Heterotrophic Plate Count (HPC)

Kentucky Division of Water

Laboratory Certification Program

TEAM KENTUCKY

ENERGY AND ENVIRONMENT CABINET
Division of Water

To Protect and Enhance Kentucky's Environment

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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 plasticware.

- 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 a 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 the sample in all wells. Air bubbles are OK.
  - Tip the plate to drain excess sample onto the absorbent pad.
  - Invert plate and incubate at **35 ± 0.5°C for 48 hours.**
  - Use 6W, 365-nm UV light to read the plate. Count fluorescent wells and use the MPN table provided by IDEXX. Record result.

SimPlate® for HPC Test Overview - YouTube

Pour Plate Method

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

Overview

- 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 a level surface, then invert.
- Incubate.
- Count colonies on the plate using the colony counter.

Quality Control

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

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)

Plate Count Agar (PCA)

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

- 5.0 g Tryptone, 2.5 g yeast extract, 1.0 g glucose, 15.0 g agar all in 1 L reagent grade water.
  - Caked or discolored medium should be discarded.
  - Discard medium by the 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 10-15 mL 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 the 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

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

- Use dilutions that will give a result of 30-300 CFU/mL.
- Aseptically pipette the sample into the middle of the plate.
- Pour 10-15 mL aliquot of PCA media onto the plate over the sample.
  - No more than 20 minutes should elapse between pipetting of the sample and pouring of media.
- Swirl plate to evenly disperse the 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.

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)