



Standard Test Method for Iron Bacteria in Water and Water-Formed Deposits¹

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1. Scope

1.1 This test method covers the determination of iron bacteria by examination under the microscope. The method provides for the identification of the following genera of bacteria found in water and water-formed deposits: *Siderocapsa*, *Gallionella* (*Dioymohelix*), *Sphaerotilus*, *Crenothrix*, *Leptothrix*, and *Clonothrix*.

1.2 *This standard does not purport to address 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 887 Practices for Sampling Water-Formed Deposits²

D 1129 Terminology Relating to Water³

D 1193 Specification for Reagent Water³

D 3370 Practices for Sampling Water from Closed Conduits³

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 The iron bacteria are generally filamentous, typically found in fresh water, and frequently surrounded by a sheath which is usually encrusted with iron or manganese, or both (1, 2).⁴ However, Starkey (3) reports another type which is classified among the true bacteria. Detection and identification is accomplished by microscopical examination of sediment from the sample. Table 1 and Figs. 1-10 (3) may be used to differentiate the various types. This test method provides an indication of the density of the iron bacteria and the severity of the clogging problem in pipes caused by these bacteria.

5. Significance and Use

5.1 Iron bacteria is a general classification for microorganisms that utilize ferrous iron as a source of energy and are characterized by the deposition of ferric hydroxide in their mucilaginous sheaths. The process is continuous with these growths, and over a period of time large accumulations of slimy brown deposits can occur. Iron bacteria may clog water lines, reduce heat transfer, and cause staining; objectionable odors may arise following death of the bacteria. The organic matter in the water is consequently increased, and this in turn favors the multiplication of other bacteria.

6. Apparatus

6.1 *Centrifuge*, complete with conical tubes.

6.2 *Microscope* that provides a magnification of 400 to 1000× and is complete with a suitable light source. A dark-field condenser is desirable.

6.3 *Pipets*, Mohr-type, 10-mL, with an opening 3 to 4 mm in diameter, for thick samples, and 1-mL Mohr-type pipets for thin samples.

6.4 *Spatula*, small and narrow, for handling thick samples.

6.5 *Membrane Filter*, with appropriate filter-holding assembly (see 9.2).

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁵ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

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

7.3 *Ammonium Oxalate-Crystal Violet Solution*—Prepare Hucker's modification of the Gram stain (4) by mixing a

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² *Annual Book of ASTM Standards*, Vol 11.02.

³ *Annual Book of ASTM Standards*, Vol 11.01.

⁴ The boldface numbers in parentheses refer to the list of references at the end of this test method.

⁵ "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."