  - B(2) 1 culture with (20mL LB, 3 colonies, 6.48 mL H2O)
  - B(3) 1 culture with 50 ppm lead( 20mL LB, 3 colonies, 3.24 mL H2O, 3.24 mL lead spiked)
  - B(4) 1 culture with 100 ppm lead (20mL LB, 3 colonies, 6.48 mL lead spiked)

FRIDAY, 7/7

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Plan:

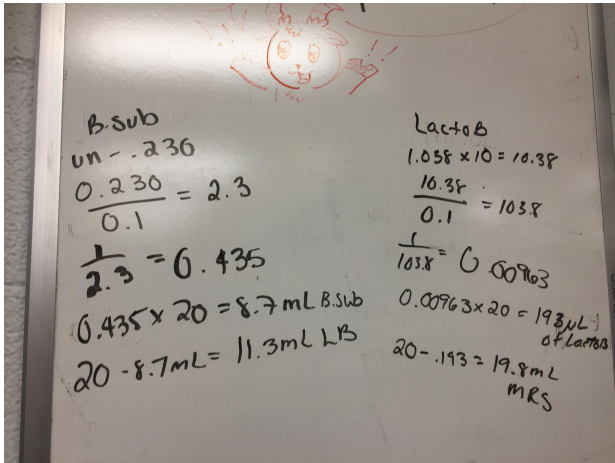
- Lead Assay
  - Try row by row, starting with 5,000ppb, use one column of control with 4 replicates
  - Start with 5,000ppb, then 2,000ppb, 1,500ppb, and 1,000ppb for LB and MRS
    - 41.5 µL of lead spiked LB, same for MRS
    - 2 µL of control LB, 4 µL of control MRS
    - 34.6 µL AuNPs, same for MRS
    - 10 µL GSH, 8 µL GSH
  - Control: 43.5 µL for LB, 45.5 µL for MRS with GSH, 53.5 µL for control without GSH

The dilutions needed to be remade. Cat did not get to finish making the MRS dilutions, but all that needs to be done is that 5mL of MRS needs to be added. It also needs to be labeled because the MRS claimed came right from the autoclave. The assay was done row by row for LB, and the 5,000ppb was done twice because too much gold may have been added to the first one. The second one was done to double check. Also the extra 2 µL of LB control was not added to the samples containing lead, but the data will be analyzed to see if the extra volume and the time differences made an impact.

 LB Lead Assay by row.xlsx

The 595nm wavelength looked promising before the 5:30 time point. Only one trial was done so error bars are not included, but the 3:30 graph has an  $R^2$  value of .9768 which is really exciting.

- Electroporation Trial 2, make sure to use both B. Subtillus and E. Coli
- Research Culturelle Clinical Trials and if other probiotics are used as LBPs or recombinant LBPs
- Redo Vector Prep:
  - minprep of pET21a
  - Digest
  - Gel Purify
- Growth curves:
  - Run 2 different experiments
  - Experiment 1: lead exposure overnight
    - Using cultures 2, 3,4 take OD every hour starting at 6am
    - Incubate shaking at 37 degrees in 50 mL conical tubes
    - Note: No bubbles were seen in any tube B.subtilis containing lead overnight, bubbles were seen in both the parent culture and culture containing just DI Water
  - Experiment 2: Lead exposure after dilution when in lag phase
    - Make 3 cultures from overnight parent culture and dilute to an OD of 0.1 in 20mL culture
      - Culture 1- Control with added H2O
      - Culture 2 - 50 ppm with added H2O and Lead
      - Culture 3 - 100 ppm with added H2O and Lead
    - Incubate at 37 degrees shaking and take OD every 1.5 hours in 50 mL conical tubes
    - Parent cultures OD
      - LactoBacillus- 10.38 - (1.038 .. 1:10)
      - B.subtilis- 0.238
      - Dilution math in picture below to get a subculture of a desired OD of 0.1



- B.subtilis didn't grow as expected too, this is a problem that has happened more than once. Are they not getting enough air? (aerobes)
  - Needed 8.7mL of parent culture for experiment 2 to reach an OD of 0.1 in subcultures.. so chose to not perform 50 ppm lead and only go through with 0ppm as a control and 100 ppm.
  - literature says that B.subtilis can survive and bioabsorb up to 800 ppm
- Serial dilutions from left over parent culture of Lactobacillus and plate each 10 fold to get individual colonies on MRS agar
- Leftover B.subtilis and Lactobacillus cultures you don't need( from parent culture) -> MAKE GLYCEROL STOCKS
  - 500 uL of culture, and 500 uL of 50% glycerol in screw cap cryotubes
  - 3 or 4 tubes of each one
  - Put in -80 freezer
- IND Application Info
  - Long Process involving interaction with the FDA before clinical trials, animal testing of some sort would need to be completed before send in approval for clinical trials, clinical trials would need to be with humans. Our project would be considered a drug because it is intended to prevent lead poisoning from drinking water, would also be considered genetically engineered because it does not say how the genetic material is altered, only if it is (which it is). We can request a formal meeting, but it would be better to contact them to try to arrange something different because the meetings are much more formal and meant for industry. We would also need to get WPI to sponsor us, and the meeting probably wouldn't be approved without the intent of involving industry right away.
  - <https://www.fda.gov/downloads/drugs/guidances/ucm079744.pdf>
  - <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070568.pdf>
- Continue Outreach to Water Testing Companies
  - Left message for Aaron Benoit at Con-Test Analytical
  - Left message (with secretary) at Northeast
  - Need to Email Timothy Begley at Analytical Balance on Tuesday: tim@h2otest.net
  - Talked to Steve Hartman at STL Westfield
  - Need to call SCILAB-Boston Inc at 9:30 to talk to Mark
- Presentation Prep
  - Made copy of Updated presentation, added slides to talk about small things that were done

# DIY Lead Assay

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## Introduction

## Materials



- > Gold Nanoparticles
- > Lead Nitrate
- > GSH
- > NaCl (1 M)
- > Phosphate Buffer (50 mM, pH=7)
- > D.I Water
- > 15 mL conical tubes
- > 1.5 centrifuge tube

## Procedure

### Make Glutathione Liquid

- ✓ 1. Make 100mM solution of L-Glutathione  
30.72 g / 100mM  
Add 0.5 g of the Glutathione powder to 16.3 mL of D.I water  
Make 1 mL aliquotes and store in freezer

### Make Phosphate Buffer

- ✓ 2. Make a 1 M stock of Phosphate Buffer
- ✓ 3. Add to a graduated cylinder 65.82 g/L of Sodium Monobasic and 93.1 g/L of sodium dibasic in 100 mL of water
- ✓ 4. Test pH with pH meter and adjust using 10 normal NaOH
- ✓ 5. Top graduated cylinder to 1 L

### Make NaCl solution

- ✓ 6. Make 1 M stock of NaCl
- ✓ 7. Add 11.86 g into 200 mL of water into a bottle

### Make GSH solution

- ✓ 8. Make dilute of the phosphate buffer to 50 mmol, add 1 mL of stock to 19 mL of D.I water
- ✓ 9. In a 15 mL conical tube add 110  $\mu\text{L}$  of NaCl, 620  $\mu\text{L}$  of Phosphate buffer, 480  $\mu\text{L}$  of L-Glu liquid and 790  $\mu\text{L}$  of D.I water.
- ✓ 10. Vortex thoroughly for 5 seconds

## Prepare Lead Spiked Water Stock

- ✓ 11. Measure out 100 mg of Lead nitrate to get 1000 ppb
- ✓ 12. Dilute as necessary to achieve concentrations of 5 ppb, 10 ppb, 15 ppb, 30 ppb, 50ppb, and 100 ppb.
  - 5 ppb - 1  $\mu\text{L}$  Pb in 20 mL of D.I
  - 10 ppb - 1  $\mu\text{L}$  Pb in 10 mL of D.I
  - 15 ppb - 1  $\mu\text{L}$  Pb in 6.67 mL of D.I
  - 30 ppb - 1  $\mu\text{L}$  Pb in 3.3 mL of D.I
  - 50 ppb- 1  $\mu\text{L}$  Pb in 2 mL of D.I
  - 100 ppb - 1  $\mu\text{L}$  Pb in 1 mL of D.I

## Prepare Cadmium Spiked Water Stock

- ✓ 13. Measure out 100 mg of Cadmium nitrate tetrahydrate to get 1000 ppb
- ✓ 14. Dilute as necessary to achieve concentrations of 5 ppb, 10 ppb, 15 ppb, 30 ppb, 50ppb, and 100 ppb.
  - 50 ppb- 1  $\mu\text{L}$  Cd in 2 mL of D.I
  - 100 ppb - 1  $\mu\text{L}$  Cd in 1 mL of D.I

## Well Preparation and Plate Reading

- ✓ 15. In wells A1-A7, place samples 5 ppb-control
- ✓ 16. In each well add 12  $\mu\text{L}$  of GSH and 34.6  $\mu\text{L}$  of AuNP along with 41.5  $\mu\text{L}$  of the lead spiked water
- ✓ 17. Mix contents with pipette by pipetting up and down
- ✓ 18. Place replicates in B1-B7, C1-C7, and D1-7
- ✓ 19. In wells A, B, C, D8-9 add 12  $\mu\text{L}$  of GSH and 34.6  $\mu\text{L}$  of AuNP along with 41.5  $\mu\text{L}$  of the cadmium spiked water (50-100 ppb)
- ✓ 20. Place in plate reader at Absorbance  $A_{610}$
- ✓ 21. Incubate for 10 min and read over period of time
- ✓ 22. Perform second well test and record color change every 1 mins f

Lead/Cadmium AuNP Assay 96-well plate

	A	B	C	D	E	F	
1	5 ppb Pb (1 uL Pb-H <sub>2</sub> O/20 mL DI H <sub>2</sub> O)	10 ppb Pb (1uL/10mL)	15 Pb (1uL/6.67mL)	30 Pb (1uL/3.33mL)	50 Pb (1uL/2mL)	100 Pb (1uL/1mL)	Cor
2	"	"	"	"	"	"	Cor
3	"	"	"	"	"	"	Cor
4	"	"	"	"	"	"	Cor
5							
6							
7							
8							

✓ 23.