Heterotrophic Plate Count (HPC)

Kentucky Division of Water

Laboratory Certification Program

To Protect and Enhance Kentucky's Environment

Overview

- Heterotrophic bacteria – bacteria that use organic carbon as a food source
  - Higher counts in water indicate the potential for ineffective disinfection
- Acceptable drinking water methods: SM 9215 B & SimPlate Method (by IDEXX)
- Colonies are counted and reported as **CFU/mL** (colony forming units)
- Purpose of running HPC:
  - Testing **Reagent Grade Water**
  - Sanitary Surveys
  - Evaluate Distribution System

Reagent Grade Water

(Laboratory Pure Water)

DWCM Chapter V; Section 4.3[1]

- Used in the preparation of media, reagents, dilution water and to rinse glass and plastic-ware.
- HPC tested **monthly** – must be ≤500 CFU/mL

  Other quality control for reagent water include:
  - **Conductivity (monthly):** >0.5 megohms resistance or <2 micromhos/cm at 25°C
  - **Pb, Cd, Cr, Cu, Ni, Zn (annually):** <0.05 mg/L per contaminant and <0.1 mg/L collectively
  - **Total Chlorine Residual (monthly):** <0.1 mg/L
  - **Bacteriological quality of reagent water [BST] (annually):** Ratio of growth rate between 0.8 and 3.0

1 – EPA Manual for the Certification of Laboratories Analyzing Drinking Water: Criteria and Procedures Quality Assurance, 5th Edition, EPA 815-R-05-004 (January 2005)

Simplate Method by IDEXX

- Collect 100 mL sample in bottle containing sodium thiosulfate
- Follow IDEXX instructions for either multi-dose or unit-dose procedure. Generally,
  - Create sample & rehydrated media mix. Using 1mL of sample and 9 mL of rehydrated media is sufficient for Reagent Grade Water testing
  - Pour 10 mL of sample/rehydrated media mix onto SimPlate and cover with lid
  - Swirl to distribute sample in all wells. Air bubbles are OK.
  - Tip the plate to drain excess sample onto absorbent pad
  - Invert plate and incubate at **35 ± 0.5°C for 48 hours**
  - Use 6W, 365-nm UV light to read plate. Count fluorescent wells and use MPN table provided by IDEXX. Record result.

SimPlate® for HPC Test Overview - YouTube

Pour Plate Method

Overview

DWCM Chapter V; Section 5[1]
SM 9215 B

- Colonies will be dispersed in and on **Plate Count Agar (PCA)**
- Use dilutions that will give a result in the range of 30-300 CFU/mL.
- Allow to solidify on level surface then invert.
- Incubate
- Count colonies on plate using colony counter

Quality Control

- Use sterile pipet
- Run a blank control
- Run a positive control
- Two plates per sample dilution

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Plate Count Agar (PCA)

DWCM Chapter V; Section 5[1]
SM 9215 A & B

- 5.0g Tryptone, 2.5g yeast extract, 1.0g glucose, 15.0g agar all in 1 L reagent grade water
  - Caked or discolored medium should be discarded
  - Discard medium by manufacturer's expiration date
- May refrigerate prepared media in tightly sealed screw cap tubes for no longer than three (3) months.
  - Maintain sterility/ temperature by keeping 10-15mL aliquots in tubes
- Melt down sterile media **one time** using boiling water.
- Keep melted agar at 44-46°C until use (no more than 3 hours)
  - **QC –** Tube with same amount of agar used as temperature blank alongside analysis media. (**can re-use temperature blank a few times**)

Record Keeping

- The following must be recorded for each new lot/batch of prepared media:
  - Date of preparation/ received
  - Type of medium
  - Lot number
  - pH verification (7.0 ± 0.2)
  - Expiration date

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Pour Plate Method

DWCM Chapter V; Section 5[1]
SM 9215 A & B

- Use dilutions that will give a result of 30-300 CFU/mL
- Aseptically pipette sample onto middle of plate
- Pour 10-15 mL aliquot of PCA media onto plate over sample
  - No more than 20 minutes should elapse between pipetting of sample and pouring of media
- Swirl plate to evenly disperse sample through media
- Allow to solidify for 5-10 minutes
- Invert plate
- Incubate at 35°C ± 0.5°C for 48 ± 3 hours
- Count plates with 30-300 CFU with darkfield colony counter

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