Standard Protocol Procedure for Using the HIMB Titrator (Gates Lab)

Last Revised: 20180619 EL Strand (Putnam Lab)

Read before proceeding:

*Since no Mercuric Chloride is being deposited into the samples and the bottles are light-penetrated, water samples MUST be run **as soon as possible** in order to be as accurate as possible. When light can reach the water sample and no mercuric chloride is used to kill any bacteria or living organisms in the sample, the potential for the water chemistry to change NOT in response to the experiment in much higher.*

Write your initials next to the date of data entries on both Excel and the Putnam Lab notebook. Thoroughly explain all entires in the lab notebook so the next person will be able to understand what you did and why. Label page numbers in the top right-hand corner of the notebook page.

Do not place water sample bottles on the counter that has had mercuric chloride since the bottles are placed in and near the tanks with live coral. Place paper towels on the workbench to sent the bottles on. Mercuric chloride is designed to kill organisms so we do not want to bring any trace back to the tanks. After handling Certified Reference Materials (CRMs), change into new gloves.

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1. **Precautions**
    a. The titrator is filled with 0.1M hydrochloric acid.
    b. The Certified Reference Materials (CRMs) have mercuric chloride in them (<1%). ***You must wear examination gloves always*** Protect your eyes with glasses or goggles. No phones or other personal devices allowed in the lab working area. Do not remove any boxes or pipettes from the lab bench or move materials between rooms.
2. **Setup**
    - Turn on the Laptop and open the LabX software
    - Turn on the Titrator and Rondolino Autosampler
        - To turn on the Titrator, press the Power Button in the front of the machine (far upper-right corner).
        - To turn on the Autosampler, push on the Power switch in the back, bottom

right of the machine (black switch).

- Using the laptop, create a new folder within the general data folder [C: /Users/Gates Lab/Desktop/Putnam_20180528/Data] for each dat. Name it by the date in yyyymmdd format. Example: 20180602 *(That is your folder of the day.)*
- ***Double check that there is an empty 80ml cup in position 00.***
- To add a cup in the 00 position, raise the Rondolino by pressing the left blue button "Rotate 180 degrees" (with no numbered tick marks). Press the same button to return the Rondolino to position 00 with the stirrer and acid dispensing tube now in an empty cup.
- PURGING (RINSING): Using the Titrator tablet, tap “Manual”, "Burette", "Rinse". Set Titrant: HCl, Concentration: 0.1 mol/L, Drive: 1, Cycles: 3, Discharge volume: 100%, Fill rate: 100%. See notes below before pressing "Start".

YOU MUST POSITION THE AUTOSAMPLER FIRST (AT POSITION 00), OTHERWISE THE ACID WILL SPILL ALL OVER THE AUTOSAMPLER

- If an acid spill ever occurs, you must use the Kim wipes with DI water to soak up the acid.
    - Rinse is used to purge any air bubbles that may occur in the acid line. If there are more air bubbles, flick both tubes until they disappear. ***DO NOT START TITRATIONS IF THERE ARE STILL BUBBLES. BUBBLES WILL RESULT IN A LOWER ACID DELIVERY TO THE TITRATION THAN EXPECTED AND WILL INTRODUCE ERROR INTO THE MEASUREMENTS***.
- After Purging/Rinsing, click "OK" to bring the titrator screen back to home.
- Raise the Rondolino and pour the clean acid into the glass bottle that is connected to the titrator by removing the small white plug.
- Using DI water and a kim wipe, clean the acid cup. Leave upside down on brown paper towel to dry.
- Located at the top right side at the back of the Titrator, there will be a pH Sensor (DGi 115-SC). Place the sensor in the Robdolino position marked with a black arrow, next to the stirrer.
    - Open the grey rubber cap on the pH sensor and leave open throughout use.
    - Check the level of pH sensor storage solution and add if necessary to be no more than 1cm lower than the open port.

3. **pH_Calibration**

- Place a 80 mL cup filled halfway with DI water into Position 00.
- Use the three 80ml cups labeled "pH 4.0", "pH 7.0", and "pH 10.0". Add the

appropriate pH buffer to each of the cups until filled to ~ 50-60 mL in each cup.
    - Change out the pH buffer standards as needed, no more than 4 days of use.
    - If a new buffer bottle is opened, write the date opened, your initials, and your lab on the bottle (ex: Opened 06/02/2018 ES (Putnam)).
- Place the three cups in the Autosampler: Position 01 - pH 4.0, Position 02 - pH 7.0, Position 03 - pH 10.0.
- Open the application "LabX" from the Desktop. Using LabX: Analysis > Released Methods: ID 100 | Calibration DG115-SC > Right click > Start > T50_GatesLab
- In Task Editor window, settings should be: No. 1: pH 4.01, No. 2: pH 7.00, No. 3: pH 10.01, T = 25 degrees celsius
    - Click "Start" to run pH Calibration.
- While calibration is running: in the LabX homepage, click "Show Workbench" > "T50_GatesLab (T50)"
    - Watch the graph for anomalies, including the graph jumping constantly.
        - *Unless the zoom scale of the graph in the y-axis is at .1, small zig-zags are acceptable, but any change >3 mV over time means that the calibration should be re-done with new buffers.*
    - Once finished, the graph should be a flat line for individual samples or a downward graph if all three points are displayed. *If the results are not as described in step [vi.a.b], replace buffers and redo calibration [i. - v.].*
- Using Excel, open the “pHCalibration.csv” file. [C: /Users/Gates Lab/Desktop/Putnam_20180528/Data]
- Record the Date, Zero Point, Slope, pH 4.0, pH 7.0, pH 10.0, and any comments in the pHcalibration.csv file. Record the same data with the same column heading format in the Putnam Lab notebook. Make sure to mark your initials next to each entry you do.
    - The Notes are used to determine the age of the buffer and any other comments. Mark the day the pH calibration solution is changed.
    - Zero Point and Slope: LabX > Data > “Result Sets” > “My Latest Result Sets” > Double Click "Origin ID: 100 | Calibration DG115-SC" > "Raw Data" > Scroll to the bottom of the report to find "SLOPECal" and "ZEROCal". *Be very careful to open the correct result set, pay attention to the date and time.*
    - pH values: "Raw Data" > Each sample will have an "E" (mV) value and "T" (degrees celsius) value.
        - The results set will state 3 samples were run and gives the report in the order of positions, position 01 is the 1st sample, position 02 is the 2nd sample, and so on.
- Once the calibrations are complete, remove the cups from the Autosampler and pour back into the appropriate used pH buffer storage tube.
- Save document and make sure the file is saved to the “Data” Folder. Ignore the

failed product activation warning that might appear with the use of Excel. Overriding the previous file with the new saved file is OK, as long the data is saved and up to date.
- Rinse the titration cups used for pH solutions with DI water and place upside down on the brown paper towel to dry.

4. **CRM_Titration**
    - *Note: CRM = Certified Reference Material. CRM qualities are known (salinity, TA, etc.), therefore they are the standards. Before running real water samples, run one "junk" seawater sample (NOT a real sample) and two CRM samples. This ensures there are no air bubbles in the acid line and based on the CRM TA values, the quality of calibration and equipment can be confirmed. All samples run will be 60g samples.*
    - "JUNK" Sample
        - Turn on the scale (to the left of the titrator) and tare an empty titration cup. Carefully pour 60g of filtered seawater. **Each sample should be between 59.00 - 60.00 g.**
        - In the Putnam Lab Notebook, write the date and "Run1" above a table with the following: Column 1: Rondolino Position, Column 2: Sample.ID, Column 3: Mass (g), Column 4: Salinity (psu).
        - Record the sample ID ("Junk") and mass value. Place in position 01 on the Autosampler.
    - CRM Samples (2 replicates)
        - Take the titration cups from the marked bin labeled "CRM Mercuric Chloride". Place on the scale and tare. Carefully add between 59.00 - 60.00g of Certified Reference Material (CRM).
        - Record the sample ID with the CRM batch number and mass value in the noteook. Label the side of the cup "CRM1" and place in position 02.
        - Repeat previous two steps. Label the side of the cup "CRM2" and place in position 03. *Note: If a new bottle of CRM is used, write the date opened, your initials, and your lab on the bottle (ex: Opened 06/02/2018 ES (Putnam)). If the new bottle is a new batch, make note in the lab notebook*
    - If facing the lab bench, extra CRM can be found in the room adjacent and to the left of the bench. There is a large grey plastic box underneath the furthest counter to the left. Take one bottle at a time when needed.
    - LabX: Analysis > Released Methods: ID 000 | URIBioMin > Right click > Start > T50_GatesLab
        - Click "Add" to add as many ID rows as needed.
        - Input ID No. with the appropriate mass value. Do not include a space between "CRM" and "1". *The R script will only read "CRM1".* (Ex: "CRM1"; 59.67)
        - Click "Start".

- Once finished, view data: LabX > Data > “Reports” > “My Latest Reports” > Double Click the latest "Origin ID 000 | URIBioMin". *The report will be the top result, but be patient while LabX creates the report. Do not export the wrong file, pay attention to the date and time.*
    - "Export as" > "CSV". Name: "yyyymmdd_CRM" (ex: 20180602_Run1).
- Create a new CSV file (or excel file and save as CSV) titled "yyyymmddmass" (ex: 20180602mass_Run1). See previous date's file for format reference. *Do not change capitalization, exact format is needed for the R script.*
    - Column 1: "sample". Below fill out each sample ID "20180602_CRM1", "20180602_Conical1", or "20180602_Tank1" for the conical and tank water samples respectively. The sample ID must match the ID given in the LabX run.
    - Column 2: "mass" (g). Add the mass from the written table in notebook of mass values.
    - Column 3: "salinity". Refer to the batch number pdf information sheet for the appriopriate CRM Batch No. salinity value. This will be the salinity value for any CRM sample used from that batch.
    - Column 4: "Sample.ID". The sample id without the date and with no spaces (e.g., Tank1)
    - Column 5: "Type". The tank type (e.g., Tank or Conical)
- Refer to Step 7 for CRM clean-up procedure.

5. **Titration_RAnalysis**

- Open RStudio and "TotalAlkCalc_wParsing.R".
- The working directory must be set to the correct folder. Ex: Gates Computer Lab setwd("/Users/hputnam/MyProjects/BioMin_HIS/RAnalysis/"). This will guide the R script to the correct Data folder.
- Change values under "#CHANGE THESE VALUES EVERY DAY".
    - "path"; change only the date in format yyyymmdd (ex: "Data/20180602").
    - "massfile"; change to "yyyymmddmass.csv" (ex: 20180602mass_Run1.csv). *Double check that the mass file created is in CSV (Comma Separated Spreadsheet) format.* ***ALL FILES MUST BE IN CSV FORMAT.***
        - If not, open the file and "Save As" to the correct folder and change the file format to CSV. Delete the version that is not CSV.
    - "titrationfile"; change to "yyyymmdd_CRM.csv" (ex: 20180602_Run1).
    - "date";'20180602'
- If a new bottle of acid was used, change information under line 107: "#CHANGE ONLY WHEN NEW BOTTLE OF ACID IS USED".
    - Change the bottle number if the titrant was changed (#Bottle A3 - acid titrant#).
    - Change the values in the density equation (#density of your titrant) and the

date the bottle was changed (ex: #bottle changed to batch A3 20180528). *Any sentence or phrase following a "#" is not part of the script and acts as a note to oneself*.
        - Change the concentration value with the date the acid was changed (ex: c<-0.099793 # 20180529 batch A3).
    - Highlight script (ctl-A) and click Run. Any errors in red font in the bottom left box on RStudio will need to be troubleshot.
    - Open the new TA file created > "20180602 TA". Displayed are the TA values for each of the samples run.
    - CRM values must be within 1% error of the theoretical TA value of the CRM batch. This information is provided on NOAA's website and batch information PDFs are saved in the "Putnam_20180528" folder. **Do not move onto real water samples until CRM TA values have an equal to or less than 1% error compared to the known TA value. Accuracy must be tested before running any samples for the day.**
6. **Samples_Titration**
    - *Note: Autosampler only has 9 sample positions, plan time accordingly. 9 samples takes approximately an hour and a half.* Loading the real water samples will be the same procedure as loading CRM samples.
    - Turn on the scale (to the left of the titrator) and tare a titration cup. Carefully pour 60g of a real water sample. **Each sample must be between 59.00 - 60.00 g.**
        - Record mass value in Putnam Lab notebook with the date and sample ID. Write the ID No. on the side of the cup on tape in sharpie (ex: Conical_8 sample - write "C8" on the cup). Place in position 01 on the Autosampler.
    - Repeat for each water sample. Write the mass values in the notebook in the order positioned in the autosampler.
    - LabX: Analysis > Released Methods: ID 000 | URIBioMin > Right click > Start > T50_GatesLab
        - Click "Add" to add as many ID rows as needed.
        - Input ID No. with the appropriate mass value. If the sample came from a conical, label it "Conical#". If the sample came from a tank, label it "Tank#". Do not include a space between "Tank" and "1". *The R script will only read "Tank1" or "Conical1".* (Ex: "T1"; 59.67)
    - Once finished, view data: LabX > Data > “Reports” > “My Latest Reports” > Double Click the latest "Origin ID 000 | URIBioMin". *The report will be the top result, but be patient while LabX creates the report. Do not export the wrong file, pay attention to the date and time.*
        - "Export as" > "CSV". Name: "yyyymmdd_all" (ex: 20180602_Run1).
    - Add the water sample mass values to the mass file (yyyymmddmass_Run1) with the correct salinity value. Refer to written notes or log in to github.com > search hputnam > BioMin_HIS > RAnalysis > Data > "Daily_Temp_pH_Sal.csv". Choose

the salinity values from the measurement set that matches the time the water was sampled. Log out of Github.com once finished collecting salinity values.

- Follow Step #5 to complete R analysis for the water samples. This procedure will be the same as R analysis for the CRM.
    - EXCEPT - change within R script: titrationfile "20180602_Run1" to "20180602_Run2".
    - Highlight script (ctl-A) and click Run. Any errors in red font in the bottom left box on RStudio will need to be troubleshooted. Ignore
    - Open the new TA file created > "20180602_TA_Run1". Displayed are the TA values for each of the samples run.
- At the end of the titration run, the date's folder should contain 6 CSV files: "yyyymmddmass_Run1", "yyyymmdd_Run1", "yyyymmddmass_Run2", "yyyymmdd_Run2", "yyyymmdd_TA_Run1", "yyyymmdd_TA_Run2"
- Email the previous four files and "pHCalibration.csv" to hputnam@uri.edu.

7. **Clean_Up**

    - CRM must be disposed of in the appropriate waste container. In Gates Lab, the waste bin lives in the furthest lab room in the fume hood. The waste container will be kept here when not being used after the CRM run.
        - After CRM is poured into the waste container, wipe out the titration cups with kimwipes and place them in the appropriate CRM bin. In Gates Lab, this is labeled with green tape and lives on the second shelf next to the Tris, CRM, and original pH calibration solutions. Change gloves when done.
    - All Seawater samples, including the "JUNK" sample, can be disposed of in the sink and rinsed with DI water. Place upside down on brown paper towel to dry.
    - Pour the DI water cup (position 00) in the sink and leave upside down on brown paper towel to dry. Place a dry empty cup in position 00.
        - Raise the Rondolino by pressing the left blue button "Rotate 180 degrees" (with no numbered tick marks). Press the same button to return the Rondolino to position 00.
    - Return the pH probe to storage solution and close the grey cap.
    - Shut off the balance, titrator, and autosampler.
    - Shutdown all computer programs: R, LabX, Internet, etc. Turn off the computer and shut screen. *Double check the correct files were emailed for the day.*
    - Wipe down the counter, and place notebook and pen to the right of the computer.
    - Throw away used gloves and wash hands.

8. **Troubleshooting_FAQ**

    - Titrator tablet will not connect to LabX.
        - Turn off titrator, autosampler, and laptop. Turn on titrator, autosampler, then laptop. Open LabX after the titrator and autosampler have been turned on.
        - Restart the laptop and repeat Step [8.i.a].
    - CRM TA values are not within 1% error of the known batch no. value.

        - Double check pH calibration values are not off.
        - Double check the correct file was exported as "yyyymmdd_Run1".
        - Look for several large liquid drops on the insides of CRM cups. This will affect the mass value but will not be included in the acid titration.
            - Re-measure mass of CRM samples (remember to tare with a dry cup and pour the sample into the new cup) and re-run titration on LabX.
    - RStudio will not run the code and errors are unclear.
        - Double check file names and working directory. Capitalization and spacing matters. Double check the date is correct.
        - Clicking Enter while holding Ctl will allow you to run through the R script line by line. Determine where the error lies and follow instruction.