Designation: D 4200 – 82 (Reapproved 2003)

An American National Standard

Standard Test Method for Evaluating Inhibitory Effects of Ink Grids on Membrane Filters [1]

This standard is issued under the fixed designation D 4200; 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 test method describes a procedure whereby the user of ink-gridded membrane filters in water quality studies can ascertain whether or not the grid lines are toxic and inhibitory to bacterial growth when the membrane and its entrapped bacteria are incubated on a suitable media.

1.2 *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:* [2]

D 1129 Terminology Relating to Water

D 1193 Specification for Reagent Water

3. Terminology

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

4. Summary of Test Method

4.1 A heavy bacterial suspension is filtered through a gridded membrane filter. The bacterial concentration employed is sufficient to cover much of the membrane with bacterial colonies.

4.2 After filtration the membrane is incubated on a suitable medium and the distribution of the colonies and shape of the colonies noted in the area around each grid line.

5. Significance and Use

5.1 This test method may be applied to determine the suitability of grid-marked membrane filters for use in bacteriological culture techniques for the detection and enumeration of bacterial organisms.

5.2 A particularly sensitive organism and growth conditions have been selected for this test method in order to maximize sensitivity to toxic materials possibly present in the inks used for grid-marking membrane filters.

6. Apparatus

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

6.2 *Membrane Filtration Units.*

6.3 *Vacuum Source* with trap vessel.

6.4 *Forceps*, blunt-nosed.

6.5 *Autoclave* or other sterilizing equipment.

6.6 *Expendables*:

6.6.1 Gridded membrane filters.

6.6.2 1-mL and 10-mL pipets.

6.6.3 Petri dishes (50-mm) containing 6 to 8 mL of agar medium or a 100-mm dish with 20 ± 2 mL of agar medium, or both.

6.6.4 Erlenmeyer flasks.

7. Reagents and Materials

7.1 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming to reagent water Type II of Specification D 1193.

7.2 *M-FC Agar with Rosolic Acid* or equivalent (henceforth referred to as agar medium), formulated, prepared, and dispensed in accordance with the manufacturer's specifications.

7.3 *Tryptone Soya Broth* or equivalent (henceforth referred to as broth medium), formulated, prepared, and dispensed in accordance with the manufacturer's specifications.

7.4 *Peptone Water*, 0.1 %, sterile.

7.5 *Broth Culture* of *E. coli* ATCC 11229, 18-h, prepared as follows: Add 1 mL of an 18 ± 2-h broth culture of *E. coli* ATCC 11229 to 99 mL of 0.1 % peptone water, mix thoroughly, then add 0.1 mL of this suspension to another flask containing 99 mL of 0.1 % peptone water. This is the working concentration and should contain approximately 10^3 bacteria per millilitre to provide contiguous but discrete growth.

---

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.08 on Membranes and Ion Exchange Materials.

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

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

NOTE 1—Hydrophobic leaching may cause growth inhibition in areas adjacent to grid lines.

8. Procedure

8.1 Assemble the sterile membrane filtration apparatus, and connect to vacuum trap and vacuum source.

8.2 Process five randomly selected membrane filters, using 1-mL aliquots of culture suspension from 7.5, and 30 mL of 0.1 % peptone water added to the filter funnel with standard membrane filtration procedures. Place the membranes in petri dishes containing M-FC agar medium and incubate at 44.5°C for at least 18 h.

8.3 After incubation, examine the overgrown membrane for inhibition of growth along the grid lines.

8.4 Examine the membrane filter for growth of "square colonies." The growth of square colonies flowing along grid lines is not necessarily an indication of toxicity, but could be an indication of either compression of the membrane or plugging of the pores, thus inhibiting nutrient feed to the colonies (see Note 1).

8.5 In this test method, toxicity is defined as the lack of growth in the area immediately adjacent to the grid lines, and if this effect is noted, repeat the test and if still present, reject the membrane filters.

9. Precision and Bias

9.1 Since this is a qualitative test method, precision and bias statements are not applicable.

10. Keywords

10.1 bacterial; filter; inhibitory ink; membrane