

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

HJ 1001-2018

**Water quality - Determination of total coliforms, fecal coliforms
and Escherichia coli - Enzyme substrate method**

水质 总大肠菌群、粪大肠菌群和 大肠埃希氏菌的测定 酶底物法

(English Translation)

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Foreword

To protect the ecology of the People's Republic of China Environmental Protection Law and the Law of the People's Republic of China on Water Pollution Prevention and Control this standard is established for the environment, to ensure human health, and to regulate the total coliform, fecal coliform and *Escherichia coli* in water.

This standard specifies the determination of total coliforms, fecal coliforms in surface water, groundwater, domestic sewage and industrial wastewater.

Enzyme substrate method for *Escherichia coli*.

Appendix A of this standard is a normative appendix, and Appendix B and Appendix C are informative appendices.

This standard is the first release.

This standard is formulated by the Department of Eco-Environmental Monitoring and the Department of Regulations and Standards of the Ministry of Ecology and Environment.

This standard was drafted. Shanghai Environmental Monitoring Center.

This standard is verified by the Jiangsu Provincial Environmental Monitoring Center, the Zhejiang Environmental Monitoring Center, and the Changzhou Environmental Monitoring Center.

Haisong District Environmental Monitoring Station, Shanghai Changning District Environmental Monitoring Station and Shanghai Qingpu District Environmental Monitoring Station.

This standard is approved by the Ministry of Ecology and Environment on December 26, 2018.

This standard has been implemented since June 1, 2019.

This standard is explained by the Ministry of Ecology and Environment. 1 Determination of total coliforms, fecal coliforms and Escherichia coli in water quality

1 Scope of application

This standard specifies the enzyme substrate method for the determination of total coliforms, fecal coliforms and Escherichia coli in water.

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

The detection limit of this method is 10 MPN/L.

2 Normative references

This standard refers to the following documents or their terms. For undated references, the valid version applies to this standard.

GB/T 6682 analysis of experimental water specifications and experimental methods GB/T 14581 Water quality lake and reservoir sampling technical guidance HJ 494 Water Quality Sampling Technical Guidance HJ/T 91 Surface Water and Wastewater Monitoring Technical Specifications

3 Terms and definitions

The following terms and definitions apply to this standard.

Total coliforms total coliforms Cultured at 37 ° C for 24 h, can produce beta-galactosidase (beta-D-galactosidase), decomposes the adjacent nitrate in selective medium Alkaloid-beta-D-galactopyranoside (ONPG) Enterobacteriaceae bacteria producing yellow

o-nitrophenol.

Fecal coliforms fecal coliforms Also known as thermotolerant coliforms. Cultured at 44.5 ° C for 24 h, can produce beta-galactosidase (beta-D-galactosidase), decomposition of o-nitrophenyl-beta-D-galactopyranoside (ONPG) in selective medium and Enterobacteriaceae bacterium producing a yellow o-nitrophenol.

Escherichia coli

Commonly known as *E. coli*. Cultured at 37 ° C for 24 h, can produce beta-galactosidase (beta-D-galactosidase), decomposition selectivity o-Nitrobenzene-beta-D-galactopyranoside (ONPG) in the medium produces yellow o-nitrophenol while producing beta-glucuronidase (beta-Glucuronidase), decomposes 4-methylumbelliferyl-beta-D-glucuronide 2 in selective medium (MUG) Enterobacteriaceae bacteria that release the fluorescent substance (4-methylumbelliferone).

The largest possible number of most probable number (MPN) Also known as dilution culture count, it is an indirect counting method based on Poisson distribution. Using statistical principles, according to certain bodies the number of target microorganisms produced by culturing samples with different dilutions, and the table to estimate the target microorganisms in a certain volume of samples the quantity present (the maximum possible number of target microorganisms per unit volume).

4 Principle of the method

When cultured at a specific temperature for a specific period of time, total coliform, fecal coliform, and *Escherichia coli* can produce beta-galactosidase, which decomposes the colorless substrate o-nitrophenyl-beta-D-galactopyranoside (ONPG) in a selective medium into yellow o-nitrophenol (ONP); *Escherichia coli* can also produce beta-glucuronidase, 4- in selective medium Methylumbelliferone-beta-D-glucuronide (MUG) is decomposed into 4-methylumbelliferone, which fluoresces under ultraviolet light.

Count the number of positive reactions, check the MPN table, and calculate the concentration of total coliform, fecal coliform, and *Escherichia coli* in the sample.

5 Interference and elimination

5.1

Active chlorine is oxidizing and can destroy the enzyme activity in microbial cells, leading to cell death.

The sodium thiosulfate solution (6.4) can be added during sample collection (8.1) to eliminate interference.

5.2

Heavy metal ions are cytotoxic and can destroy the enzyme activity in microbial cells, leading to cell death.

Add (2.8) to the disodium edetate solution (6.5) to eliminate interference.

6 Reagents and materials

Unless otherwise stated, analytically pure reagents that meet national standards are used for analysis, and laboratory water should meet the requirements for tertiary water in GB/T 6682.

6.1

Medium.

This standard uses Minimal Medium ONPG-MUG medium. Use medium powder per 100 ml sample 2.7 ± 0.5 g, the basic ingredients are as follows.

6.2

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

6.3

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

6.4

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

Weigh 15.7 g of sodium thiosulfate (6.2), dissolve in an appropriate amount of water, and dilute to 100 ml.

6.5

Ethylenediaminetetraacetic acid disodium solution. $\rho(\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8\text{Na}_2 \cdot 2\text{H}_2\text{O}) = 0.15$ g/ml.

Weigh 15 g of disodium edetate (6.3), dissolve in an appropriate amount of water, and dilute to 100 ml. The shelf life of this solution is 30 d.

6.6 96-well tray. 49 large holes and 48 small holes. Among them, each hole can hold 0.186 ml sample, large hole the 48 large holes each hold 1.86 ml of sample and one top large hole accommodates 11 ml of sample. Commercially available finished products sterilized by ethylene oxide can also be used.

6.7

Standard positive colorimetric plate.