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.

1.4. The air quality check does not receive a LIMS test ID. It is entered into LIMS Laboratory Log Book.

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 B205 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 nitrogen 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. 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 DI water and air quality plates are turned upside down and are incubated for 48±3 hrs and 20±4 hrs respectfully at 35° C.

2.3. 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.

2.4. Interferences

2.4.1. TSA and PCA are non-selective medium 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.

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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 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, 11 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), 2 bottles 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.

6.2. In the Room B205 turn on the DI water. Let it run in a low, slow, steady stream for approximately 5 min.

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 120 mL plastic container with an orange cap labeled DW-PLATECT, seal unbroken, one 500 mL plastic bottle labeled W-NH3/W-TKN/W-TOC, 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.

6.3.1.2. The 500 mL bottle should also have a small, yellow sticker that says, "Sulfuric Acid, H_2SO_4."

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), **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, along with the field sheets, to the Receiving Room (Lab C-201).

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

6.13. 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 PCA in a 1000 mL beaker and fill with water to just below the necks of the bottles.

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

7.4. 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.

7.5. On a clean bench, lay out twelve 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 9 plates are for the air quality check and labeled as the following: **Control 1, Control 2, , Hood, Bench, Door, Sink, Filtration Station, Microscope,** and **pH Meter**. Write the **date** and media SN used on every plate.

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

7.6. On every plate, except the controls, randomly write **C1** or **C2**. C1 and C2 correspond to the Control 1 and Control 2 plates. The control plates correspond with 1 bottle of media each. Because there are 9 plates used for air quality, there should be 4 plates using 1 bottle and 5 plates using the other (may not get the full 5 out of 1 bottle so may have to melt a third TSA bottle).

7.7. On both Control plates and both DI water plates, also write the PCA Serial Number, and write **F:** (filter time), **I:** (incubation time), and **R:** (read time). Leave space to fill in times.

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7.8. Designate one bottle of TSA for Control 1 and C1 plates, the other for Control 2 and C2 plates. Pour about 10-12 mL of the melted TSA into each of the corresponding plates, except for the DI water plates which PCA will be used. Allow the leftover media to cool, but not solidify.

7.8.1. 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.

7.9. Cover the plates and allow the media to solidify.

7.10. Add 1 mL Sample DI Water to each of the two plates labeled "DI Water." Pour cooled media into the plates, swirling the plates as the media is poured. After the media has been poured, continue swirling clockwise, counter-clockwise, forward and back, and side to side to ensure even mixture and distribution of the DI water. Take care to not splash the media over the side of the dish.

7.10.1. The time the media is added to the sample is the **F:** (filter) time.

7.11. Cover the DI Water plates and allow the media to solidify.

7.12. Set a certified timer for 15 minutes. Place the solidified agar plates around the micro lab at the locations written on them (Hood, Door, Sink, Filtration Station, Microscope, and pH Meter) and remove their lids, resting them on the edge of their Petri dishes (inside facing down, outside facing up). Start the timer when the first lid is removed. Invert the Control plates, the DI Water plates, and the Bench plate.

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

7.14. 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.

7.15. Invert all of the plates and incubate at 35 ± 0.5 °C for 48 hours ± 3 hours (PCA plates) and 20±4 hrs (TSA plates). Record the incubation time (**I:**) on the Controls of both the DI Water and air quality plates.

7.16. 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."

8. SAMPLE ANALYSIS

8.1. After incubation, remove the plates from the incubator.

8.2. Count every colony on the agar.

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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. Also, 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 DI Water sample Prep Worksheet, record the "Read Date/Time" and "Plates Read By". "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.

9.4 Document all support equipment and consumables used in Micro-QC Preparation Worksheet.

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.

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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

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