



Standard Test Method for Isolation and Enumeration of *Pseudomonas aeruginosa* from Water

This standard is issued under the fixed designation D 5246; 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 covers the isolation and enumeration of *Pseudomonas aeruginosa* (*P. aeruginosa*) from surface waters; recreational waters; ground water, water supplies; especially rural nonchlorinated sources; waste water; and saline waters. The detection limit of this test method is one microorganism per 100 mL.

1.2 This test method was used successfully with reagent water and it is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

1.3 The values stated in SI units are to be regarded as the standard.

1.4 *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.* Specific hazard statements are given in Section 10.

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water¹

D 1193 Specification for Reagent Water¹

D 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water¹

D 3370 Practices for Sampling Water from Closed Conduits¹

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 *Pseudomonas aeruginosa*—an aerobic, motile, gram negative rod that produces fluorescent pigments and pyocyanin. It is oxidase and caseinase positive, is able to grow at 42°C, is relatively resistant to many antibiotics, and may utilize acetamide.

3.2.2 *refrigeration*—storage at 2 to 8°C.

4. Summary of Test Method

4.1 A water sample is passed through a 0.45 mm or equivalent membrane filter. The filter carrying the retained organisms is placed on a selective medium (M-PA-C)² and is incubated at 41.5 $\pm$ 0.5°C for 48 to 72 h. The resulting pink-brown to black colonies of *Pseudomonas aeruginosa* are counted and reported per 100 mL of the sample. Colonies may be verified on skim milk agar.

5. Significance and Use

5.1 *Pseudomonas aeruginosa* is an opportunistic pathogen, and has been linked as the causative agent of numerous infections that may be transmitted through a contaminated water supply to a susceptible host. In addition to its direct pathogenicity, the association of *P. aeruginosa* with human fecal waste indicates that where elevated levels of *P. aeruginosa* are found, a serious health hazard may exist due to the presence of other pathogens.

5.2 The membrane filtration procedure described is a rapid and reliable test method of detecting *P. aeruginosa* in water.

6. Interferences

6.1 For certain samples, bacterial cells may have been exposed to adverse environmental factors that lower their probability for survival and growth on a membrane filter medium. This effect may be pronounced in this test method due to the presence of antibiotics and the elevated incubation temperature.

6.2 The selection of an appropriate dilution volume is essential. Too small a dilution volume may fail to detect any *P. aeruginosa* organisms, while too large a volume may cause an overabundance of colonies that would interfere with an accurate count.

6.3 Chemicals or a combination of chemicals in certain samples can have a toxic effect upon *P. aeruginosa* when concentrated.

¹ Annual Book of ASTM Standards, Vol 11.01.

² Available from BBL Microbiological Systems, Division of Becton Dickinson and Co., Cockeysville, MD 21030. Other suppliers may be utilized if equivalent.