

HJ

INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

HJ 347.1-2018

Water quality - Determination of fecal coliform - Membrane filtration

水质 粪大肠菌群的测定 滤膜法

(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...	14
14 Precautions...	15
Appendix A (Informative) Test record and report format of fecal coliform...	16

1 Scope

This standard specifies the membrane filtration method, 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: When the inoculation volume is 100 ml, the detection limit is 10 CFU/L; when the inoculation volume is 500 ml, the detection limit is 2 CFU/L.

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. It is and Enterobacteriaceae which, after cultured at 44.5 °C for 24 hours, can grow on MFC selective medium, ferment lactose to produce acid, form blue or blue-green colonies.

Bile salt No.3: 1.5 g

1% aniline blue aqueous solution: 10 ml

1% rose red acid solution (dissolved in 8.0 g/L sodium hydroxide solution): 10 ml

Dissolve the components in the above medium (except aniline blue and rose red acid), in 1000 ml of water. Adjust the pH to 7.4. Distribute them in conical flasks. Sterilize them, at high pressure steam at 115 °C, for 20 min. Store them in a cold and dark place, to prepare for use. Before use, according to the above formula proportions, use a sterilized straw, to respectively add 1 ml of 1% aniline blue aqueous solution, that has been boiled and sterilized, AND 1 ml of 1% rose red acid solution (dissolved in 8.0 g/L sodium hydroxide solution). Mix well. If there are not many bacteria in the culture, it is also possible not to add rose red acid. Before heating to dissolve, add 1.2% ~ 1.5% agar to make solid medium. It may also use the commercially available media. The prepared medium shall be protected from light and stored in a dry place. If necessary, it shall be stored in a refrigerator, at 5 °C ± 3 °C. The culture medium, which is dispensed into plates, can be stored for 2 ~ 4 weeks. The prepared medium cannot be thawed many times; it shall prepare a small amount of it frequently. When the color of the medium changes, OR the dehydration is obvious, it shall be discarded.

6.2 Sterile filter membrane: Cellulose acetate filter membrane, which has a diameter of

50 mm and a pore size of 0.45 µm. It is wrapped according to aseptic operation requirements; sterilized by high pressure steam at 121 °C, for 20 min; dried naturally for later use. OR put the filter membrane into a beaker; add the experimental water;

make it boil and sterilize for 3 times, 15 min/time. After the first 2 times of boiling, it needs to be rinsed 2 ~ 3 times, by changing water.

6.3 Sterile water: Take an appropriate amount of experimental water; sterilize it by high pressure steam at 121 °C, for 20 min. Prepare for use.

6.4 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$).

6.5 Disodium EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}$).

6.6 Sodium thiosulfate solution: $\rho(\text{Na}_2\text{S}_2\text{O}_3) = 0.10 \text{ g/ml}$

Weigh 15.7 g of sodium thiosulfate (6.4). Dissolve it in an appropriate amount of water.

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

6.7 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 (6.5). Dissolve it in an appropriate amount of water.

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

~ 5 minutes. Then close the faucet. Burn it with flame for about 3 minutes for sterilization.
OR use 70% ~ 75% alcohol to sterilize the faucet. Turn on the faucet to the maximum flow, to let the water run for another 1 min, to fully remove the retained impurities in the water pipe. When sampling, control the water flow rate and carefully collect it into the bottle.

When collecting surface water, wastewater samples, samples at a certain depth, it may also use sterilized special sampling devices, 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 at different levels.

If samples containing active chlorine are collected, add sodium thiosulfate solution (6.6), 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 taking the sample, which has high heavy metal ion content, add disodium EDTA solution (6.7), 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 (6.4) can remove 1.5 mg of active chlorine in the sample;

the consumption of sodium thiosulfate can be adjusted, according to the actual active chlorine content of the sample.

8.2 Sample preservation

After sampling, it shall be tested within 2 hours. Otherwise, it shall be refrigerated below 10 °C, but not more than 6 hours. After the laboratory receives the sample, if the testing cannot be carried out immediately, the sample shall be refrigerated below 4 °C

AND tested within 2 hours.

9.1 Sample filtration

Judge the inoculation volume according to the type of sample. The minimum filtration volume is 10 ml. If the inoculation volume is less than 10 ml, it shall be diluted level by level. First estimate the volume, which is suitable for counting on the filter membrane. Then take 1/10 and 10 times of this volume. Filter it, respectively. The ideal sample inoculation amount is that, the number of fecal coliform colonies, which grow on the filter membrane is 20 ~ 60; the total number of colonies shall not exceed 200.

When the minimum filtration volume is 10 ml AND the colony density on the filter is still too high, the sample shall be diluted. The method for 1:10 dilution is as follows:

pipette 10 ml of the sample; inject it into a conical flask, which contains 90 ml of sterile water (6.3). Mix well. Prepare a 1:10 diluted sample. See Table 1 for the reference table of sample inoculation volume.

11.2 Accuracy

Six laboratories carry out six repeated determinations of standard samples, which contain fecal coliforms, at a concentration of 3670 MPN/L (acceptable range of 330 ~

7710 MPN/L): the relative error range is -19% ~ -32%; the final value of relative error is: $-23\% \pm 10\%$.

Note: The microbiological detection data are skewed distribution. All the measurement results are calculated, after logarithmic transformation with base 10.

12.1 Culture medium inspection

When changing different batches of medium, it shall inspect the positive and negative strains. Prepare the positive strains (such as *Escherichia coli*) and negative strains (such as *Enterobacter aerogenes*), into appropriate concentrations. According to the requirements of sample filtration (9.1), make the number of colonies, which grow on the filter membrane, be 20 ~ 60. Then operate according to the requirements of culture (9.2). The positive strains shall grow into blue or blue-green colonies; the negative strains shall grow gray, pale yellow, colorless or no colony growth. Otherwise, the test result of this sample is invalid. It shall find out the reasons and make determination again.

12.2.1 Blank control

For each test, sterile water shall be used for laboratory blank determination (9.3.1).

There shall be no colony growth, on the cultured medium. Otherwise, the test result of this sample is invalid. It shall find out the reasons and make determination again.