Pour Plate Method for Heterotrophic Plate Count

1. SCOPE AND APPLICATION

1.1. The heterotrophic plate count estimates the number of viable heterotrophic bacteria in water. The pour plate method can be used for 0.1-2.0 mL of sample. This method is used in the laboratory to conduct several quality control tests including, water suitability test, use test and monthly water quality tests.

2. SUMMARY OF METHOD

2.1. Small aliquots of sample are pipetted into duplicate Petri dishes. 10-12 mL of Plate Count Agar (PCA) is added to each plate. The plates are carefully swirled to mix the sample with the agar. After the media solidifies at room temperature, the plates are inverted and incubated for 48 ± 3 hours. The ideal colony count should be between 30 and 300 colonies for at least one dilution of sample. The reporting limit is <1000 CFU/mL.

2.2. Interferences

2.2.1. PCA is not selective and can support the growth of many types of bacteria. Aseptic techniques must be used to ensure that there is no contamination of the sample via the media, reagents, supplies, or the analyst.

2.2.2. PCA 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 could potentially die. This would cause the results to be less reliable.

3. EQUIPMENT AND SUPPLIES

3.1. Petri dishes, sterile, 100 mm x 15 mm, 4-8/sample

3.2. Volumetric pipets, 1 mL and 10 mL – each new lot is checked per SOP MB-03.3

3.3. Hot plate

3.4. 1000 mL beaker

3.5. Heratherm IMH 60 L (SN 42511812) or Isotemp 60L (SN 43315553) maintained at 35 ± 0.5°C

3.6. Thermo Scientific water bath (S/N 300561398 or 300536247) maintained at 44.5 ± 0.2°C.

4. REAGENTS AND STANDARDS

4.1. Plate Count Agar (PCA), 1-2 bottles per sample (prepared per SOP MB-02.33)

4.2. Dilu-Lok 99 mL sterile buffered water bottle, 2 per sample

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5. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1. Samples must be stored at <4˚C until analyzed. See SOP FS 1000 and Bureau of Labs Quality Manual for general sample collection and preservation.

5.2. Holding time

5.2.1. Potable: within 30 hours of collection

5.2.2. Non-Potable: received and filtered within 8 hours of collection

6. SAMPLE PREPARATION

6.1. See SOP BB-030 for sample custody and label instructions.

6.1.1. Label duplicate Petri dishes (two plates per sample dilution) with bar code label and dilution of sample.

6.1.2. Sample dilutions should be based on expected pollution levels. The ideal range should be 30-300 colonies for at least one dilution. For samples where no growth is expected, analyze as a potable water sample.

6.1.2.1. Potable water sample: use 1 mL and 0.1 mL dilutions

6.1.2.2. Non-Potable water sample: use 0.01 mL and 0.001 mL dilutions

6.1.2.3. Polluted Non-Potable water sample: use 0.0001 mL dilution

6.2. Melt PCA

6.2.1. Place two pre-filled PCA bottles into a 1 L beaker. Add de-ionized water until the water level reaches just below the caps of the bottles (≈ the necks). Because each sample needs one-to-two bottles of media (including its control), you may have to use more than one beaker for a batch.

6.2.2. Place beaker(s) on a hot plate(s) and bring water to a boil until agar is completely melted. Do not allow the water to dry out; monitor continuously.

6.2.3. Remove bottles from the beakers and allow them to cool to 44.0 - 46.0˚C before pouring. This can be done by placing the bottles in a water bath. Media can be kept at 44 - 46 °C for up to 3 hours in the waterbath. Media should still be liquid, but cool enough to handle. Prepare only as many samples as you can complete the dilution and plating steps within 20 minutes, to avoid agar solidifying prematurely.

Note: Prepared agar should be melted only once. Any unused melted agar should be discarded.

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7. SAMPLE ANALYSIS

7.1. Vigorously hand-shake the bottle containing the sample approximately 25 times.

7.2. Potable water samples

7.2.1. Use sterile pipettes to transfer 1 mL of undiluted (100%) sample into duplicate Petri dishes (1 pipette per plate, 2 plates total). Pour an additional plate per bottle used as a control.

7.2.2. Add 10-12 mL of the melted PCA to each dish

7.2.3. Mix the inoculated medium carefully to prevent spilling. Swirl the plate 5 times in each direction (counterclockwise, clockwise, and back-and-forth motion).

7.2.4. Allow medium to solidify at room temperature (about 10 minutes).

7.2.5. Invert plates and incubate in an incubator at 35.0±0.5˚C for 48±3 hours.

7.2.6. After incubation, select the plates with 30-300 colonies. Use a colony counter to count all the colonies on each of those plates. For each plate, record the count on the lid with a permanent marker.

7.3. Non-Potable water samples

7.3.1. Prepare a 1:99 dilution of the original 100% sample.

7.3.1.1. Use a sterile pipette to transfer 1 mL of the original sample into a Dilu-Lok 99 mL sterile buffered dilution water bottle.

7.3.1.2. Mix thoroughly by shaking vigorously approximately 25 times.

7.3.2. Use sterile pipettes to plate 1 mL and 0.1 mL of the 1:99 sample dilution into duplicate Petri dishes (1 pipette per plate, 4 plates total). The new dilutions will be 0.01 mL and 0.001 mL, respectively.

7.3.3. Continue with steps 7.2.2.-7.2.6.

7.4. Polluted Non-Potable water samples

7.4.1. Further dilute the original sample by using a sterile pipette to transfer 1 mL of the 1:99 sample dilution into another Dilu-Lok 99 mL sterile buffered dilution water bottle and mix thoroughly by vigorous shaking approximately 25 times.

7.4.2. Use sterile pipettes to plate 1 mL of the second 1:99 sample dilution into duplicate Petri dishes (1 pipette per plate, 2 plates total). The new dilution will be 0.0001 mL.

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7.4.3. Continue with steps 7.2.2.-7.2.6.

8. DATA ARCHIVAL

8.1. Document the supply QC record number (QC ID) of all disposable supplies used in the preparation worksheet.

8.1.1. For all lab QC tests such as Use Test, monthly DI water test, and IRT test, document all information including QC IDs of supplies in a LIMS Micro-QC preparation worksheet.

8.2. Record counts and sample data in appropriate columns in the **Micro_Data_Log**, found in **LIMS/Laboratory/Technical/Laboratory Logbook**.

8.3. Print a Micro Data Log Report. Go to **LIMS/Laboratory/Technical/Laboratory Logbook**. Click on **Reports** in the top left corner of the window. Choose the appropriate report and print.

8.4. Verify the colony counts and dilutions. Refer to SOP BG-01.

8.4.1. After verification, place dishes in the hazardous waste bin.

8.5. Upload according to SOP BB-31.

9. QUALITY CONTROL

9.1. Perform sterility checks of each lot of disposable supplies by swirling a small amount of TSB 2X (See SOP MB-02.33) to contact all surfaces. Also incubate a separate container with an aliquot of TSB 2X to confirm the sterility of the media. Incubate at 35.0 ± 0.5 deg C for 24 ± 2 hours.

9.1.1. Document sterility checks in MICRO-QC worksheet accordingly.

9.2. For each set of samples, pour enough PCA into sterile Petri dishes (1 per bottle of media used) to cover the bottoms of the dishes. Incubate with the sample dishes.

9.2.1. Growth indicates contamination.

9.2.2. No growth indicates no contamination.

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

10. SAFETY/HAZARDOUS WASTE MANAGEMENT

10.1. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management.

10.2. Read MSDS information for reagent handling and disposal.

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11. REFERENCES

11.1. Standard Methods Online Edition, Section 9215A-00 and 9215B-00

11.2. FDEP Laboratory Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

11.3. Laboratory Health and Safety Manual https://www.floridadep.net/resources/health-safety-manual

11.4. DEP SOP MB-03.3 *Calibration and Use of Mechanical and Disposable Pipettes*

11.5. DEP SOP FS-1000 *General Sampling Procedures*

11.6. DEP SOP MB-02.33 *Preparation of Dehydrated Culture Media*

11.6. DEP SOP BB-030 *Sample Custody, Preparation Labels and Worksheet Instructions for Bench Biology Samples*

11.7. DEP SOP BG-01 *Data Entry Verification*

11.8. DEP SOP BB-031 *LIMS QC Manager Data Uploading Instructions for Bench Biology and Microbiology Samples*

12. TABLES, DIAGRAMS

Table12.1 Dilution Preparations

Step	Potable	Non-Potable	Polluted
Initial Sample	Water Sample	1 mL into 99 mL Dilution Water	1 mL into 99 mL Dilution Water
Dilution Step 1	1 mL	1 mL	1 mL
Dilution Step 2	1 mL	0.01 mL	0.0001 mL
Dilution Step 3	1 mL	0.01 mL	0.0001 mL

Note: Potable samples are expected to have low microbial loads and are processed with undiluted serial 1 mL transfers.

Non-potable samples undergo a 1:100 dilution followed by smaller transfers to account for moderate contamination.

Polluted samples require greater dilution due to higher microbial concentrations and the need for accurate enumeration.

Appendix of Changes

08/11/2008 Updated to SM 9215B Online Edition and added a references section.

08/12/2010 Added Introduction and updated reference to new version of safety plan.

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08/08/2011	Corrected reference to quality manual, added specific incubator used in materials section.
07/20/2012	Updated format; added DEP SOP FS-1000, DEP SOP BB-030, and DEP SOP BB-032 to References section.
07/16/2013	Changed references to PCA to HPC.
11/16/2015	Remove reference to the old water bath and updated reference to Health and Safety Manual.
09/15/2017	Updated incubator information.
09/01/2019	Corrected Heratherm Incubator Model number. Added tempering in water bath to 6.2.3. Added Precision water bath information to Section 3: Equipment and Supplies list (3.6).
09/30/2019	Replaced references of HPC with PCA. Updated Data Archival and Quality Control sections.
09/29/2021	Updated reference to FDEP Laboratory Quality Manual and Laboratory Health and Safety Manual in References Section.
11/02/2022	Updated sections 1.1 and 7.2.1. Updated Figure 12.1 to reflect the use of one dilution for the test.
10/12/2023	Updated sections 6.2 and 9.1. Updated water bath serial numbers
10/31/2024	Updated section 3.5 with new incubator information.
09/22/2025	Updated 6.2.3 to set a 3-hour limit on agar tempering at 44-46 °C, replaced figure 12.1 with a concise table that includes notes on potable, non-potable and polluted samples, and reworded key steps for clearer readability.

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