



Designation: D 4412 – 84 (Reapproved 2002)

Standard Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits¹

This standard is issued under the fixed designation D 4412; 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 procedure for the detection and enumeration by the most probable number (MPN) technique of sulfate-reducing bacteria in water or water-formed deposits.

1.2 Two media preparations are provided. Medium A which is prepared with reagent grade water, and Medium B which is prepared using the water to be sampled as the water source. Medium B is offered for those special conditions where sulfate-reducing bacterial strains have adapted to atypical non-fresh water environment.

1.3 For the isolation and enumeration of thermophilic sulfate-reducing bacteria encountered in waters associated with oil and gas production, all broths, dilution blanks, and incubations must be maintained at temperatures of at least 45°C and preferably within 5°C at the sample temperature.

1.4 The sensitivity of these test methods can be increased by purging the dilution blanks and tubes of media with nitrogen immediately prior to use.

1.5 The analyst should be aware that adequate collaborative data for precision and bias statements as required by Practice D 2777 are not provided. See Section 11 for details.

1.6 *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 Water²

D 1193 Specification for Reagent Water²

D 2777 Practice for Determination of Precision and Bias of Applicable Methods of Committee D19 on Water²

D 3370 Practices for Sampling Water from Closed Conduits²

2.2 APHA Standard:

Standard Methods for the Examination of Water and Wastewater, Fifteenth Edition³

3. Terminology

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

3.2 *Definitions of Terms Specific to This Standard*: —For a description of the term MPN used in these test methods, refer to literature.⁴

4. Summary of Test Methods

4.1 Water and water deposit samples and dilutions of these samples are dispensed into tubes of Starkey's medium (A or B) following five tube MPN procedures. The tubes are sealed with liquid paraffin, and incubated at 20°C for 21 days.⁴ Positive reactions are indicated by the deposit of a black precipitate.

5. Significance and Use

5.1 Sulfate-reducing bacteria are widely distributed in marine and fresh water muds which, in consequence, frequently are laden with the hydrogen sulfide produced by these organisms during dissimilatory sulfate reduction.

5.2 It has been reported that *Desulfovibrio* can form as much as 10 g of sulfide per litre during active multiplication. Sulfate-reducing bacteria can cause the external or internal corrosion of water or wastewater pipelines and pipelines for petroleum and natural gas. The formation of galvanic cells by massive growth of sulfate-reducing bacteria under suitable conditions makes the corrosion much worse than just the effect of the hydrogen sulfide on the metal or concrete.

6. Apparatus and Materials

6.1 *Anaerobic Incubator*, 20°C, if available, or conventional 20°C incubator.⁵

6.2 *Pipets*, sterile, 1 mL and 10 mL, "calibrated" to deliver.

6.3 *Test Tubes*, with close fitting or airtight caps; 16 by 150 mm and 20 by 150 mm.

¹ These test methods are under the jurisdiction of ASTM Committee D19 on Water and are the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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

³ Available from American Public Health Association, 1015 18th St. N.W., Washington, DC 20036.

⁴ Bonde, G. J., "Bacterial Indicators of Water Pollution," *A Study of Quantitative Estimation*, Teknisk Forlag, Copenhagen, 1963.

⁵ For thermophilic organisms use a 45°C incubator.