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SAMPLE PREPARATION INSTRUCTIONS

BENCHMARK PERFORMANCE AUDIT
WATER SUPPLY (WS) SERIES

WS/NS/OS Coliform Colony Count by Membrane Filter and MPN Methods Culture Recovery Instructions

Description of the Sample Set: This standard consists of one sealed black plastic pouch and one sterile, ≈ 99 mL phosphate buffered re-hydration blank. The black pouch contains one sealed clear plastic pouch. The pouch contains one plastic vial and the vial contains one colored tablet (disc) and desiccant. The bacteria are contained in the disc residing on top of the cotton plug. **The material below the cotton plug is a silica gel desiccant pouch to protect the sample from moisture. The desiccant should not be analyzed and should be disposed of properly after sample use.**

Storage of the Sample Set: Upon arrival the vials containing the bacteria should be stored unopened at or below -10°C. The phosphate buffered re-hydration blank should be stored at room temperature.

Before You Begin:

- We recommend that the quality control guidelines in *Standard Methods for the Examination of Water and Wastewater*, Section 9020B be followed to determine media acceptability prior to analysis.
- "EPA strongly recommends that laboratories evaluate the false-positive and negative rates for method(s) they use for monitoring total coliforms . . . with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used." - 40 CFR 141.21 f.3.12.
- To avoid reporting any false positive results, follow the coliform verification steps as indicated in the method you are using. Both typical and atypical colonies need to be verified.
- The bacteria are in a lyophilized form in the tablet and each sample must be re-hydrated per the following instructions prior to analysis.
- This standard contains viable microorganisms and should be analyzed within 30 minutes after being hydrated.

Preparation Instructions:

Note: All steps must be performed at room temperature

1. Remove the sample vial(s) from the pouch. Leave unopened and allow to equilibrate to room temperature for 10 – 15 minutes.
2. Open the bacteria sample vial and aseptically transfer the colored disc into a re-hydration blank bottle.
    a. **NOTE:** The disc may occasionally stick to the vial. If this occurs, use sterile tweezers to gently remove the disc. Usually the disc may simply be added to the phosphate buffer by inverting the sample vial over the buffer and tapping the vial.
3. You may add 1 – 5 ml of sterile dilution water to ensure the sample volume is adequate for your method, since your results are to be reported per 100 ml volumes.
4. Swirl the sample and allow it to stand for 10 – 15 minutes. The disc should completely dissolve. After it is dissolved, mix by inverting the vessel 10 times
5. Use this sample within 30 minutes of hydration if you are performing microbial count.
6. Choose sample volumes that will yield verifiable results through your laboratory technique. The sample will fall somewhere within the set range of 20 to 200 CFU/100ml. The method used and its limits will determine if any dilution of the sample is required.
7. Analyze this sample using your everyday normal laboratory procedures and personnel.
8. Report your results as CFU or MPN/100 ml.
9. Properly disinfect any spills and sterilize all wastes according to appropriate regulations before disposal.
10. **Please note these instructions are for Total/Fecal/E.Coli Coliforms by Membrane Filter or Most Probable Number analysis.**