



Designation: D4412 – 84 (Reapproved 2009)

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

This standard is issued under the fixed designation D4412; 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 D2777 are not provided. See Section 11 for details.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *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.*

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. Referenced Documents

2.1 *ASTM Standards*:²

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

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 D1129.

3.2 *Definitions*—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

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.

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.