Microbiology
Revision Date: Dec 20, 2019

Heterotrophic Plate Count (HPC) in Water - Prescriptive

Parameter | Heterotrophic Plate Count
Analytical Methods | 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 and are authorized for use.

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 applicable prescriptive test method options may be used for HPC testing:

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 mL. 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 mL 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 sample. This test produces no heat shock and is comparable in performance to the pour plate method.
**MDL(s) and EMS Method & Analyte Code(s)**

<table>
<thead>
<tr>
<th>Method Version / Analyte</th>
<th>Approx. MDL</th>
<th>EMS Analyte / Method Codes*</th>
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| Pour Plate
Heterotrophic Plate Count | 1 CFU / mL | SPCN / not available |
| Spread Plate
Heterotrophic Plate Count | 1 CFU / mL | SPCN / not available |
| Membrane Filtration
Heterotrophic Plate Count | 1 CFU / mL | SPCN / X385 |
| Enzyme Substrate
Multi-well / Quanti-tray
Heterotrophic Plate Count | 1 CFU / mL | SPCN / not available |

*Refer to EMS Parameter Dictionary on the ministry website for all current EMS codes.

**Matrix**

- Water

**Interferences and 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.
- Refer to Method Summaries for interferences particular to each method option.

**Sample Handling and Preservation**

- The sample is collected in the field and submitted unfiltered in a sterilized bacteriology bottle containing sufficient sodium thiosulfate to neutralize up to 15 mg/L residual chlorine, or a minimum of 10 mg anhydrous / 120 mL container or 15 mg of the pentahydrate form. Sodium Thiosulfate is effective in neutralizing the bactericidal effect of chlorine, neutralizing residual halogens, and preventing continuation of bactericidal action during sample transit.
- **Holding Time:** Incubation must begin within 24 hours of sample collection for results to be valid (9215A, 2016). Minimum volume required for analysis is 100 mL (APHA 9060A 2013).
- **Storage:** The sample should be kept cool (at <10°C) during transport and storage until analysis. Do not freeze samples (APHA 9060B 2013).

**Procedure**

- Consult the following reference methods / instructions for detailed procedures for the four applicable test method options:
  i) APHA 9215 Section A Introduction.
  ii) APHA 9215 Section B Pour Plate Method.
  iii) APHA 9215 Section C Spread Plate Method.
  iv) APHA 9215 Section D Membrane Filter Method
  v) Enzyme Substrate Multi-well / Quanti-tray Method: Refer to manufacturer's instructions.

  The APHA guidance and the manufacturer’s guidance for the enzyme substrate method are prescriptive and must be followed without modification.
  Where subsampling occurs, be sure to homogenize the sample well prior to subsampling.
  Incubation temperatures and times are important to prevent false positive and false negative reactions. Incubation instructions are provided by the media manufacturer and must be followed.
  For data analysis, refer to reading instructions in the applicable reference section.
## Quality Control

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<th>Summary of QC Requirements</th>
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<td>Method Blank (MB)</td>
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<td>Lab Duplicates (DUP)</td>
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<td>Positive &amp; Negative Controls</td>
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If DQOs are not met, repeat testing or report qualified test results.

**Method Blank:** The method blank is 100 mL sterile water that proceeds through the same sample handling processes as test samples, (including sodium thiosulfate if used with test samples; recommend preparing Method Blank in a sample bottle).

**Laboratory Duplicates:** Sample duplicates are prepared when sufficient sample is received to subsample for laboratory duplicates. Homogenize the sample well prior to subsampling. Process both aliquots through the same sample handling processes as test samples.

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

Proofing of sample bottles, organisms, reagents 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.

### References
4. Enzyme Substrate Multi-well / Quanti tray Manufacturer’s Instructions.

### Revision History
- **Feb 14, 1994**  
  Publication in 1994 Lab Manual
- **Nov 14, 2002**  
  SEAM codes replaced by EMS codes
- **Dec 20, 2019**  
  Updated to BC Lab Manual Prescriptive Method format. Prescriptive nature of test is confirmed. APHA 9215 was revised in 2015. APHA 9060 was revised in 2013. APHA 9020 was revised in 2015. QC Section updated to include Method Blanks and Duplicate Samples. Changed sample storage temperature to <10°C as per APHA 9060 (2013).