

HJ

INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

HJ 347.2-2018

Water quality - Determination of fecal coliform - Manifold zymotechnics

水质 粪大肠菌群的测定 多管发酵法

(English Translation)

Issued on: December 26, 2018

Implemented on: June 1, 2019

Issued by: Ministry of Ecological Environment

Table of Contents

Foreword...	4
1 Scope...	6
2 Normative references...	6
3 Terms and definitions...	6
4 Principles of the method...	7
5 Interference and elimination...	7
6 Reagents and materials...	7
7 Instruments and equipment...	9
8 Samples...	9
9 Analytical procedures...	10
10 Result calculation and presentation...	12
11 Precision and accuracy...	13
12 Quality assurance and quality control...	14
13 Waste disposal...	15
Appendix A (Informative) Most probable number (MPN) table...	16

1 Scope

This standard specifies the manifold zymotechnics, for the determination of fecal coliforms in water.

This standard applies to the determination of fecal coliforms, in surface water, groundwater, domestic sewage, industrial wastewater.

The detection limit of this method is 3 MPN/L for the 12-pipe method; 20 MPN/L for the 15-pipe method.

2 Normative references

This standard refers to the following documents or clauses thereof. For undated references, the valid edition applies to this standard.

GB/T 14581 Water quality - Guidance on sampling techniques from lakes, natural and man-made

HJ 494 Water quality - Guidance on sampling techniques

HJ/T 91 Technical specifications requirements for monitoring of surface water and waste

water

3 Terms and definitions

The following terms and definitions apply to this standard.

3.1

Fecal coliforms

Also known as thermotolerant coliforms. The aerobic and facultative anaerobic

Gram-negative A. budding bacteria, that can ferment lactose to produce acid and gas, after being cultivated at 44.5 °C for 24 h.

3.2

Most probable number (MPN)

Also known as dilution culture counting, which is an indirect counting method, based on Poisson distribution. Using statistical principles, according to the positive number of target microorganisms, which are produced by a certain volume of samples, at different dilutions after culturing, look up the Table to estimate the number of target microorganisms, in a certain volume of samples (the most probable number of target microorganisms per unit volume).

4 Principles of the method

The samples are added to the test tube, which contains lactose peptone medium. The initial fermentation and enrichment culture is carried out at 37 °C. The coliform bacteria grow and reproduces in the medium; decomposes lactose to produce acid and gas. The generated acid changes the bromocresol purple indicator from purple to yellow; the gas produced goes into the reverse tube, indicating gas production. After re-fermentation at

44.5 °C, the bile salt No.3 in the medium can inhibit the growth of Gram-positive bacteria; the bacteria, that produced gas at the end, are determined to be fecal coliforms.

By checking the MPN Table, the fecal coliform concentration value is obtained.

5 Interference and elimination

5.1

Active chlorine has oxidative properties, which can destroy the enzyme activity in microbial cells AND cause cell death. Sodium thiosulfate solution (6.7) can be added, during sample collection (8.1), to eliminate the interference.

5.2The metal ions have cytotoxicity, which can destroy the enzymatic activity in the

microbial cells AND cause cell death. The disodium EDTA solution (6.8) can be added, during sample collection (8.1), to eliminate the interference.

6 Reagents and materials

Unless otherwise stated, analytical reagents or biological reagents, that meet national standards, are used in the analysis. The experimental water is distilled or deionized water.

6.1

Lactose peptone medium.

Peptone: 10 g

Beef extract: 3 g

Lactose: 5 g

Weigh 15.7 g of sodium thiosulfate (6.5). Dissolve it in an appropriate amount of water.

Make its volume reach to 100 ml. Prepare it before use.

6.8 Disodium EDTA solution: $\rho(\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}) = 0.15 \text{ g/ml}$

Weigh 15 g of disodium EDTA (6.6). Dissolve it in an appropriate amount of water.

Make its volume reach to 100 ml. This solution can be stored for 30 days.

7 Instruments and equipment

7.1

Sampling bottle

500 ml wide-mouth glass bottle with screw cap or ground stopper.

7.2

Autoclave

115 °C, 121 °C adjustable.

7.3 Constant temperature incubator or water bath: The allowable temperature deviation

is $37\text{ °C} \pm 0.5\text{ °C}$, $44\text{ °C} \pm 0.5\text{ °C}$.

7.4 pH meter: Accurate to 0.1 pH unit.

7.5 Inoculation loop: 3 mm in diameter.

7.6 Test tubes: 300 ml, 50 ml, 20 ml.

7.7 Common laboratory instruments and equipment.

Note: Glassware and sampling equipment shall be wrapped, according to the requirements of aseptic operation before the test; sterilized by high pressure steam at 121 °C for 20 minutes, for later use.

8.1 Sample collection

The point layout and sampling frequency shall be implemented, in accordance with the relevant provisions of GB/T 14581, HJ/T 494, HJ/T 91.

When collecting microbial samples, the sampling bottle (7.1) shall not be washed by the sample. The sample shall be collected in a sterilized sampling bottle. The sampling volume of clean water bodies is not less than 400 ml. The sampling volume of other water bodies is not less than 100 ml.

When collecting surface water samples, such as rivers, lakes, reservoirs, it may hold the bottom of the bottle, to directly insert the stoppered sampling bottle into the water, at about 10 ~ 15 cm away from the water surface, with the bottle mouth facing the direction of water flow. Pull out the stopper, to let the sample flow into the bottle. Then cap the bottle. Remove the sampling bottle from the water. If there is no water flow, it may hold the bottle, to push it horizontally forward. The sampling volume is generally about 80% of the capacity of the sampling bottle. After the samples are collected, they are quickly wrapped with sterile wrapping paper.

When collecting samples from the faucet device, do not select a leaking faucet. Before collecting water, open the faucet to the maximum flow, to let the water run for 3 ~ 5 minutes. Then close the faucet. Use flame to burn it, for about 3 minutes, for sterilization; OR use 70% ~ 75% alcohol to sterilize the faucet. Turn on the faucet to the maximum, to let the water run for 1 min, to fully remove the retained impurities in the water pipe. When sampling, control the water flow rate to carefully let it flow into the bottle.

When collecting surface water, wastewater samples, samples at a certain depth, sterilized special sampling devices can also be used for sampling.

When stratified sampling is carried out, at the same sampling point, it shall be carried out from top to bottom, to avoid disturbance of different levels.

If a sample containing active chlorine is collected, add sodium thiosulfate solution (6.7), before sterilizing the sampling bottle, to remove the inhibitory effect of active chlorine on bacteria (add 0.1 ml of sodium thiosulfate solution per 125 ml volume). If the sample with high heavy metal ion content is collected, add disodium EDTA solution (6.8), before sterilizing the sampling bottle, to eliminate interference (add 0.3 ml of disodium EDTA solution per 125 ml volume).

Note: 15.7 mg of sodium thiosulfate (6.5) can remove 1.5 mg of active chlorine in the sample; the consumption of sodium thiosulfate can be adjusted, according to the actual active