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(JIWA/JSA)

**Testing methods for standard plate
counts of heterotrophic bacteria
in industrial water and wastewater**

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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 the Japan Industrial Water Association (JIWA)/the Japanese Standards Association (JSA), with a draft of Industrial Standard based on the provision of Article 12 Clause 1 of the Industrial Standardization Law.

JIS K 0350 consists of the following four parts.

- JIS K 0350-10-10 *Testing methods for standard plate counts of heterotrophic bacteria in industrial water and wastewater*
- JIS K 0350-20-10 *Testing methods for detection and enumeration of coliform organisms in industrial water and wastewater*
- JIS K 0350-30-10 *Testing methods for detection and enumeration of heterotrophic bacteria in industrial water and wastewater*
- JIS K 0350-40-10 *Testing method for enumeration of total bacteria in industrial water and wastewater*

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Testing methods for standard plate counts of heterotrophic bacteria in industrial water and wastewater

1 Scope This Japanese Industrial Standard specifies testing methods for standard plate counts of general (heterotrophic) bacteria in industrial water and wastewater.

2 Normative references The standards given 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 shall be in accordance with **JIS K 0101**, **JIS K 0102**, **JIS K 0550** and **JIS K 0211**, and the following definition shall apply.

3.1 general (heterotrophic) bacteria General (heterotrophic) bacteria refer to all bacteria which form colonies in the culture medium when culturing at 36 ± 1 °C for 24 ± 2 h using the standard agar culture medium.

4 Matters in common Matters in common 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 used in this Standard shall be **A2** water or **A3** water⁽¹⁾ specified in **JIS K 0557**.

Note ⁽¹⁾ The metal-made still shall not be used.

4.3 Reagents

- a) The reagent specified in **JIS** shall be used. When there is no reagent specified in **JIS**, the reagent giving no hindrance to the test shall be used.
- b) For the concentration of the solution of reagents, in general, the mass concentration shall be expressed in the unit of g/L or mg/L and for the molality, in the unit of mol/L or m mol/L.

In addition, on treating compounds, the mass as anhydride shall be used.

- c) The concentration indicated in parentheses following the name of the solution of reagents refers to the rough value of concentration except in the case of a reference solution. For example, sodium hydroxide solution (1 mol/L) refers to sodium hydroxide solution of approximately 1 mol/L.
- d) The concentration of liquid reagents shall be expressed in the mixture ratio [reagents ($a + b$)] with water (or other liquid reagents). This expression indicates that reagents a ml and water (or other liquid reagents) b ml are mixed.

In addition, when liquid reagents (for example, hydrochloric acid) specified in Table 3 of **JIS K 0050** are used without dilution, only the names of reagents shall be stated.

- e) Water to be used for the preparation of reagents shall be that of 4.2.
- f) The names of reagents shall be coordinated as much as possible with those mentioned in the nomenclature of compounds specified by the Chemical Society of Japan and in **JIS** reagents, which are based on the nomenclature system for inorganic compounds and for organic compounds prescribed by International Union of Pure and Applied Chemistry (IUPAC).
- g) Care shall be sufficiently taken in accordance with the related laws and regulations for treating reagents and wastewater and the like.

4.4 Sterilizing procedures of implement and the like Sterilizing procedures of implement and the like shall be carried out as follows:

- a) **Dry heat sterilization** This is used for sterilization of glass-made and metal-made implements. Sterilize them at approximately 170 °C for approximately 1 h.
- b) **High-pressure steam sterilization** This is used for sterilization of culture media, dilution water, a sample container, culture media that have been used, etc. Sterilize them at 121 °C for 15 min to 20 min.
- c) **Flame sterilization** This is used for the sterilization of a test tube and the mouth part of a flask. Sterilize them by keeping the mouth part aslant in flame and turning for a while, after and before cultivating operation.

4.5 Disinfection operations Disinfection operations shall be carried out as follows:

- a) Before and after test operation, disinfect fingers and a testing bench. Use cresol soap solution (10 g/L), cationic soap solution (1 g/L to 10 g/L), or alcohol for disinfection (which is specified in the Japanese Pharmacopoeia) or ethanol (80 vol%) [prepared using ethanol (95) specified in **JIS K 8102**], for disinfection of fingers.

For the disinfection of a testing bench, either spray cationic soap solution (10 g/L), alcohol for disinfection or ethanol (80 vol%), etc. or wipe with the cloth moistened with these solutions.

- b) Employed implements such as a pipette, a sample container, a water sampler, and so on, shall be immersed in disinfection solution such as cresol soap solution (30 g/L to 50 g/L) and the like for 1 day (or shall be sterilized by high-pressure steam), and washed with water sufficiently until disinfection solution is completely removed.
- c) Sterilize the test tube, petri dish or the like⁽²⁾, which has been used for culture test, by high-pressure steam method in 4.4 b) together with culture media, then throw away the culture media, and wash sufficiently with water.

Note ⁽²⁾ For the plastic petri dish or the like, discard it after adequate sterilizing.

4.6 Glass ware implements For glass ware implements, those which are specified in **JIS R 3503** and **JIS R 3505**, shall generally be used. However, when special implements are required, one example of them for each item shall be illustrated or explained. Moreover, when heating operations are involved, the borosilicate glass-1 specified in **JIS R 3503** shall be used.