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Heterotrophic Plate Count (HPC) Pour Plate Method Standard Method 9215B

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1 Scope and Application

This method is used to determine the number of live heterotrophic bacteria in a sample. Samples are plated on plate count agar, incubated for 48 hours, and counted. This was formerly known as the Standard Plate Count. The pour plate method can accommodate volumes of sample or diluted sample ranging from 0.1 to 2.0 ml. The resulting number of colonies is recorded in CFU's or colony forming units. In preparing plates, plate sample volumes that will give from 30 to 300 colonies per plate. The goal is to have at least one dilution giving colony counts between these limits. Primarily, HPC is used as a quality control tool. Monthly, it is performed to test the quality of the deionized water.

2 Definitions

2.1 Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control Definitions. (SOP Reference 13.2)

3 Interferences

3.1 Method interferences may be caused by contaminants in reagents, media, bottles, or glassware. To abstain from interferences, all reagents and media are tested for sterility prior to use. Also, all bottles and glassware are washed, sterilized, and tested prior to use.

3.1.1 All glassware must be washed, sterilized, and put in the hot air oven at 180°C for 2 hours. A pH check is performed on all batches of glassware using a 0.04% solution of bromothymol blue. After drying and cooling, store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Purchased transfer pipettes are received with a certificate of analysis which ensures sterility of product and accuracy within a 2.5% tolerance.

3.1.2 Dilution water that is prepared commercially is tested for sterility upon receipt. Dilution water that is prepared in house is aseptically prepared and sterilized according to prescribed methods. Each batch of water is tested for sterility.

4 Safety

4.1 Refer to Laboratory Chemical Hygiene Plan and Fire Safety Plan, online revision. (SOP Reference 13.3)

5 Apparatus and Equipment

5.1 35º ± 0.5º C Incubator
5.2 Round – 120 ml or 250 ml (4 or 8 oz) bottles
5.3 Hot Plate
5.4 Metal Pan (to melt agar)
5.5 Thermometer
5.6 Pipettes (glass or plastic)
5.7 Pipet Holder
5.8 100 x 15 mm Petri Dishes (glass or plastic)
5.9 Wire Baskets
5.10 Colony Counter
5.11 125 ml amber narrow mouth glass bottle
5.12 Free Chlorine Strips

6 Reagents

6.1 Plate Count Agar
6.2 Dilution Water
6.3 Deionized Water

7 Sample Collection

7.1 Refer to Chapter 5 of the Georgia EPD Laboratory Quality Assurance Manual for Sample Container, Sample Preservation and Sample Holding Times.

8 Calibration

8.1 There are no calibrations associated with this method. As with any test for bacteria, all equipment and reagents must be sterile to ensure accuracy. This includes the glass or disposable plates and pipettes, and dilution water.

9 Quality Control

9.1 Refer to Table 14.1 Quality Control Acceptance Criteria associated with this method.

10 Procedure

10.1.1 Collect a sample of deionized water in a 250 ml round bottle that does not contain sodium thiosulfate. (**ALLOW THE WATER TO RUN FOR 2-3 MINUTES BEFORE COLLECTION**). Shake sample 25 times. Immediately collect another sample in the 125 ml amber glass bottle.
10.1.2 Test sample for free chlorine using the strips. Record the result.
10.1.3 Label ten (10) glass or plastic plates as follows:
2 - 1.0 ml
2 - 0.1 ml
2 - .01 ml
2 - .001 ml
1 - Control

1 - Dilution Water

10.1.4 Melt four (4) bottles of plate count agar and maintain the water in which it's tempered between 44°C and 46°C until ready for use. Note: Melted agar should not be held longer than 3 hours.

10.1.5 Select two (2) bottles of leveled dilution water: one to be used for the actual dilution and one to be used as a control.

10.1.6 Pipette 1.0 ml and 0.1 ml respectively from the original sample bottle of deionized water into two (2) plates for each dilution (1.0 and 0.1).

10.1.7 From the original bottle of deionized water pipette 1.0 ml of water into a leveled bottle of dilution water and label as bottle "A".

10.1.8 Pipette 1.0 ml and 0.1 ml respectively from bottle "A" into two (2) plates each for the last two dilutions (.01 and .001).

10.1.9 Add at least 10 – 12 ml of tempered agar to each plate and swirl to mix. Allow plates to cool and solidify. Turn plates upside down and place in a wire basket. Do not stack plates more than 4 high and arranged in the incubator to allow proper air circulation. Incubate plates for 48 hours at 35º ± 0.5º C, then read.

10.1.10 Upon completion of the test take the amber sample bottle and orange capped bottle of water to Receiving to be distributed for further testing of TOC, pH, and conductance.

Record the results.

10.2 To Read Plates:

After 48 hours of incubation, remove plates and prepare to read under the colony counter.

10.2.1 Examining each plate individually, count each colony and record the total number of colonies under the proper dilution.

11 Calculations

11.1 Calculations are used when reporting results (i.e. PT reporting). No calculations are used for QC, just final counts are reported.

11.2 To compute the heterotrophic plate count, CFU/ml,

11.2.1 **Plates with 30 to 300 colonies**, take the average count of both plates (must be the same dilution), then divide total number of colonies by the sample volume.

$$\frac{\text{Total number of colonies}}{\text{Total volume of tested}} = \text{CFU/ml}$$

If two or more consecutive dilutions are used, independently carry each calculation of plate count to a final count per ml, then calculate the mean of these counts/ml.

$$\frac{280}{.01} = 28{,}000/\text{ml} \qquad \frac{34}{.001} = 34{,}000/\text{ml}$$

Reporting value: $\frac{28000 + 34000}{2} = 31{,}000$ CFU/ml

11.2.2 **Plates with less than 30 colonies –** If there are less than 30 colonies on **all plates**, record the actual number of colonies on the plate that contains the largest sample volume (lowest dilution). For example, if volumes of 0.1, 0.01, and 0.001ml were plated and produced counts of 22, 21 and 0 colonies respectively, the colony count of 22 from the largest sample volume (0.1 ml) would be selected. These results should be reported as Estimated Value

Reporting value: $\frac{22}{0.1} =$ 220 CFU/ml (Estimated Value)

Note: If 1ml volume of original sample is used and produces less than 30 colonies the actual counts are reported.

11.2.3 **Plates with no colonies** - If all dilutions have no colonies, report the count as less than 1 divided by the corresponding largest sample volume used. For example, if 0.1, 0.01 and 0.001 ml sample volume is used with no colonies.

The largest sample vol or lowest dilution is 0.1 ml

Reporting value: $\frac{1}{0.1} =$ <10 CFU/ml estimated value

11.2.4 **Plates with greater than 300 colonies** – When counts per plate in the highest dilution is greater than 300 colonies, multiply the mean count by the dilution used as a greater than (>) CFU/ml. For example, if the duplicate plates volumes are 1.0, 0.1, and 0.01 and produced a mean count of >500, >500 and 340 colonies respectively, the result is 34,000 CFU/ml

Reporting value: $\frac{340}{0.01} = 34{,}000$ CFU/ml

When colonies on duplicate plates and /or consecutive dilutions are used and averaged before being recorded, round off counts to two significant figures only when converting to CFUs. To report two significant numbers, record only the first two left-hand digits. Raise the second digit to the next higher number when the third digit from the left is ≥ 5; use zero for each successive digit toward the

right from the second digit. For example, report a count of 142 as 140 and a count of 155 as 160, but report a count of 35 as 35.

12 Waste Management

12.1 See GA EPD Laboratory SOP – EPD Laboratory Waste Management Standard Operating Procedures, SOP 6-015, online revision.

13 References

13.1 Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association: Washington, D.C., 1998 or later.

13.2 GA EPD Laboratory Quality Assurance Plan, online revision.

13.3 GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

13.4 GA EPD Laboratory SOPs – Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.5 Microbiological Methods for Monitoring the Environment Water and Wastes. EPA-600/8-78-017.

13.6 Manual for the Certification of Laboratories Analyzing Drinking Water, Fifth Edition. EPA 815-R-05-004.

14 Practical Quantitation Limits (PQLs), Precision and Accuracy Criteria and Quality Control Approach

Table 14.1 Summary of Data Quality Objectives

Method	Parameter	QC Check	Min. Frequency	Accepted Criteria	Corrective Action
SM 9215B- Heterotrophic Plate Count (HPC)-Pour Plate Method	Agar Control	Agar Plated with no Inoculum	1 per set Monthly	No Growth	Discard Agar
	Dilution Water Control	1 ml of Dil. water plated	1 per set Monthly	No Growth	Discard Dilution water
SM 9215B- Heterotrophic Plate Count (HPC)-Pour Plate Method	Deionized Water	Deionized water inoculated agar in the volumes of 1.0 ml, 0.1 ml, 0.01 ml & 0.001 ml.	2 plates per dilution (Total of 8) Monthly	< 500 CFU/mL	Check filters and replaced if necessary. Repeat test.

Updates to previous version:
Updated online revision