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.

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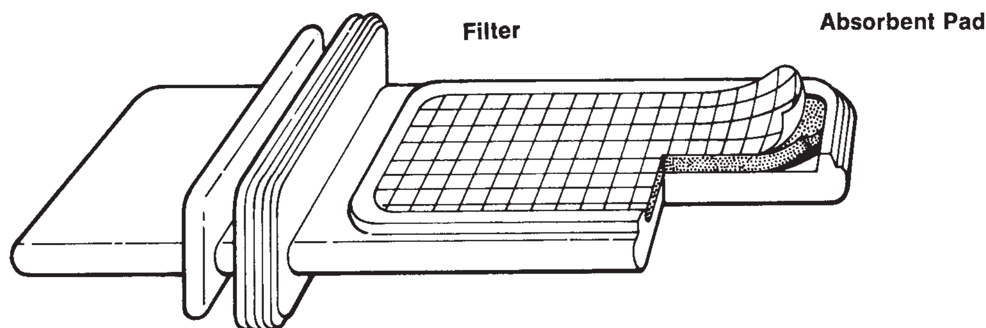


FIG. 1 SPC Sampler

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.

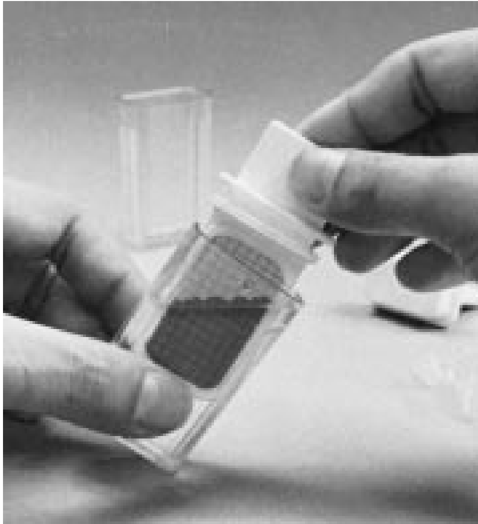
7.3.4 *Peptone Dilution Water*—Prepare a 10 % solution of peptone in water. Dilute a measured volume to provide a final 0.1 % solution. The final pH should be 6.8.

8. Sampling

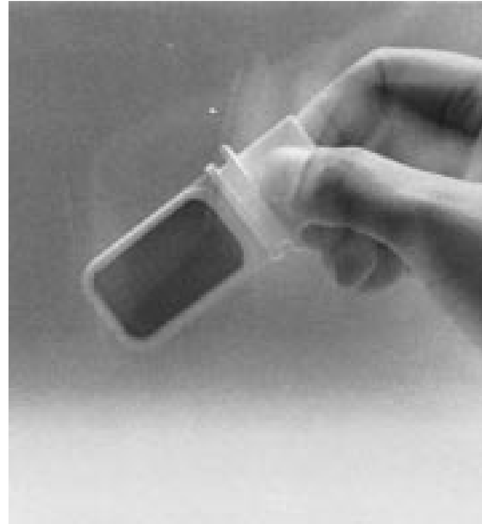
8.1 *Dynamic System*:

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

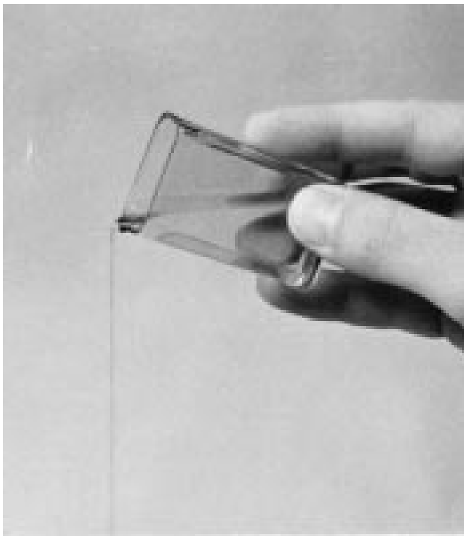
[5] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States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



(a) Insert the sample all the way into the filled case for 30 s (except for still water samples).



(b) Remove the sampler and shake to remove excess water.



(c) Empty the case.

(d) Insert the sampler into the empy case and then place in the incubator.

FIG. 2 Testing the Sample

8.1.1 Open the outlet valve on the system containing the water to be tested and flush the outlet valve with a minimum of 500 mL of water while the system water is flowing through the valve. Allow the system water to flow through the valve a few seconds after flushing is completed; then directly fill the water sampler plastic case to the upper line inscribed on the case (see Fig. 2).

8.1.2 When bacterial counts are greater than 160, as indicated by overgrown or confluent colonies on the filter in initial tests, repeat the test by filling the plastic case only to the lower line with the system water. Then add sterile phosphate buffer dilution water or sterile peptone dilution. Water to the upper line of the plastic case to produce a 1 to 10 dilution of the sample.

8.1.3 If overgrown or confluent colonies still result after running the 1 to 10 dilution as described 8.1.2, employing the apparatus described in 6.3, prepare a 1 to 100 dilution and a 1 to 1000 dilution of the sample.

8.1.3.1 To prepare a 1:100 dilution, add 1 mL of the test sample to a dilution bottle containing 99 mL of sterile phosphate buffered dilution water (see 7.3.3) or peptone dilution water (see 7.3.4). Screw bottle cap tightly. Immediately prior to pouring this diluted sample to the upper mark inscribed on the water tester case, shake the bottle vigorously about 30 times.

8.1.3.2 A 1:1000 dilution is prepared in the same manner as shown in 8.1.3.1 but only 0.1 mL of the test sample is added to 99.9 mL of the dilution water.

8.2 *Static System*:

8.2.1 Pour or draw a sample directly into the water sampler plastic case to the upper line on the case.

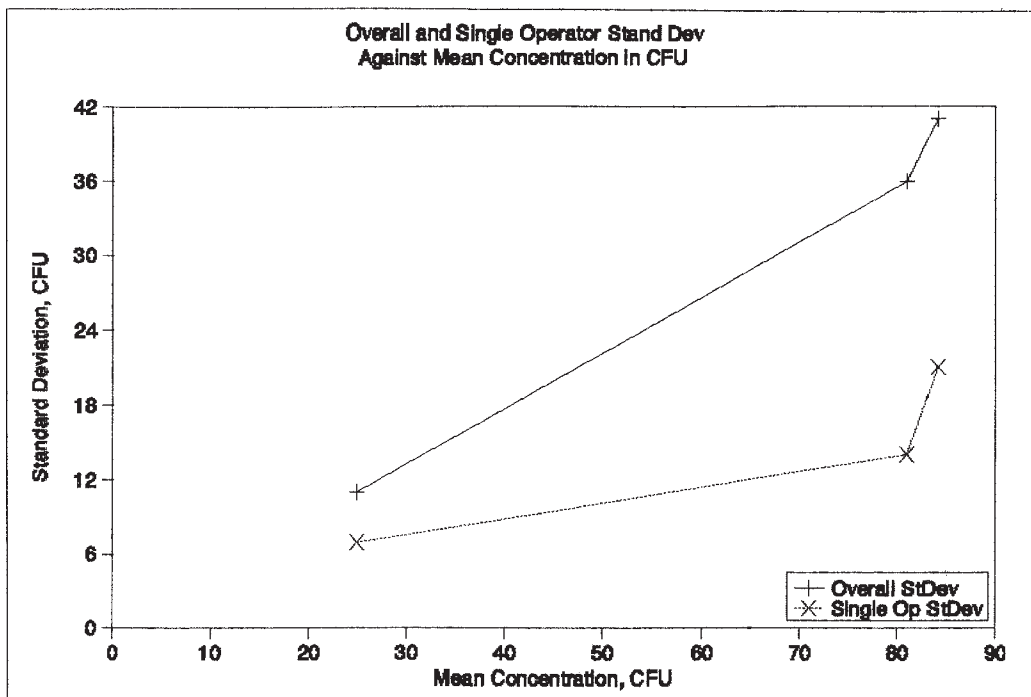


FIG. 3 Overall and Single-Operator Standard Deviation

8.2.2 If dilution is required because of high bacterial counts, follow the procedure for dilution as described in 8.1.2 or 8.1.3.

8.3 Analyze the sample as soon as possible after collection; do not exceed 4 h between collection and analysis of the sample.

9. Procedure

9.1 Separate the paddle-shaped membrane filter and pad holder from its case and immerse it completely for 30 to 60 s in the water sample prepared in Section 8. Remove the holder from the water sample and shake off the excess water. Empty the plastic case and replace the holder firmly in its case as shown in Fig. 2 Fig. 3.

NOTE 1—Hydration of the nutrient pad will result in the automatic filtration of a 1.0-mL aliquot of the sample water.

9.2 Place the plastic case containing the sampler with the filter side up horizontally in the incubator, and incubate at 30 ± 2°C for 48 ± 2 h, then remove the sampler from the incubator. An extended incubation period is preferable if time permits. In any case, whatever incubation period is used, one must always be consistent and repeat this period when monitoring each type of condition repeatedly. The temperature used must also be consistent.

9.3 Count all the bacterial colonies on the filter using direct light illumination and record the number of colonies counted.

NOTE 2—Bacteria colonies appear as 1 to 2-mm diameter round, clear, white, or colored spots that are easily seen against the darker filter. Each colony represents one bacterium collected from the 1-mL water aliquot sampled.

NOTE 3—Low-power magnification of 5 to 10× will facilitate the counting. Magnification is advisable when the colonies are small or crowded on the filter.

10. Calculation

10.1 If a 1 to 10 dilution was used, multiply the number of colonies counted in 9.3 by 10 and record the result. If a 1 to 100 dilution was used, multiply colonies counted by 100.

11. Precision and Bias

11.1 *Collaborative Test*—This test method was evaluated by seven operators in four independent laboratories. Two samples of raw lake water and one bacterial culture were used in the study. Each operator performed triplicate tests on each sample. The outlier test indicated that all data could be used in the analysis of precision.

11.2 The overall precision (St) and single-operator (Op) precision for these samples are as follows:

	Final Statistics		
	Sample 1	Sample 2	Sample 3
Mean (CFU)	84	25	81
Overall St	41	11	36
Single Op	21	7	14

11.3 No bias statement is possible since comparisons of this test method were not evaluated against a known concentration of bacteria.

12. Keywords

12.1 dilution; field method; heterotrophic bacteria; screening; SPC sampler

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