Monthly DI Water and Air Quality Check

1. SCOPE AND APPLICATION

1.1. Laboratory deionized (DI) water is analyzed monthly to ensure its bacterial counts are within the reporting limits of <1000 CFU/mL, and its nitrogen and total organic carbon levels are acceptable (NH_3<0.10 mg/L, TKN<0.10 mg/L, and TOC <1.0 mg/L). Air quality is checked monthly to ensure that the air in Room B212 (the micro lab) and a cleaned laboratory bench have a bacterial count of <15 colonies for every 15 minutes of air exposure and per cleaned area swabbed (EPA 600-878-017; Standard Methods 9215).

1.2. Annually, the DI water is also checked for metals: SiO2 (<0.10 mg/L), Zn, Ni, Pb, Cu, Cr, and Cd (<0.05 mg/L).

1.3. The monthly DI water samples are collected by the microbiology group under the LIMS test IDs DW-PLATECT, W-NH3, W-TKN, and W-TOC. The annual metals also include LIMS test IDs W-SI-ICP-F and W-ICPMS. The DI water check is entered in a MICRO-DW worksheet and the LAB_WATER_QC_LOG in the LIMS Laboratory Logbook.

1.4. The air quality check does not receive a LIMS test ID. It is entered in the AIR_QUALITY_LOG in the LIMS Laboratory Logbook.

2. SUMMARY OF METHOD

2.1. A cooler containing sample collection containers and sample preservatives is retrieved from the Receiving group. The DI water spigot in Room B212 is turned on to allow any contaminants that have entered the nozzle to be expelled. Field Sheets are filled in and sample containers are labeled. DI water is collected in the DW-PLATECT container and the W-NH3/W-TKN/W-TOC container. The sample taken for the chemistry tests is preserved with Sulfuric Acid (H2SO4). The samples are then preserved on ice and taken to Receiving for log-in.

2.2. The DW-PLATECT sample is picked up for analysis by a MICRO/BENCH analyst. Plate Count Agar (PCA) is melted and poured into labeled Petri dishes, two of which have had 1 mL DI water sample added to them. Plates are covered and left to solidify. Once solid, the plates are turned upside down and incubated for 48±3 hrs at 35° C.

2.2.1 Once a year, additional samples are taken for metals testing and are preserved with ice and Nitric Acid (HNO_3) before being taken to Receiving.

2.3. Tryptic Soy Agar (TSA) is melted and poured into labeled Petri dishes for the air quality check. The air quality plates are placed around the microbiology lab, opened (except for the “Bench surface” plate) and allowed to sit undisturbed for 15 minutes before being closed. The solidified air quality plates are turned upside down and are incubated for 20±4 hrs at 35° C.

2.4. Interferences

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2.4.1. TSA and PCA are non-selective media and can support the growth of many types of bacteria. Aseptic techniques must be used to ensure that there is not contamination of the sample via the media, reagents, supplies, or the analyst.

2.4.2. Human activity (leaning, sneezing, coughing, laughing, talking) directly over or very near the open-air quality plates can cause an overestimation of bacterial density.

2.4.3. PCA and TSA should be tempered to 44-46°C after melting. If it is too warm when mixed with the DI sample, bacteria in the sample could undergo heat shock and potentially die. This would cause the results to be less reliable.

3. EQUIPMENT AND SUPPLIES

3.1. Sterile gloves, latex or nitriles

3.2. Petri dishes, sterile, 100 mm x 15 mm, 12 total

3.3. Glass serological disposable pipette (1 mL), sterile and individually wrapped, with pipette pump

3.4. Polyester-Tipped Applicators, 6 inch, sterile and individually wrapped

3.5. Hot plate

3.6. 1000 mL beaker

3.7. Autoclave gloves and/or silicone mitts

3.8. Low Temp Incubator (S/N 0162177801170112), maintained at 1- 4 °C

3.9. Heratherm 60L Incubator (S/N42511812) or 650F (S/N 303N0034), maintained at 35 ± 0.5 °C

3.10. NIST Traceable Timer

4. REAGENTS AND STANDARDS

4.1. DI water

4.2. Tap water

4.3. Ice

4.4. 95% Ethanol

4.5. Plate Count Agar (PCA), 1 bottle and Tryptic Soy Agar (TSA), 2-3 bottles (prepared per SOP-MB 02.33)

5. CALIBRATION AND STANDARDIZATION

5.1. See FDEP Bureau of Labs Quality Manual, Section 5.5 and 5.6.

5.2. Check temperatures of incubators twice daily at least 4 hours apart. Record in LIMS logbook

5.3. Check thermometers annually against a NIST certified thermometer

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6. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1. Retrieve a prepared sample collection kit from the Receiving group.

6.1.1. Prepared coolers are placed in the hallway outside of Lab Room C 301. Check the label for the correct Project (BIO-QC) description (Monthly Micro QC) or the correct RQ number.

6.2. In the Room B212 turn on the DI water. Let it run in a low, slow, steady stream for approximately 5 min. This allows any contaminants that have collected in the tube and faucet to be expelled.

6.3. Unpack the cooler. Make sure all of the bottles and preservatives have been included and that the labels have the correct test IDs.

6.3.1. Monthly, the kit should include one 250 mL plastic bottle labeled DW-PLATECT, seal unbroken; one 500 mL plastic bottle labeled W-NH3/W-TKN/W-TOC, with a yellow sticker that says "Sulfuric Acid, H_2SO_4"; 1 small vial of H_2SO_4; 1 field sheet with bottle groups (bottle groups sheet is sometimes separate from the field sheet), one black plastic bag, and one packing slip.

6.3.1.1. If everything has been packed correctly, the packing slip can be recycled and the black plastic bag can be stored in the micro lab.

6.3.2. Once a year, the kit should also include 2 additional 500 mL plastic bottles and a small vial of HNO_3. One bottle should be labeled as W-ICPMS and the other as W-SI-ICP-F.

6.3.2.1. The bottle labeled W-ICPMS should also have a small, red sticker that says, "Nitric Acid, HNO_3."

6.4. Complete the field sheet. Fill in the boxes labeled **Collected by:** (collector's name), **Field Report Prepared By:** (preparer's name), **Sampling Agency:** (FDEP), **Location** (FDEP Labs), **Field ID** (DI Water), **Matrix** (DI Water), **Date**, and **Time** sample was collected. Also, check the box next to "**Grab**" and write in the letter "A" in the box labeled **Bottle Group(s).**

6.5. On the bottle labels, write the **Location**, **Field ID**, **Date**, **Time**, and the **Sampler's initials**. (The information on the bottles should match the information on the field sheet.)

6.6. Uncap one of the bottles. Place the opening of the bottle in the DI water stream. (The water can be turned up a little to speed up the collection process.) Fill the bottle completely with DI water (up to the bottle neck). When the bottle is full, remove it from the water stream and cap. Do not turn off the water! Uncap the next bottle and fill it completely with DI water. Continue until all of the bottles have been filled.

6.6.1. If the bottle is too full (above the bottle neck), pour out as much as needed to bring the volume down to the correct level.

6.7. After all of the bottles have been filled, turn off the water.

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6.8. Add the vials of H_2SO_4 and HNO_3 to the bottles with corresponding labels. Cap the bottles and invert the sample several times to mix.

6.9. Place the samples in one of the Ziploc bags that the bottles came in and place in the cooler.

6.10. Cover the samples with ice from the ice machine located in the hallway between the walk-in refrigerator and the walk-in freezer.

6.11. Take the cooler containing the samples, along with the field sheets, to the Receiving Room (Lab C-201) to have the samples logged in and receive a label with sample number.

6.12. On the field sheet, fill in the boxes labeled **Relinquished by:** (your signature) and **Date/Time:** (date/time relinquished). Fill in the box labeled **Shipping Method:** with "hand delivery."

6.13. Sign out the DW-PLATECT bottle by writing on the field sheet, "took 1 micro bottle", along with the date, time, and your initials. Bring the bottle to the lab to conduct the test.

6.14. Samples must be stored at <4 °C until analyzed.

7. SAMPLE PREPARATION

7.1. See SOP BB-30 for sample custody and labels.

7.2. Place 2 bottles of TSA in a 1000 mL beaker and fill with water to just below the necks of the bottles. Do the same for 1 bottle of PCA.

7.3. Place beaker on a hot plate and bring water to a boil until agar is completely melted.

Using silicone mitts/autoclave gloves, remove bottles from the beaker. If not using immediately, place them in a 44.5±0.2 ˚C water bath to cool until ready to use (no longer than 3 hours).
__Note:__ Prepared agar should be melted only once. Any unused melted agar should be discarded.

7.4. On a clean bench, lay out 12 100 x 15 mm Petri dishes. For monthly DI water, PCA is poured into three plates labeled **Control, DI water 1 mL** and **DI water 1 mL dup**. The other nine plates will use TSA for the air quality check and should be labeled **Control 1, Control 2, Hood, Bench, Door, Sink, Filtration Station, Microscope,** and **pH Meter**. On all 12 plates, write the **date**, **media Serial Number**, **S:** (start time), **I:** (incubation time), and **R:** (read time). Leave space to fill in times.

7.4.1. If sample labels have been printed for the DI water, place those on the two designated DI Water plates.

7.5. Add 1 mL Sample DI Water to each of the two plates labeled "DI Water." Pour 10-12 mL of cooled PCA into all three of the plates, gently swirling the plates as the media is poured. The time the media is added to the sample is the **S:** (start) time. Cover the DI plates and allow the media to solidify. Continue gently swirling clockwise, counter-clockwise, forward and back, and side to side to ensure even mixture and

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distribution of the DI water. Take care to not splash the media over the side of the dish.

7.5.1. Media must be cooled to 44.0-46.0 ˚C before it is poured into the DI water sample dishes to prevent killing any bacteria that may be present in the water. An easy test is to place a **cooled** bottle against the wrist. It should be close to body temp. (**If the bottle is too hot to handle with bare hands, it is too hot to place against the wrist!**) Another option is to place the leftover media in a 44.5±0.2 ˚C water bath to cool until ready to use (no longer than 3 hours), as in step 7.4.

7.6. Invert the DI plates and incubate at 35 ± 0.5 °C for 48± 3 hours. Record the incubation time (**I:**) on the Control plate.

7.7. In the DI Water sample Prep Worksheet created in Step 7.1., fill in the boxes labeled "Prep Start Date/Time," "Prepared By," "Media 1" (enter PCA Serial Number), and "Date/Time Incubated", "Pipette QC ID", "Petri Dish QC ID" and "Incubator ID".

7.8. Designate the TSA bottles as "C1" and "C2". These will correspond with the Control 1 and Control 2 plates. Including controls, there should be 4 plates using one bottle and 5 plates using the other bottle. Write "C1" or "C2" on each plate to indicate which bottle was used.

7.8.1 If there is not enough TSA in either bottle to pour 5 plates, a third TSA bottle can be melted, an extra "Control 3" plate must be set up, and "C3" must be written on the air quality plates that were used with that bottle.

7.9. Pour about 10-12 mL of the melted TSA into each of the nine air quality plates. Cover the plates and allow the media to solidify.

7.10. After all plates have solidified, set a certified timer for 15 minutes. Place the plates around the micro lab in their corresponding locations (Hood, Door, Sink, Filtration Station, Microscope, and pH Meter). Start the timer while removing the first lid and immediately remove all other lids, resting them face-up beside their respective Petri dishes. The time the plates are uncovered is the **S:** (start) time. Invert the Control plates and the Bench plate.

7.11. After 15 min, replace the lids to cover the Petri dishes.

Clean a small area of the bench with prepared ethanol. Carefully remove the polyester-tipped applicator from its wrapper. Touch only the handle of the applicator, not the tip. Swab the disinfected bench with the tip. Swipe the applicator tip onto the solidified agar in the dish labeled "Bench." Discard the applicator in a biological waste container. Replace the lid on the dish.

7.12. Invert all of the plates and incubate at 35 ± 0.5 °C for 20 ± 4 hrs. Record the incubation time (**I:**) on each Control plate.

7.13. In the Prep Worksheet, fill in the boxes labeled "Prep Start Date/Time", "Prepared By", "Media 1" (enter TSA Serial Number), "Date/Time Incubated", "Petri Dish QC ID" and "Incubator ID".

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8. SAMPLE ANALYSIS

8.1. After incubation, remove the plates from the incubator. Record the read time (**R**:).

8.2. Count every colony on the agar.

8.2.1. If there are "spread" colonies, not every individual colony must be counted. If there is a chain of colonies that look as though they originated from the same bacterial clump, but were spread due to mixing of the sample and agar, count the chain as one colony. If the spread colonies have created a film of growth between the agar and the bottom of the Petri dish, count the film as one colony. If the spread colony is caused by bacteria growing in a film of water over the surface of the agar, count that as one colony.

8.2.2. Do not count "fuzzy" looking growth (fungi).

8.3. Record the count on the lid. Draw a circle around the number.

8.4. In the appropriate Prep Worksheet, record the "Read Date/Time" and "Plates Read By". Fill in the "Date/Time Reviewed" and "Reviewed By" boxes, as well as any other missing information. "Complete" the Worksheet.

9. DATA ARCHIVAL

9.1. Record the DI Water colony counts in **LIMS/Laboratory/Technical/Laboratory Logbook/Micro_Data_Log**.

9.1.1. Enter the Sample ID into the "**Sample ID**" box.

9.1.2. Average the colony counts from the two DI Water plates. Round up to the next whole number and enter the result in the "**Entry Value**" box. Record the results of each individual plate, and both Control plates, in the "**Comments**" box.

9.1.3. "**Entry Type**" should be "1 mL Count." The PCA serial number should be entered in the "**Lot Number**" box. "**Batch Number**" is the date the sample was prepped. The format is MMDDYY-DW (ex: 042915-DW). "**Test ID**" is DW-PLATECT and "**Date Entered**" is the analysis date. "**Entered By**" is the analyst.

9.1.4. The Location box should automatically be filled with "B212," the Category box with "Microbial Analysis Result," the Sub Category box with "Sample Result," the Entry Units box with "CFU," and the Date Received and Expiration Date boxes with "12/30/1899." All other entry boxes should be automatically filled with "N/A."

9.2. Upload the DI Water sample into QC Manager following SOP BB-31.

9.3. Enter the Air Quality plates in **LIMS/Laboratory/Technical/Laboratory Logbook/Air_Quality_Log**. Select an entry with the location of the plate to be entered. All entry boxes should be automatically filled with the correct information, except for **Entry Value**. In that box, enter the number of colonies counted. In the **Lot Number** box, enter the media (TSA) Serial Number. In **Entry Comments**, enter

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control results and corresponding Micro-QC Worksheet number. Repeat until all seven sample locations have been entered.

10. QUALITY CONTROL

10.1. For each bottle of media used, pour enough agar to cover the bottom of its corresponding Control plate. Incubate with the Sample/Air Quality plates.

10.1.1. Growth indicates contamination.

10.1.2. No growth indicates no contamination.

10.2. Refer to QA manual for corrective action and handling out-of-control data.

11. SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1. All media/bacteria must be autoclaved for 45 minutes before disposal.

11.2. Refer to the Laboratory Health and Safety Manual.

11.3. Read MSDS information for reagent handling and disposal.

12. REFERENCES

12.1. Standard Methods, Sections 9215A and 9215B

12.2. NELAC Quality Systems Manual

12.3. Bureau Quality Manual http://www.dep.state.fl.us/labs/library/progplan.htm

12.4. Laboratory Health and Safety Manual http://depnet/burlabs/safety.htm

12.5. FDEP SOP-001/01 FS 1000, *General Sampling Procedures*

12.6. FDEP SOP BB-30: *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

12.7. FDEP SOP MB-02.33, *Preparation of Dehydrated Culture Media*

Appendix of Changes

04/22/2020	Replaced HPC with PCA or TSA and made adjustments throughout SOP in accordance to the use of these agars. Added timer documentation requirements to section 7.12
04/02/2021	Edited Sample Preparation section for clarity. Changed room to B212 and added Matrix to field sheet instructions.

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