Designation: F 488 – 95

An American National Standard

Standard Test Method for On-Site Screening of Heterotrophic Bacteria in Water [1]

This standard is issued under the fixed designation F 488; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This screening test method covers the detection and enumeration of bacteria contained in a water sample employing a commercial device specifically designed for that purpose. This test method applies only to the enumeration of those viable bacteria that will grow under the test conditions specified (for example, medium, temperature, time, etc.). It is not applicable to the detection of anaerobic bacteria.

1.2 No bacterial culture technique can enumerate all the viable bacteria in a sample, since bacteria occur singly, in pairs, chains, or clusters and no single set of growth conditions or media can satisfy the physiological requirements of all bacteria in a sample. Therefore, this test method cannot provide a total bacterial count, but can only strive to achieve a relative count of viable aerobic and facultative anaerobic bacteria present in a sample.

1.3 The test method applies to samples in which the number of culturable bacteria per millilitre exceeds at least 10 and no more than 160 bacteria/mL in the sample or sample dilution.

1.4 This test method is intended to be used as a simplified field method where bacteriological laboratory facilities are not readily available.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water [2]

D 1193 Specification for Reagent Water [2]

D 3370 Practices for Sampling Water from Closed Conduits [2]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this test method, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *dynamic system*—a system or container in which the water contained is in motion.

3.2.2 *estimated bacterial count*—the number of bacteria present in a 1.0-mL sample that can be cultured into individual, countable colonies by the technique described in this test method.

3.2.3 *static system*—a system or container in which the water is not in motion. Water held in a bottle or storage tank is an example of a static system.

3.2.4 *CFU*—colony forming units.

3.2.4.1 *Discussion*—Examples are a pump-driven water circulating system and a flowing-water purification line.

4. Summary of Test Method

4.1 A commercially available water sampler device [3] (SPC Sampler) is immersed in a water sample. A1.0-mL volume is automatically drawn through a 0.45-um pore size bacteria retentive membrane filter into a backing pad of absorbent material. The absorbent pad, sealed to the back of the filter contains a dehydrated nutrient medium which hydrates and diffuses through the filter. The water sampler is then incubated, and the bacteria trapped on the filter surface grow into visible colonies. The colonies may be counted directly or preferably with low-power magnification.

4.2 With high bacterial count samples, a suitable dilution is prepared prior to conducting the test described in 4.1.

5. Significance and Use

5.1 This test method provides a means for locating the source of bacterial contaminations in a system.

5.2 This is a screening test method that should be limited to use in estimating levels of bacterial contamination in a system. This test method is intended to provide a simple field technique toward estimating the bacteria count in samples of water. Since the method employs a 1-mL sample, it is not statistically significant unless the culturable bacteria are present in greater than 10 cfu/mL.

[1] This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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[2] *Annual Book of ASTM Standards*, Vol. 11.01.

[3] A SPC Sampler, available from Millipore Corp., Bedford, MA 01730, or its equivalent, has been found suitable for this purpose.

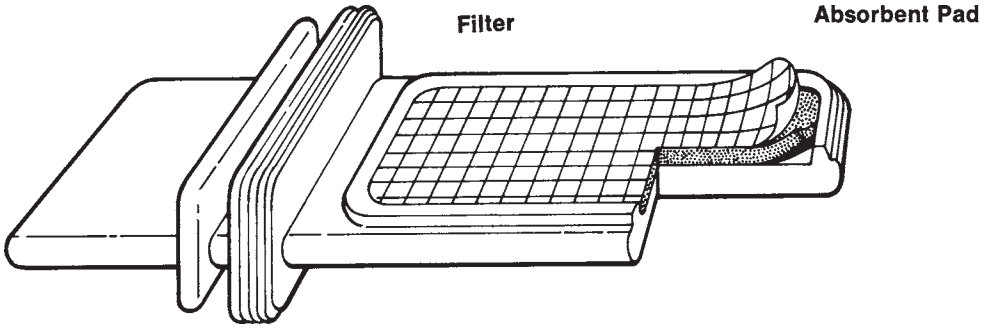

FIG. 1 SPC Sampler

5.3 This test method is applicable to the detection of culturable, aerobic, and facultative anaerobic bacteria in water.

6. Apparatus

6.1 *Expendable Apparatus*:

6.1.1 *SPC Sampler*—a presterilized bacteriological field-test device comprised of a 0.45-um pore size membrane filter and absorbent pad with a dehydrated nutrient medium of the type described as follows. The filter and nutrient medium-containing pad are sealed together in a paddle-shaped plastic holder (see Fig. 1), with a self-contained incubation chamber.

6.1.1.1 *M-HPC Nutrient Formulation (SPC Sampler)*:

(1) Peptone[4], 2.0 g,

(2) Gelatin, 2.5 g,

(3) Glycerol, 1.0 mL, and

(4) Water, 100 mL.

6.2 The following apparatus is required only if dilutions are necessary for evaluating high bacteria count water samples (counts in excess of 100/mL).

6.2.1 *Wide Mouth Sample Bottle*—A vessel with the capacity of at least 300 mL with a screw-cap closure. The bottle shall be capable of protecting the contents from bacteriological contamination and shall be of borosilicate glass or other material capable of withstanding conventional sterilizing procedures.

6.2.2 *Dilution Bottles*, borosilicate glass, screw cap, autoclavable bottles of a 100-mL capacity.

6.2.3 *Graduates*, borosilicate glass, 10-mL and 100-mL, sterile.

6.2.4 *Pipettes*, sterile, disposable, graduated plastic or glass, of 1-mL capacity.

6.3 Clean and sterilize the wide-mouth sample bottles and dilution bottles in accordance with 13.2.1 and 13.2.2 of Practices D 3370.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the committee on Analytical Reagents of the American Chemical Society.[5]

7.2 *Purity of Water*—Unless otherwise specified, references to water shall mean reagent water conforming to Specification D 1193, Type III.

7.3 *Dilution Water*:

7.3.1 *Phosphate Buffer Solution, Stock*—Dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of water. Adjust pH to 7.2 with NaOH solution (40 g/L) and bring to 1000 mL with water. Sterilize by filtration through a 0.22-um filter, or autoclave for 15 min at 121°C. Store in a refrigerator and handle aseptically. If cloudiness or other evidence of contamination appears, discard the stock. A marked change of pH indicates stock solution contamination. Confirm that the pH is 7.2 ± 0.1.

7.3.2 *Magnesium Chloride Solution (81.4 g/1000 mL)*—Dissolve 81.4 g of hexahydrate magnesium chloride ($MgCl_2 \cdot 6H_2O$) in 1000 mL of water. Mix well and sterilize by filtration or autoclave for 15 min at 121°C. Store in a refrigerator. If cloudiness or other evidence of contamination occurs, discard the stock.

7.3.3 *Phosphate Buffered Dilution Water*—Add 1.25 mL of stock phosphate buffer solution and 5 mL of magnesium chloride solution to 1000 mL of reagent water in a volumetric flask and mix well. The final pH should be 7.2. Dispense buffered dilution water in amounts that will provide 99 ± 2 mL after sterilization, in screw-cap dilution bottles, or in larger-volume containers for use as rinse water if desired. Sterilize immediately.

7.3.4 *Peptone Dilution Water*—Prepare a 10 % solution of peptone in water. Dilute a measured volume to provide a final 0.1 % solution. The final pH should be 6.8.

8. Sampling

8.1 *Dynamic System*:

[4] Bacto Peptone, or its equivalent, has been found suitable for this purpose.

[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.