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**Testing method for detection and
enumeration of sulfate-reducing
bacteria in industrial water**

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Reference number : JIS K 0350-60-10 : 2005 (E)

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Foreword

This translation has been made based on the original Japanese Industrial Standard established by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee according to the proposal of establishing a Japanese Industrial Standard from Japan Industrial Water Association (JIWA)/Japanese Standards Association (JSA), with a draft of Industrial Standard based on the provision of Article 12 Clause 1 of the Industrial Standardization Law. Attention is drawn to the possibility that some parts of this Standard may conflict with a patent right, application for a patent after opening to the public, utility model right or application for registration of utility model after opening to the public which have technical properties. The relevant Minister and the Japanese Industrial Standards Committee are not responsible for identifying the patent right, application for a patent after opening to the public, utility model right or application for registration of utility model after opening to the public which have the said technical properties.

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Testing method for detection and enumeration of sulfate-reducing bacteria in industrial water

1 Scope This Japanese Industrial Standard specified the testing method for the qualitative and quantitative judgement of sulfate-reducing bacteria in industrial water by culture method.

2 Normative references The standards listed in attached table 1 contain provisions which, through reference in this Standard, constitute provisions of this Standard. The most recent editions of the standards (including amendments) shall be applied.

3 Definitions For the purposes of this Standard, the definitions given in **JIS K 0101**, **JIS K 0102**, **JIS K 0211**, **JIS K 0550** and the following definition apply.

3.1 sulfate-reducing bacteria obligately anaerobic bacteria, which produce hydrogen sulfide by reducing sulfate. They are (0.5 to 1.0) μm $\times$ (2.5 to 5.0) μm in size, indicated by curved bacillary or spiroid forms and move actively.

Remarks : Sulfate-reducing bacteria mostly inhabit the more polluted rivers apt to lack oxygen, the ooze in lakes and marshes, the intestines of warm-blooded animals and the like. These are often detected from industrial water, and the hydrogen sulfide produced from sulfate-reducing bacteria causes problems such as it giving an offensive smell to water and corroding the metallic parts of conduit pipes. *Desulfovibrio* genus are often detected.

4 Common matters The common matters shall be as follows:

4.1 General rules The general matters common to chemical analysis shall be in accordance with **JIS K 0050**.

4.2 Water Water of A2 or A3⁽¹⁾ specified in **JIS K 0557**.

Note (1) Water purified by distillation apparatus of quartz glass or borosilicate glass-1.

4.3 Reagents The reagents shall be as follows:

- a) The reagents of the highest quality among those specified in **JIS** shall be used. In the case of no reagent specified in **JIS**, the reagent without causing any hindrance to the test shall be used.
- b) Unless otherwise specified, the concentration of reagents solution shall be indicated by g/L or mg/L for mass concentration and by mol/L for mol concentration.

For the compounds, the mass as anhydride shall be used.

- Remarks 1 The concentration indicated by parenthesis at the back of the name of reagent solution means an approximate concentration.
- 2 The designations of reagents are made to conform with the naming scheme of compounds established by the Chemical Society of Japan and the designation of reagents in **JIS** as much as possible based on the nomenclature of inorganic chemistry and the nomenclature of organic chemistry of the International Union of Pure and Applied Chemistry (IUPAC).
- 3 The handling of reagents, wastewater, etc. shall follow the related laws and ordinances, and care shall be sufficiently taken.

4.4 Glassware The glassware, which are specified in **JIS R 3503** and **JIS R 3505**, shall generally be used. When special instruments are required, an example of them is illustrated or explained respectively. When there is an accompanying heating operation, the borosilicate glass-1 specified in **JIS R 3503** shall be used.

4.5 Sterilization of instruments The sterilization of instruments, etc. shall be as follows.

- a) **Dry heat sterilization** This can be used for the sterilization of instruments of glass, metal, etc. The instruments to be sterilized are wrapped with vegetable parchment or aluminium foil, etc. or placed in a metallic box for sterilization, etc., then put in a sterilizer for dry heat. After the inside temperature of the sterilizer reaches approximately 170 °C, it is heated approximately 1 h.
- b) **Autoclaving** This can be used for the sterilization of culture medium, dilution water, used culture medium, etc. And this can be used for the sterilization of fragile instruments by dry heat sterilization such as glassware of comparatively complicated structure, glass bottle with thick wall.

When large glass bottle, flask, etc. are autoclaved, a little water is poured in the inside beforehand. The sterilization time shall be 15 min to 20 min after the inside temperature of autoclaving sterilizer has reached 121 °C.

- c) **Flame sterilization** This can be used for the sterilization of the neck of test tube, etc. at the time of transference operation. This can be also used for the sterilization of water faucet, etc. at the time of sampling.

4.6 Disinfection operation The disinfection operation shall be as follows:

- a) Prior to and after tests, hands, fingers and laboratory benches shall be disinfected. For disinfection of hands and fingers, use cresol soap water (10 g/L), cationic soap solution (1 g/L to 10 g/L), ethanol for disinfection (specified in the Japanese Pharmacopoeia) or ethanol [80 % (volume percentage)] [prepared by using ethanol (95) specified in **JIS K 8102**].

Laboratory benches shall be disinfected by spraying cationic soap solution (10 g/L), ethanol for disinfection, ethanol [80 % (volume percentage)], etc., or by wiping them with a cloth moistened with these solutions.

- b) The instruments such as used pipets, sample container, water sampler shall be immersed in disinfectant solution such as cresol soap solution (30 g/L to 50 g/L) for 24 h (or autoclaved), and then rinse them with water enough to remove the disinfectant completely.

5 Sample

5.1 Sampling The sample shall be taken by using a sample container or a water sampler.

5.1.1 Instruments The instruments shall be as follows:

- a) **Sample container** A 100 ml to 1 000 ml glass bottle with grounding stopper. A sample container shall be sterilized⁽²⁾ by dry heat or by autoclaving after it has been washed with water and dried beforehand and its neck and stoppered place have been wrapped with aluminium foil (or vegetable parchment). Otherwise, a polyethylene bottle for bacterial test which has been sterilized may be used. Care shall be taken so as not to contaminate it until it is used for sampling.

Note ⁽²⁾ When the sample containing oxidative substance such as residual chlorine is taken, 20 mg to 30 mg of sodium thiosulfate pentahydrate (powdered) specified in **JIS K 8637** is put in the sample container and autoclaving sterilization or oxirane (ethylene oxide) sterilization is carried out beforehand. The sterilized polyethylene bottle for bacterial test on the market containing sodium thiosulfate may be used.

- b) **Water sampler** Heyroth type water sampler. The water sampler is set in a portable container and then sterilized by dry heat. It is exemplified in figure 1.

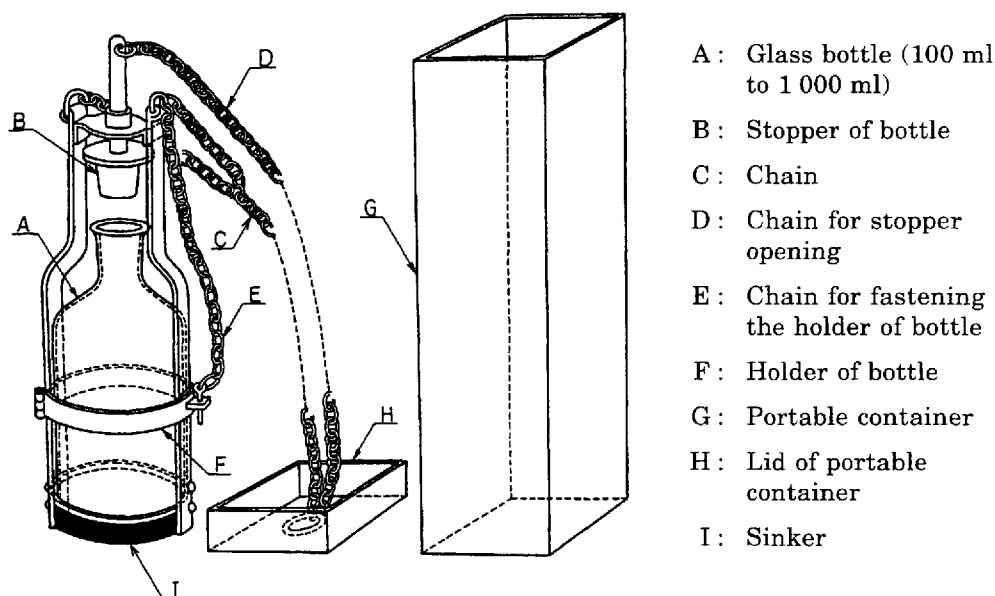


Figure 1 An example of Heyroth type water sampler and portable container