Determination of Heterotrophic Plate Count (HPC) in Water by Pour Plate, Spread Plate, Membrane Filtration and Enzyme Substrate Test Methods

Parameter(s)

Heterotrophic Plate Count

Analytical Method

Pour Plate
Spread Plate
Membrane Filtration
Enzyme Substrate

Introduction

The heterotrophic plate count (HPC), formerly known as the standard plate count, is a procedure for estimating the number of live culturable g-heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools

Four prescriptive test method options are described in the Method Summary section below. Choose the procedure that best complies with the application of the information.

A licence must be obtained from the Public Health Agency of Canada (PHAC) to purchase the control organisms required for these tests. Refer to the PHAC website.

Method Summary

Four prescriptive test method options are described in the reference method:

a) **Pour Plate:** The procedure is simple to perform and can accommodate volumes of sample or diluted sample ranging from 0.1 to 2.0 mLs. The colonies produced are relatively small and compact, and less likely to encroach on each other than those produced by surface growth. However, submerged colonies can be slower growing and difficult to transfer. A thermostatically controlled water bath is essential for tempering the agar, and care is needed to prevent heat shocking the bacteria when dispensing the hot agar. Replicating every volume and dilution plated analyses is not required.

b) **Spread Plate:** This procedure causes no heat shock and all colonies are on the agar surface where they can be easily distinguished from particles and bubbles. Colonies can be quickly transferred and morphology easily discerned. However, this method is limited by the small volume of sample or diluted sample that can be absorbed by the agar: 0.1 to 0.5 mLs depending on the degree to which the pre-poured plates have been dried. A supply of pre-dried, absorbent agar plates must be maintained to use this procedure.

c) **Membrane Filtration:** This procedure permits testing of large volumes of low-turbidity water. It produces no heat shock. Disadvantages include the expense for the membrane filtration equipment, the smaller display area of the filter, the need to detect colonies by reflected light against a white

background if coloured filters or contrast stains are not used, possible damage to cells by excessive filtration pressures, and possible variations in membrane filter quality.

d) **Enzyme Substrate:** This procedure can be used with samples having a wide range of bacterial concentrations. The method uses a substrate-based medium in which the substrates are hydrolyzed by microbial enzymes causing the release of 4-methylumbelliferone maximally after 48 hours of incubation at 35˚C. 4-Methylumbelliferone fluoresces when exposed to long-wavelength (365 nm) ultraviolet light. The number of fluorescing wells corresponds to a most probable number (MPN) of bacteria in the original sample. This test produces no heat shock and is comparable in performance to the pour plate method.

MDL(s) and EMS Analyte Code(s)

Analyte	Approx. MDL (units)	EMS Analyte Code
Pour Plate		
Heterotrophic Plate Count	1 CFU/mL	
Spread Plate		
Heterotrophic Plate Count	1 CFU/mL	
Membrane Filtration		
Heterotopic Plate Count	1 CFU/100 mL	SPCN X385
Enzyme Substrate		
Heterotrophic Plate Count	1 MPN/100 mL	

Matrix

Water

Sample Handling and Preservation

The sample is collected in the field and submitted unfiltered and unpreserved in a sterilized water bacteriology bottle containing sufficient sodium thiosulfate to neutralize up to 15 mg/L residual chlorine, or a minimum of 10 mg/container. Sodium Thiosulfate is effective in neutralizing the bactericidal effect of chlorine, neutralizing residual halogens, and preventing continuation of bactericidal action during sample transit.

Stability

Holding Time: Incubation must begin within 24 hours of sample collection for results to be valid. Minimum volume required for analysis is 100 mL ± 2.5 mL (APHA 9060 B.1. 2006).

Storage: The sample should be kept cool (at <8°C) during transport and storage until analysis. Do not freeze samples (APHA 9060 B.1 2006).

Procedure

PRECAUTIONS

Work aseptically to prevent contamination of lab personnel and the lab area, and to prevent cross-contamination between samples. Refer to the *Government of Canada Canadian Biosafety Standard* for more information.

Incubation temperatures and times are important to prevent false positive and false negative reactions. The incubation details are provided by the manufacturer and must be followed.

Where subsampling occurs, be sure to homogenize the sample well prior to sub-sampling.

TEST PROCEDURE

The procedures are described in detail in APHA 9215:

- Section A introduction
- Section B Pout Plate Method.
- Section C Spread Plate Method
- Section D Membrane Filter Method
- Section E Enzyme Substrate Method

Refer to APHA 9020 for guidance on quality control testing practices for the evaluation and maintenance of equipment, media and organisms.

Data Analysis

Refer to reading instructions in the applicable APHA 9215 section.

Quality Control

Proofing of sample bottles, organisms, and supplies by lot is recommended to demonstrate sterility and performance prior to use. Refer to APHA 9020 for more information on recommended Quality Control practices for this test.

Summary of QC Requirements		
QC Component	**Minimum Frequency**	**Minimum Data Quality Objectives**
Method Blank (MB)	One per batch (max 20 samples)	Less than reported DL
Lab Duplicates (DUP)	1 per batch (max 20 samples)	± 65% RPD
Positive Control	One per day per incubator	Expected reaction to confirm proper operation of incubator and performance of the test.
If DQOs are not met, repeat testing or report qualified test results. If Analyst precision criteria is not met additional training may be needed.		

Method Blank: The method blank is 100 mL sterile water poured into a 120 mL sample bottle, (containing sodium thiosulfate if used with test samples).

Laboratory Duplicates: Sample duplicates are prepared when sufficient sample is received to subsample for laboratory duplicates. Homogenize the sample well prior to subsampling into individual 120 mL sample bottles.

Positive Control: Any organism that will provide a positive reaction is suitable t demonstrate that the incubator is operating as expected (gets to the right temperature at the right rate). *Enterobacter aerogenes* has been shown to have good performance characteristics for this test. Refer to APHA 9020 for more information.

References

1. APHA 9215 (2004) Heterotrophic Plate Count.
2. APHA 9060 (2006) Samples.
3. APHA 9020 (2005) Quality Control

Revision History	November 14, 1994:	Publication in 1994 Lab Manual
	November 14, 2002:	SEAM Codes replaced by EMS codes
	December 8, 2017:	New Format (BC Lab Manual Prescriptive Method 2016), APHA 9215 revised in 2004. APHA 9060 revised in 2006. APHA 9020 revised in 2005. Prescriptive nature to tests are confirmed. QC section includes Method Blanks and Duplicate Samples.