Designation: D 4198 – 82 (Reapproved 1998)

Standard Test Methods for Evaluating Absorbent Pads Used with Membrane Filters for Bacteriological Analysis and Growth [1]

This standard is issued under the fixed designation D 4198; 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 These test methods cover the determination of the nutrient-holding capacity and the toxic or nutritive effect on bacterial growth of organisms retained on a membrane filter, when the absorbent pad being tested is used as a nutrient reservoir and medium supply source for the retained bacteria.

1.2 The tests described are conducted on 47-mm diameter disks, although other size disks may be employed for bacterial culture techniques.

1.3 *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 3508 Method for Evaluating Water Testing Membrane Filters for Fecal Coliform Recovery[3]

3. Terminology

3.1 *Definitions*—For definitions of terms used in these test methods, refer to Terminology D 1129.

4. Summary of Test Methods

4.1 Test Method A involves saturating a 47-mm absorbent pad with water and determining the volume of water held by the pad by weighing the pad dry and when fully saturated.

4.2 Test Method B involves culturing micro-organisms from suspensions of pure cultures on a 0.45-µm membrane filter, which is placed on the test absorbent pad saturated with the appropriate growth medium. The resultant cultures are compared to cultures grown on spread plates and to membrane filters placed directly on agar with no absorbent pad.

5. Significance and Use

5.1 These test methods are appropriate for qualifying absorbent pads used with membrane filters for bacteriological enumeration.

5.1.1 The test methods described are applicable to quality control testing of absorbent pads by the suppliers and users of these pads and to specification testing of absorbent pads intended for use with membrane filters in bacteriological enumeration.

5.2 Other pure culture organisms and their appropriate culture medium may be substituted for the *E. coli* and M-FC media for specification testing, as required.

6. Apparatus

6.1 *Filtration Units* for membrane filters with side-arm flask and tubing.

6.2 *Vacuum Source*.

6.3 *Vortex Mixer* or similar mixer.

6.4 *Forceps*, flat-bladed.

6.5 *Incubator* capable of maintaining temperatures of 44.5 ± 0.2°C.

6.6 *Stereoscopic Microscope and Illuminator*.

6.7 *Illuminated Magnifying Stand* for counting colonies on agar spread plates.

6.8 *Hand Tally Counter*.

6.9 *Autoclave*.

6.10 *Analytical Balance* readable to the nearest 1 mg.

6.11 *Petri Dish*, 50-mm, nonsterile.

6.12 *Expendable Equipment*:

6.12.1 Filters (gridded, 0.45-µm, 47-mm) sterile, for water testing.

6.12.2 Absorbent pads (47-mm), sterile for the growth test.

6.12.3 Petri dishes, sterile 50-mm and 100-mm.

6.12.4 Pipets, sterile, 10-mL, 0.1 mL graduations, accuracy of ± 5 %.

6.12.5 Test tubes, sterile, 20-mL, with screw caps.

6.12.6 Bent glass rod, sterile, for spreading bacterial cultures.

6.12.7 Burner, for flame sterilization.

7. Reagents and Materials

7.1 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming

[1] These methods are under the jurisdiction of ASTM Committee D-19 on Water and are the direct responsibilities of Subcommittee D19.08 on Membranes and Ion Exchange Materials.

Current edition approved Oct. 29, 1982. Published March 1983.

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

[3] Discontinued; see *1994 Annual Book of ASTM Standards*, Vol 11.02.

to Specification D 1193, Type II, with 0.2-µm membrane filtration. In addition, suitability tests for determining the bactericidal properties of the reagent grade water should be performed.

7.2 *M-FC Agar with Rosolic Acid*—Fecal coliform medium specific for the membrane filter technique.

7.3 *M-FC Broth with Rosolic Acid*—Broth nutrient for bacterial growth.

7.4 *Peptone Water*, 0.1 %.

7.5 *E. coli* (ATCC 11229).

8. Preparation of Equipment and Materials

8.1 *Washing and Cleaning*—Clean all glassware and filtration equipment thoroughly, using a suitable detergent in hot water, rinse with hot water, and then rinse in reagent grade water. Dry the equipment thoroughly prior to sterilization.

8.2 *Sterilization*—Follow standard microbiological laboratory practices for preparing glassware and filtration equipment prior to placing in the autoclave. Autoclave at 121°C for 15 min. Refer to Method D 3508 for details.

8.3 *Incubator*—Set incubator at 44.5 ± 0.2°C.

9. Media Preparation

9.1 *M-FC Broth with Rosolic Acid*—Dissolve in reagent grade water in accordance with the manufacturer's instructions.

9.2 *M-FC Agar with Rosolic Acid*—To a solution of M-FC, add agar (15 g/1000 mL), mix while heating in accordance with the manufacturer's instructions, cool, and dispense into 100-mm petri dishes.

10. Culture Preparation

10.1 Resuspend culture in accordance with the supplier's instructions.

10.2 Using a sterile loop, streak an agar plate with culture of *E. coli.*

10.3 Incubate 24 h at 44.5°C.

10.4 Using a sterile needle, inoculate M-FC agar with organisms from a single colony on the streak plate.

10.5 Let the culture incubate for 5 to 6 h at 35°C.

10.6 Plate out a series of dilutions and store the remainder of the culture in the refrigerator. Incubate the plates for 18 ± 2 h at 44.5°C.

10.7 Based on the 24-h plate count, dilute a portion of the culture to give a solution with 200 to 800 microorganisms per millilitre.

11. Procedure

11.1 *Method A—Water Retention*:

11.1.1 Weigh three dry 50-mm plastic petri dishes to the nearest 1 mg, for each lot of pads to be tested.

11.1.2 Randomly select three absorbent pads from each lot, place a dry pad in each of the dishes, cover, and weigh again.

11.1.3 To each pad, add an excess of water.

11.1.4 After the pads are fully saturated (20 to 30 s), pour off the excess water and shake out any remaining excess.

11.1.5 Cover the dishes and weigh again.

11.2 *Method B—Culture Technique*:

11.2.1 Prepare a set of ten 100-mm sterile petri dishes with 16 ± 1 mL of M-FC agar. Make sure the agar plates are at room temperature and that the surfaces are dry before using.

11.2.2 Prepare a set of five 50-mm sterile petri dishes with the sterile pads. To each pad add 1.8 mL of sterile M-FC broth and pour off the excess.

11.2.3 Test five replicate sets of three aliquots. Each replicate shall include *(a)* two membrane-filtered samples (one on agar, one on a pad), and *(b)* one spread plate.

11.2.4 Add 0.1 mL of the diluted culture solution from 10.7 to the agar plate from 11.2.1 and using a sterile bent glass rod, spread over the surface of the agar. Cover the plate.

11.2.5 Set up two sterile filter funnels with flasks so that the two membrane samples in the set can be run concurrently with the spread plate.

11.2.6 Place a sterile gridded 0.45-µm membrane onto each of the two filtration bases and assemble the funnels.

11.2.7 Add 0.1 mL of the diluted culture (20 to 80 organisms/0.1 mL) from 10.7 to each of the two tubes, which contain 20 mL of sterile 0.1 % peptone.

11.2.8 Cap the tubes and mix on a vortex mixer.

11.2.9 Add the contents of one tube to each funnel and turn on the vacuum.

11.2.10 After the liquid has filtered through the membrane, carefully wash the sides of the funnel with about 20 mL of sterile 0.1 % peptone solution.

11.2.11 Turn off the vacuum and remove the funnel tops.

11.2.12 Using sterile forceps, carefully remove one membrane and place on an agar plate. Remove the other membrane and place on the pre-soaked test absorbent pad. Be careful not to trap air under the membranes as this will inhibit growth in these areas.

11.2.13 Repeat 11.2.4-11.2.12 four more times.

11.2.14 Cover each plate after completing 11.2.12.

11.2.15 Store 100-µm plates inverted in sealed plastic bags with a wet paper towel in each bag. Fifty-millimetre plastic petri dishes do not require sealed plastic bags or wet towels, but should also be inverted.

11.2.16 Place all of the plates into an incubator at 44.5 ± 0.2°C for 22 to 24 h.

11.2.17 Remove the plates and count the number of colonies on each plate.

12. Calculation

12.1 *Method A*—Calculate the weight of water retained as follows:

$$C = (B - T) - (A - T)$$

$$C = B - A$$

where:

C = weight of water retained,
T = tare weight of dish,
A = weight of dry pad plus dish, and
B = weight of wet pad plus dish.

12.2 *Method B*:

12.2.1 Average the five replicates for each of the three test sets and calculate the standard deviation.

12.2.2 Compute percent recovery for the filter culture on the absorbent pad (R_p) and the filter culture on the agar medium (R_m) as follows:

$$\% R_p = (N_p/N_a) \times 100$$

$$\% R_m = (N_m/N_a) \times 100$$

where:

N_p = the number of colonies recovered with the filter on the pad,

N_a = the number of colonies recovered with the agar spread plate, and

N_m = the number of colonies recovered with the filter on agar directly.

13. Results

13.1 Compare % R_p with % R_m.

13.2 Report the volume of water retained by the absorbent pad, after converting weight of water to volume.

14. Precision and Bias

14.1 No statement is made about either the precision or bias of these test methods for evaluating absorbent pads since the results merely indicate whether there is conformance with the requirements for the intended use.

15. Keywords

15.1 absorbent pads; bacteriological; filter membranes