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(JIWA/JSA)

Testing methods for detection and enumeration of coliform organisms in industrial water and waste water

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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)/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.

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Testing methods for detection and enumeration of coliform organisms in industrial water and waste water

1 Scope This Japanese Industrial Standard specifies testing methods for detection and enumeration of coliform organisms in industrial water and waste water.

2 Normative references The Standards given in Attached Table 1 contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the Standards (including Amendments) shall apply.

3 Matters in common

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

3.2 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.

- a) **coliform group bacteria** Gram's stain negative sporeless bacillus, that is, aerobic or facultative anaerobic bacteria which can decompose lactose to generate acid and gas.

3.3 Water Water used in this Standard shall be **A2** water or **A3** water⁽¹⁾ specified in **JIS K 0557**.

Note ⁽¹⁾ Water which is refined with the still made from silica glass or borosilicate glass-1.

3.4 Reagents Reagents shall be as follows:

- a) When the name of reagent is specified, the most excellent grade specified in **JIS** shall be used, and when there is no reagent specified in **JIS**, the reagent giving no hindrance 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 or the molality in the unit of mol/L or mmol/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 in other case of the 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 of a ml and water (or other liquid reagents) of b ml are mixed.

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

- e) Water to be used for the preparation of reagents shall be water of **3.3**.
- f) Care shall be sufficiently taken in accordance with the related laws and regulations for treating reagents and waste water and the like.

Informative reference : The name of reagents shall be coordinated 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**) as much as possible.

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

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

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

- a) Before and after test operations, 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 %) [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 sampling bottle, 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 wash with water sufficiently enough to remove disinfection solution completely.
- c) Sterilize the test tube, petri dish or the like, which has been used for culture test, by the high-pressure steam method in **3.5 b)** together with culture media, then throw away the culture media, and wash sufficiently with water.

3.7 Glass ware implements For glass ware implements, those which are specified in **JIS R 3503** and **JIS R 3505**. 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.

4 Sample

4.1 Sampling and collection of bacteria In case the number of coliform group bacteria in the sample is anticipated to be many, sampling shall be carried out using a sampling bottle or water sampler. In case the number of coliform group bacteria in the sample is anticipated to be few, filter a fixed quantity of sample with the membrane filter of 0.45 μm in pore size and collect coliform group bacteria on the membrane filter.

a) **Implements** Implements shall be as follows:

- 1) **Sampling bottle** 100 ml glass bottle with a ground stopper. Carry out the dry heat sterilization in **3.5 a)** or the high pressure steam sterilization in **3.5 b)** on the sampling bottle⁽²⁾ whose stopper part and neck part have been covered with aluminium foil (or parchment paper), etc. Alternatively, a sterilized polyethylene bottle for a bacteria test may be used. Take care so that the sampling bottle is not subjected to contamination until sampling begins.

Note ⁽²⁾ When sampling the water which contains oxidizing material as residual chlorine, place 20 mg to 30 mg of sodium thiosulfate pentahydrate (powdered) specified in **JIS K 8637** into the sampling bottle, and then sterilize it by the high pressure steam method in **3.5 b)** or by the oxirane (ethylene oxide) method.

- 2) **Water sampler** Fig. 1 shows an example. Heyroth type water sampler. The water sampler, put in a portable box, shall be sterilized by the dry heat sterilization in **3.5 a)**⁽³⁾.

Note ⁽³⁾ When using a sampling bottle which has been sterilized in accordance with **1)** for a water sampler, the sterilization of the sampling bottle may be omitted.

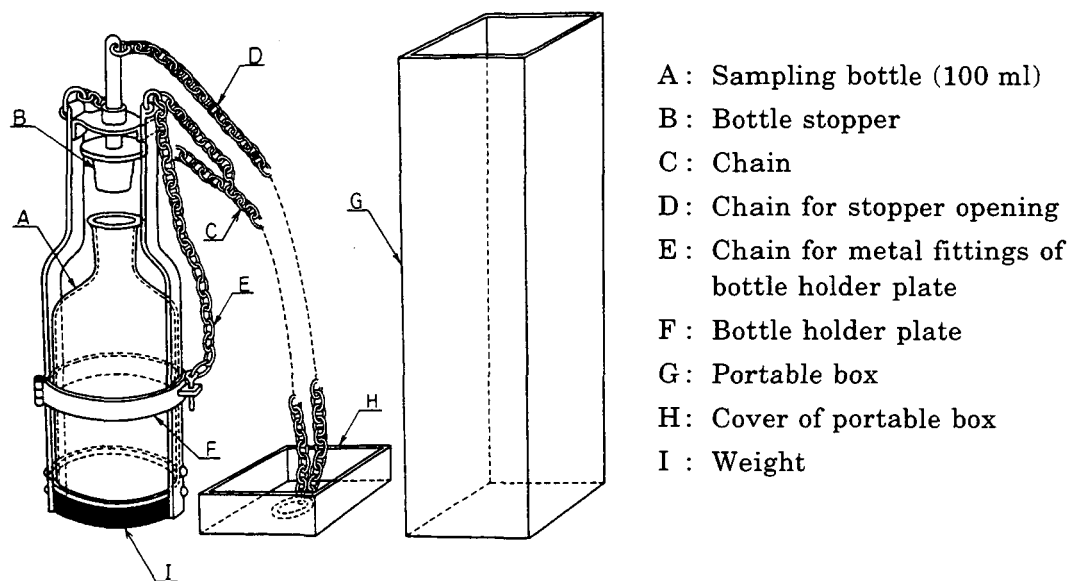


Fig. 1 An example of water sampler

- 3) **Filter (separate type)** Fig. 2 shows an example. Wrap each part of a filter in aluminium foil (or parchment paper etc.), and carry out the sterilization suitable for materials.

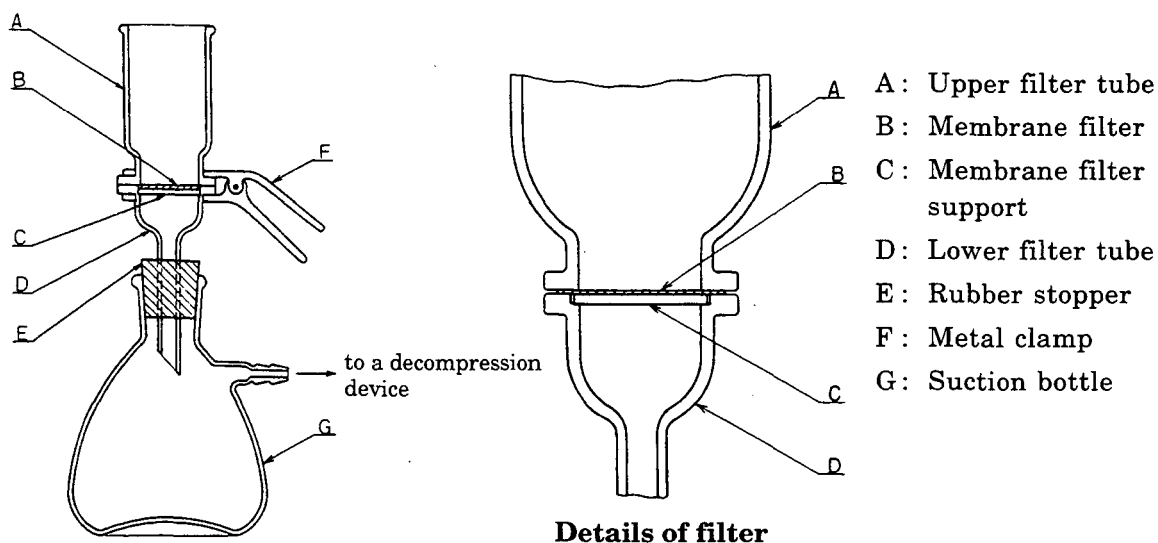


Fig. 2 An example of filter (separate type)

- 4) **Membrane filter** The membrane filter of 0.45 μm in pore size, approximately 50 mm in diameter. Wrap it in aluminium foil (or parchment paper, etc.), place it in a glass petri dish and carry out the high-pressure steam sterilization in 3.5 b). Or commercial products which have been sterilized may be used. For handling a membrane filter, a pincette shall be used.
- 5) **Pincette** The pincette whose tip is flat and smooth. Carry out the flame sterilization in 3.5 c) just before use.
- b) **Operations** Sampling and collection of bacteria shall be carried out as follows:
- 1) In case the number of coliform group bacteria in the sample is anticipated to be many, take the sample into a sampling bottle.