



Designation: D 932 – 85 (Reapproved 2002)

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

This standard is issued under the fixed designation D 932; 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 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.

¹ This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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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. 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

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."

TABLE 1 Key for Identification of Bacteria

I.	TRUE BACTERIA: Capsulated coccoid or short rods	Genus: <i>Siderocapsa</i> (Fig. 1)
	The organisms are coccoid or short rods, occurring in groups of 1 to 30 but generally less than 10, surrounded by a mucoid capsule. The deposit surrounding the capsule is rust-brown due to the presence of hydrous ferric oxide.	
II.	STALKED BACTERIA: Twisted or straight bands resembling a ribbon or a row of beads. Bacteria are rod-shaped and borne at the top of the stalk.	Genus: <i>Gallionella</i> (<i>Didymohelix</i>) (Figs. 2 and 3)
	The stalks are slender (1 to 3 μm), dichotomously branched, composed of colloidal hydrous ferric oxide. The bacteria are frequently overlooked and the stalk considered as the bacterium.	
III.	FILAMENTOUS BACTERIA:	
	A. Not encrusted with iron:	Genus: <i>Sphaerotilus</i> (Fig. 4)
	The filaments are attached, colorless, may show false branching. The cells are rod-shaped or oval, 1.5 to 4 μm in diameter, surrounded by a firm sheath which is entirely organic and not impregnated with iron.	
	B. Encrusted with iron:	
	(1) Not branched:	Genus: <i>Crenothrix</i> (Figs. 5, 6, and 10)
	The filaments are usually attached to a firm substrate, and are differentiated into a base and a tip. The sheath is plainly visible and is thin and colorless at the tip, becoming thick and encrusted with iron oxide at the base. The cells vary from cylindrical to spherical, the diameter being between 2 and 9 μm . Spherical, nonmotile reproductive bodies are formed. False branching may occur due to germination of spores within the sheath.	
	(2) May be branched:	
	(a) Cells from 0.5 to 1 μm in diameter	Genus: <i>Leptothrix</i> (Figs. 7 and 8)
	The filaments contain colorless, cylindrical cells which first have a thin colorless sheath that later becomes encrusted with iron oxide.	
	(b) Cells 2 μm or more in diameter	Genus: <i>Clonothrix</i> (Fig. 9)
	Filaments attached, show false branching. The sheaths are organic and encrusted with iron hydroxide or manganese, are broader at the base, and taper to the tip, varying from 7 to 2 μm . The cells are colorless, cylindrical, 2 by 10 μm . The filaments are colorless when young, becoming dark, yellowish-brown with age. Forms spherical reproductive cells on the short branches of the younger portions of the filaments.	

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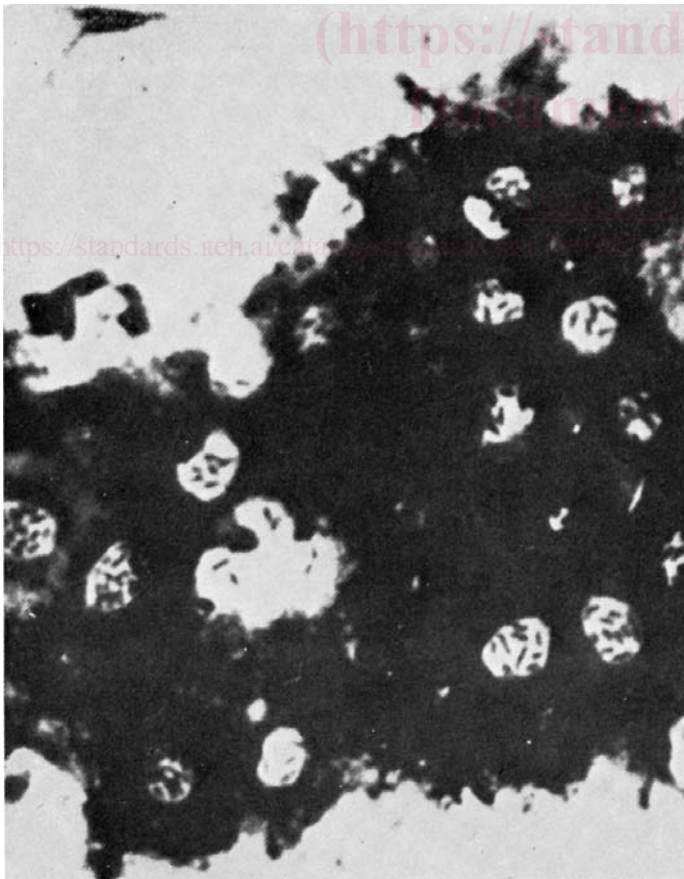


FIG. 1 *Siderocapsa treubii*. Multiple colonies surrounded by ferric hydrate. Magnification about 500 \times . Fig. 4 of Ref (5)

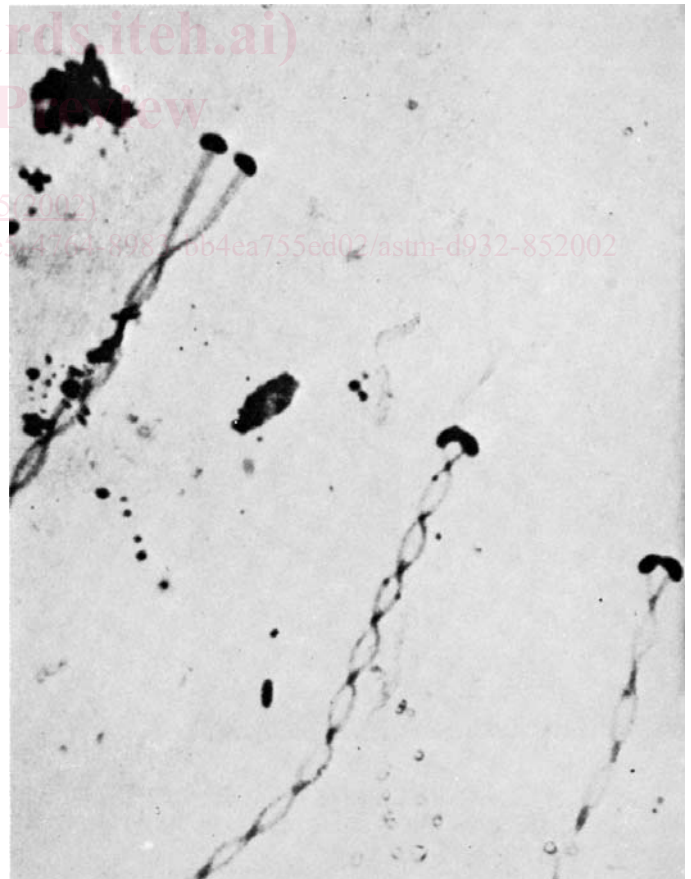


FIG. 2 *Gallionella major*. Cells at the ends of excretion bands undergoing division. Magnification about 1180 \times . Fig. 3 of Ref (6)

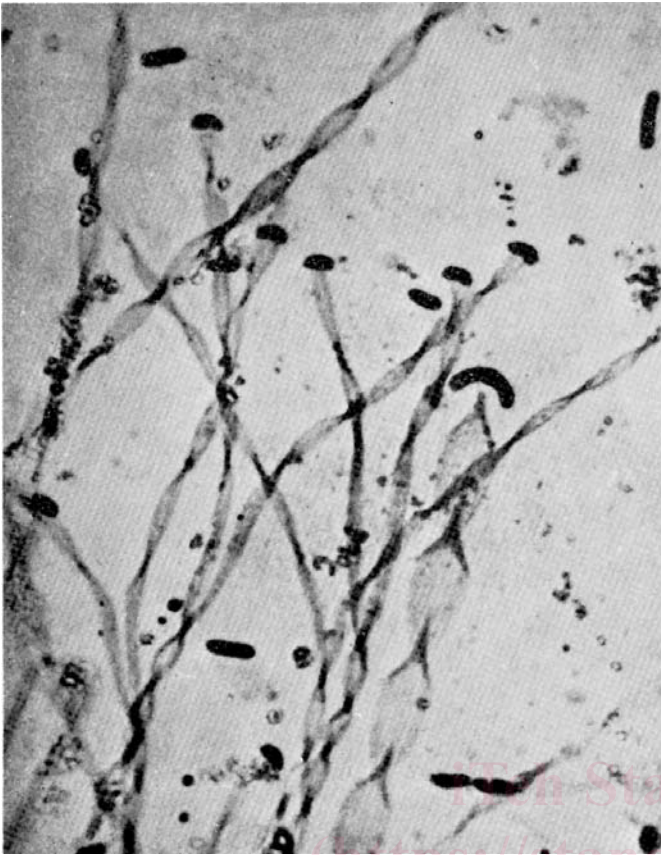


FIG. 3 *Gallionella major*. Curved cells at the ends of excretion bands. Magnification about 1120 × . Fig. 6 of Ref (6)

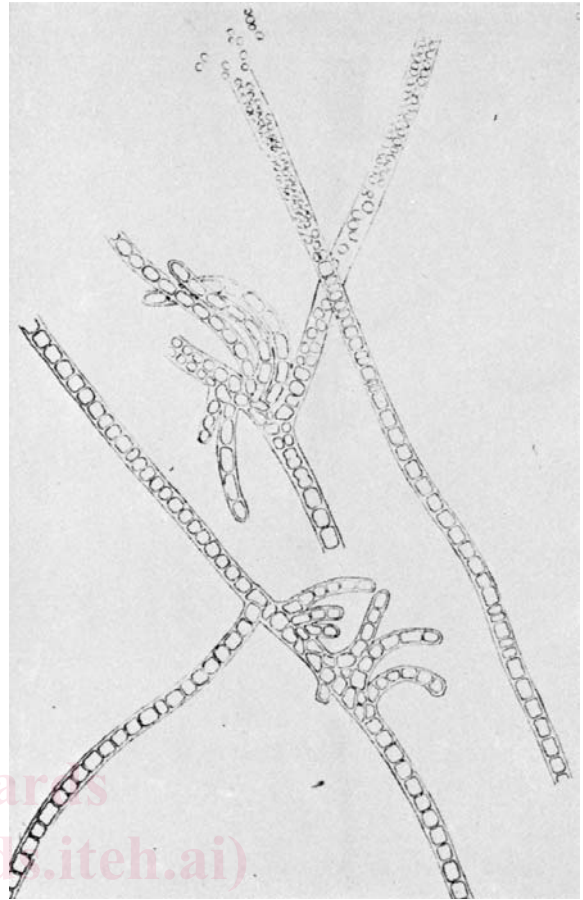


FIG. 5 *Crenothrix polyspora*. Sketch showing details of false branching of cells within sheath. Magnification about 380 × . Plate 1, Fig. A of Ref (8)



FIG. 4 *Sphaerotilus dichotoma*. Sketch showing false branching. Magnification about 230 × . Fig. 3b of Ref (7)

solution of 2.0 g of crystal violet (90 % dye content) in 20 mL of ethyl alcohol (95 % with a solution of 0.8 g of ammonium oxalate monohydrate (NH₄)₂C₂O₄·H₂O) in 80 mL of water.

7.4 *Hydrochloric Acid (1 + 4)*—Mix 1 volume of hydrochloric acid (HCl, sp gr 1.19) with 4 volumes of water.

7.5 *Iodine Solution*— Prepare Gram's modification of Lugol's solution (4) by dissolving 1 g of iodine in a solute containing 2 g of potassium iodide (KI) in 10 mL of water and diluting the resulting solution to 300 mL with water.

7.6 *Filter Paper or Blotter*.

7.7 *Slides*, standard type, 25 by 76-mm (1 by 3 in.) with either plain or frosted end.

7.8 *Cover Glasses*, round or square type, 19 mm (¾ in.) in diameter.

8. Sampling

8.1 Collect the samples in accordance with either Practices D 887 or D 3370, whichever is applicable.

8.2 Obtain a 500-mL (1-pt) sample of water, using a sterile 1-L (1-qt) bottle. The bottle should not be more than half-filled because of the oxygen demand of suspended matter; filling the bottle may cause the sample to become anaerobic.

8.3 If the number of iron bacteria are very low or that they are just becoming established in the system, use a small side stream filter to collect the sample to be examined. The water suspected of containing iron bacteria should be filtered through a highly retentive filter paper (or some other comparable media) for 24 h. Centrifugation or membrane filtration is